

CFTR: Mechanism of Anion Conduction

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Dawson, David C., Stephen S. Smith, and Monique K. Mansoura. CFTR: Mechanism of Anion Conduction. *Physiol. Rev.* 79, Suppl.: S47–S75, 1999.—The purpose of this review is to collect together the results of recent investigations of anion conductance by the cystic fibrosis transmembrane conductance regulator along with some of the basic background that is a prerequisite for developing some physical picture of the conduction process. The review begins with an introduction to the concepts of permeability and conductance and the Nernst-Planck and rate theory models that are used to interpret these parameters. Some of the physical forces that impinge on anion conductance are considered in the context of permeability selectivity and anion binding to proteins. Probes of the conduction process are considered, particularly permeant anions that bind tightly within the pore and block anion flow. Finally, structure-function studies are reviewed in the context of some predictions for the origin of pore properties.

I. CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR: A CHANNEL AND A CHANNEL REGULATOR

In the years B.C., that is before cloning in 1989 of the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), it was well established

that the inherited disease cystic fibrosis was characterized by a defect in ion transport in both secretory and absorptive epithelial cells (116–119). When the gene was identified by means of a positional cloning strategy that was not driven by any presumption about the function of the gene product, the determination of the primary structure of CFTR set off an explosion of studies aimed at defining

the function of this protein. Early investigations of CFTR function were dominated by the question of whether CFTR was itself an ion channel or if, instead, it served to regulate other channels in the cell. Now, 10 years A.C., there is a substantial amount of evidence in support of the notion that CFTR functions as a Cl channel, but there is, in addition, a growing body of evidence that the presence of CFTR can exert a regulatory influence on other Cl-selective channels and cation-selective channels (6, 10, 48, 56, 61, 135, 144).

This review focuses exclusively on the anion-selective channel function of CFTR and, in particular, on the mechanism of ion conduction through the anion-selective pore. Our understanding of CFTR anion conduction mechanisms is in a primitive state. Whereas the primary structure (80, 126, 128) of the cytosolic domains of CFTR and their homology to other proteins accurately foreshadowed the regulation of channel activity by protein kinase A and ATP, the amino acid sequence of the predicted membrane-spanning segments did not provide many clues as to how the pore might be formed. Important leads have emerged from several sources, however. One is the large number of mutations that have been identified in the gene, now over 700. Mutations associated with disease of varying severity point the way to residues in the protein that may be important for conduction (107, 137, 138). Amino acid conservation across vertebrates may also identify "critical" residues (101) and, as always, we are free to apply all manner of guesses based on presumptions about the underlying physics of the conduction process.

The aim of this review is to gather together the existing information about CFTR anion conduction and to examine what all of this may suggest about the mechanisms that underlie the conduction process and are responsible for the ion selectivity of the channel. Two features of anion conduction through CFTR have emerged as particularly important. First, it appears that the entry of anions into the channel from the adjacent aqueous solution is strongly influenced by the ion-water interactions. Second, there is substantial evidence that some anions, e.g., SCN, bind tightly in the pore and that altered anion binding is a sensitive index of structural changes in the conduction path. The review focuses on these properties. We begin with a review of the measurements that are used to define channel properties and a brief consideration of the models that are used to interpret them. In the process, we will have the opportunity to address some general questions about the design of an anion-selective pore.

II. CONDUCTANCE AND PERMEABILITY

For the purpose of this review, an ion channel is defined as a purely dissipative, conducting element, i.e., one that can be represented by a resistor in an equivalent

circuit and that functions in a purely permissive fashion. Like a valve, it permits ions to flow when it is open, but that flow must be driven by an external driving force, i.e., an ion concentration gradient or an electrical potential difference. A purely dissipative element has no ability to couple free energy, derived from ATP hydrolysis or an ion gradient for example, to the flow of something else. This expectation for an ion channel was a source of some consternation for those who attempted to predict the function of CFTR from its primary structure and apparent topology. The presence of two domains (NBF1 and NBF2) that were likely to hydrolyze ATP provoked an image that was more that of a pump or transporter than that of an ion channel (see Fig. 1). It now seems likely, however, that the hydrolysis of ATP at NBF1 and NBF2 is a crucial step in regulating the opening and closing of the CFTR Cl channel (5, 9, 72, 141, 158). This review focuses on the conduction properties of the open channel, but the reader should not lose sight of the fact that ATP hydrolysis could have functions other than opening and closing a Cl-conducting pore, particularly with regard to the emerging role of CFTR as a regulator of other channels, and as yet poorly defined relationships to ATP transport (1, 60, 122, 123, 135). In addition, Linsdell and Hanrahan (94) recently reported an apparent ATP-dependent asymmetry in the conduction of organic anions through CFTR that may point to some additional mode of ion transport by CFTR.

A. Ohm's Law

The most economical description of the behavior of an ion channel is Ohm's law in which the flow of ions, expressed as an electric current (i), is written as the product of the conductance of the channel (γ) and the total electrochemical driving force, i.e.

$$i = \gamma(V_m - E_{\text{rev}}) \quad (1)$$

where γ is the conductance of a single channel (in pS), V_m is the membrane potential (in mV) referenced to the outside of the cell, and E_{rev} is the reversal potential defined as the value of V_m (in mV) at which $i = 0$. The value of E_{rev} is determined by the gradients of permeant ions across the membrane as described below. The dissipative or passive nature of the flow process is captured in the driving force term, $V_m - E_{\text{rev}}$; if the net electrochemical force is zero, then ion flow is zero. In the presence of a net driving force, the magnitude of flow is determined by the conductance γ , a concise description of the series of events that results in ion translocation, i.e., 1) the movement of the ion from the aqueous solution into the channel, 2) translocation of the ion through the channel pore, and 3) the exit of the ion on the other side. In any investigation of channel conduction properties, the behavior of

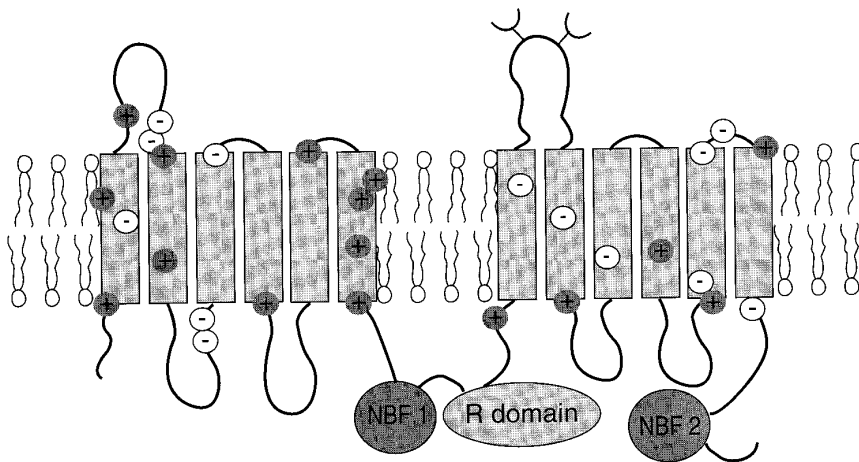


FIG. 1 Diagrammatic representation of domain topology of cystic fibrosis transmembrane conductance regulator (CFTR) showing location of charged residues in predicted transmembrane segments.

these two parameters, channel conductance and the E_{rev} for single-channel current, are the principal basis for inferring ion translocation mechanisms. Both can provide important information about the environment of the pore as experienced by permeating ions.

B. Conductance

Conductance is measured by determining the i - V_m relation for the channel, a plot of the single-channel current at different voltages as indicated in Figure 2. If this

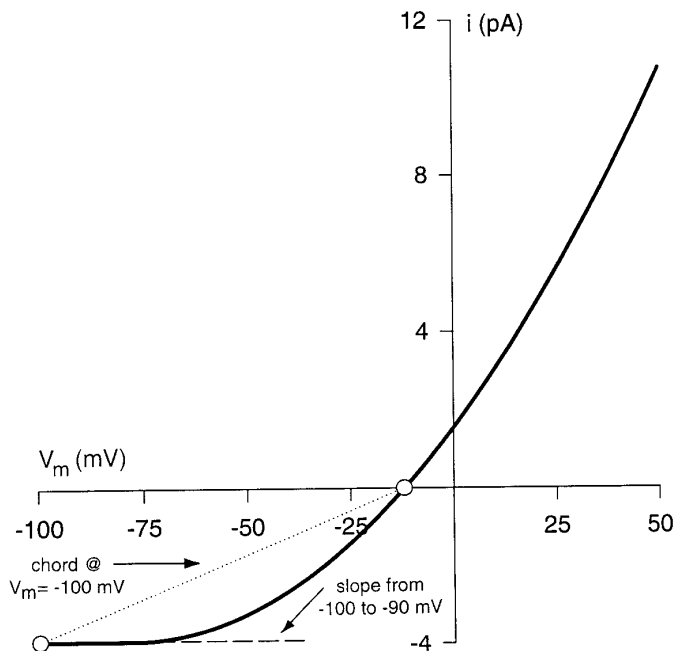


FIG. 2. Plot of current (i) vs. voltage (V_m) for a hypothetical single channel that exhibits strong outward rectification such that inward current becomes voltage independent at hyperpolarized potentials. Lines represent conductance as defined by slope (dashed line) or chord (dotted line) at $V_m = -100$ mV.

plot were a straight line, the definition of γ would be straightforward; it would simply be the voltage-independent slope of the i - V plot. This is rarely the case in general, however. Most i - V relations will, under some ionic condition, exhibit nonlinearities described as either inward or outward rectification. For a nonlinear i - V plot, the slope varies with voltage; hence, the “slope conductance” is voltage dependent. (It is important to remember that this means that the conductance of the open channel varies with voltage as distinct from the voltage dependence of macroscopic conductance arising from voltage-dependent gating.) For a nonlinear i - V plot, the slope conductance is a useful description of the shape of the i - V plot, but it does not meet the criterion for an Ohmic conductance (38). This is easily seen if one considers an i - V relation such as that shown in Figure 2 that exhibits marked outward rectification such that the slope of the i - V plot in the lower left quadrant approaches zero. The slope conductance of the channel is thus zero, despite the fact that the flow of inward current indicates that the Ohmic conductance must be nonzero. The Ohmic or “chord conductance” is the most appropriate measure of channel conduction properties and is defined by measuring the slope of a chord connecting any operating point of interest on the i - V relation with E_{rev} . This chord would describe the “instantaneous” trajectory of i and V if, for example, a perturbation in V was made around the operating point and i was recorded during a time during which γ did not vary. Clearly, at $V_m = E_{rev}$, the slope conductance and the chord conductance are identical.

C. Reversal Potential

The reversal potential for the current flowing through a single channel is the voltage at which the total driving force for ion flow is zero so that the current is zero. In the presence of a single permeant ion, the current must

reverse when V_m is equal to the equilibrium potential for that ion. Thus, for a Cl-selective channel, in the presence of a single permeant anion (Cl), Ohm's law can be written as

$$i_{\text{Cl}} = \gamma_{\text{Cl}}(V_m - E_{\text{Cl}}) \quad (2)$$

where $E_{\text{Cl}} = (RT/zF) \ln ([\text{Cl}]_o/[\text{Cl}]_i)$, where $[\text{Cl}]_o$ and $[\text{Cl}]_i$ are extracellular and intracellular Cl concentrations, respectively, so that E_{rev} is that value of V_m at which the electrochemical potential difference for Cl ($\Delta\mu_{\text{Cl}}$) is zero, i.e., thermodynamic equilibrium.

If more than one permeant ion is present, E_{rev} is the voltage at which the net current due to the algebraic summation of all permeant ion fluxes is zero. Hence, E_{rev} in this condition depends on the concentration of all of the permeant ions and their relative abilities to permeate the channel. In the context of studies of anion permeation, the parameter of interest is most often the shift in E_{rev} when all or a portion of the Cl bathing the channel is replaced by a substitute ion. The behavior of E_{rev} when a permeant ion is substituted for Cl is most commonly interpreted in terms of an expression for E_{rev} that assumes the following form if there are two permeant anions, for example, Cl and a "substitute" anion S

$$E'_{\text{rev}} = (RT/zF) \ln \left(\frac{[\text{Cl}]'_o + \alpha[\text{S}]_o}{[\text{Cl}]_i} \right) \quad (3)$$

E'_{rev} is the new value of E_{rev} after equimolar substitution of S for Cl outside the cell ($[\text{Cl}]'_o + [\text{S}]_o = [\text{Cl}]_o$). $[\text{Cl}]'_o$, $[\text{Cl}]_i$, and $[\text{S}]_o$ represent the respective concentrations of Cl and the substitute anion outside and inside the cell, and α may be thought of as an empirical parameter that provides a quantitative measure of the "similarity" of the substitute ion to Cl. It can be seen that the new value of the E_{rev} is determined by the concentrations of both permeant ions and that the contribution of the substitute ion is weighted by the "similarity factor" α . Clearly, in the limit $\alpha \rightarrow 0$, E_{rev} approaches the equilibrium potential for Cl (E_{Cl}), and S is judged to be rather unlike Cl in its permeation.

The determination of α is relatively straightforward; one measures the shift in E_{rev} , ΔE_{rev} , associated with the equimolar substitution of the ion S for Cl. When Cl is the only permeant anion, the E_{rev} is given by E_{Cl} . Now, if a portion of the external Cl is replaced by the substitute ion S, then the new value of E_{rev} , E'_{rev} , before S has entered the cell in any significant amount, is given by Equation 3, so that the substitution of S for Cl in the external bath produces a shift in E_{rev} , ΔE_{rev} given by the difference between E'_{rev} and E_{Cl} (assuming that $[\text{Cl}]_i$ is constant)

$$\Delta E_{\text{rev}} = (RT/zF) \ln \left(\frac{[\text{Cl}]'_o + \alpha[\text{S}]_o}{[\text{Cl}]_o} \right) \quad (4)$$

and α can be calculated directly from ΔE_{rev} , $[\text{Cl}]'_o$, and $[\text{S}]_o$.

The mechanistic interpretation of α , however, is complicated by the fact that its value is not completely independent of the mechanism of ion permeation through the channel. Generally, α is defined as being equal to the ratio of the "permeabilities" for Cl and the substituted ion, i.e.

$$\alpha = \frac{P_{\text{S}}}{P_{\text{Cl}}} \quad (5)$$

where P_{S} and P_{Cl} are the respective permeabilities. But, what are the permeabilities and how are they related to the underlying physics of the permeation process?

For channels in which the fluxes of permeant ions are not coupled, i.e., those that obey the Ussing flux-ratio criterion (149–151), permeabilities can be thought of as tracer rate coefficients that would be measured if the unidirectional flow of a labeled form of Cl or S were measured in the condition at $V_m = 0$ (38). This corresponds with the general notion of permeability as applied to both electrolytes and nonelectrolytes and would apply to channels that contain, at most, one ion at a time. For channels that can be occupied by more than one ion at a time, however, the flows of permeant ions can be coupled. That is to say, the gradient of S can drive the flow of Cl, and vice versa. In such channels, the mechanistic interpretation of permeability is more complicated. Nevertheless, the operational definition of the permeability ratio provided by Equation 3 is empirically useful and is widely used to evaluate anion selectivity. In the context of macroscopic, multichannel i - V relationships, the E_{rev} has the important property of being independent of channel gating (the number of open channels), because it is defined as the voltage at which the ionic current is zero.

III. MODELS FOR ION PERMEATION: INTERPRETATION OF PERMEABILITY AND CONDUCTANCE

The goal of measurements of channel permeability and conductance is to obtain information about the mechanism of ion permeation. Permeant ions serve as probes or "reporters" of channel properties because the nature of their interaction with the channel will depend on their physical properties as well as the structure and physical properties of the pore. Thus the interpretation of permeability or conductance, or the relation between these two parameters, is dependent on adopting some physical model that describes the three-step process of perme-

ation: leaving water and entering the channel, translocating within the channel, and exiting on the other side. The state of an ion in the aqueous solution bathing the channel is perhaps best depicted using the language of coordination chemistry (8, 26, 30, 103). The ion is coordinated or stabilized by a layer of water molecules with which it interacts strongly. The water molecules in this primary or "inner" hydration sphere, because of their association with the central ion, interact more strongly with other water molecules that form a secondary or "outer" hydration sphere. The energy of interaction between the ion and the inner sphere water molecules is appreciable, but the complex is nevertheless kinetically labile, and individual water molecules exchange with rates on the order of 10^{-12} s. For an ion to enter a channel, the highly favorable interaction with coordinating water molecules must be partly or completely replaced by interactions with the channel. The crystal structure of the bacterial K channel, for example, suggests that within the selectivity filter the K ion is completely (or nearly completely) dehydrated and is tetrahedrally coordinated by carbonyl oxygens contributed by the peptide backbones of the four subunits (45). It has been suggested that such dehydration could occur in a stepwise fashion so that the overall rate of the process is increased (24, 32, 40, 49). Andersen and Koeppe (4) envisioned the process by which an ion enters a channel in the following way. A hydrated ion diffuses through the aqueous bath up to the mouth of the channel where it forms an "encounter complex" and then an outer sphere complex with the channel mouth. The next step involves loss of some or all of the waters of hydration in exchange for solvation of the ion by polar groups within the channel (43, 115, 131). This is followed by diffusional translocation within the pore and exit on the other side, involving again the exchange of ion-channel interaction for ion-water interaction. The essence of the permeation process, as suggested by Doyle et al. (45), is that the energy of interaction of the ion with the channel must be sufficient to balance the energy required to dehydrate the ion, but the ion must, nevertheless, remain mobile within the channel. In K channels (45, 108) and perhaps CFTR (147), the limitation on mobility induced by tight binding is overcome by ion-ion repulsion due to multiple ion occupancy. The description of the three-step process of ion permeation has been dominated by two types of model, one based on simple electrodiffusion and the other based on the theory of absolute reaction rates, which we outline here only in their simplest forms.

A. Electrodiffusional Anion Channel

In its simplest form, the electrodiffusion model views the process of ion translocation within the channel as identical to the diffusion of ions in water so that it can

be described by the Nernst-Planck (N-P) equation (38, 37). The movement of ions between the aqueous solution and the "ends" of the channel is viewed as an equilibrium distribution in which the ion "partitions" between water and the channel interior. The permeability of the channel to an ion i is defined operationally as the rate coefficient for the unidirectional flow of a labeled form of i (tracer) through the channel in the condition $V_m = 0$ given by the rate of unidirectional tracer flow (J_i) divided by the tracer concentration (C_i) (see Ref. 38 for detailed discussion). The N-P equation, together with the equilibrium boundary assumptions, predicts that the permeability (P_i) is given by

$$P_i = A\beta_i D_i / l \quad (6)$$

where A is the cross-sectional area of the channel, β_i is the equilibrium partition coefficient between the aqueous solution and the channel interior, D_i is the diffusion coefficient for i in the channel, and l is the length of the channel (so that the units of P are cm^3/s) (38). Note that the units for P_i reflect the fact that the area is incorporated in the definition. If the area is factored out, the units become centimeters per second. The permeability of the channel to ion i is proportional to the partition coefficient (propensity to partition into the channel) and the diffusion coefficient (mobility within the channel) but is independent of the concentration of i in the bath. The concentration independence of the permeability is a reflection of the fact that the simple N-P model views the interior of the pore as similar to a dilute aqueous solution in which ions do not interact. In practical terms, this means that the channel is rarely occupied by an ion (37) and never by more than one ion. Thus there is no "competition" between ions for entry into the pore, nor is there any coupling between the flows of permeant ions.

The conductance of the N-P channel in the presence of symmetric solutions and in the condition $V_m = 0$ can be derived in several ways (38, 37) and is given by

$$(\gamma_i)_o = [(zF)^2/RT]P_i[i]_o \quad (7)$$

where $(\gamma_i)_o$ is the single-channel conductance, P_i is the permeability as defined by Equation 6, and $[i]_o$ is the symmetric bath concentration of the permeant ion i . As might be anticipated for a channel in which ions move independently, the conductance of the channel is directly proportional to the permeability multiplied by the ion concentration. This relationship prompts us to view permeability and conductance of the N-P channel as being related parameters that measure different things. Permeability is best thought of as describing the experience of a single ion as it traverses the channel, whereas conductance is a measure of the rate of ionic throughput per unit

driving force. Thus, if the concentration of i in the bath were reduced to zero, the permeability of the N-P channel measured using radiotracer flow would be unchanged, but the conductance would vanish. Conductance is proportional to permeability, but also depends on the abundance of the permeating ion in the conduction path.

It is possible to get an idea of the sort of prediction that this type of model makes for CFTR conduction properties by letting the CFTR pore be a right circular cylinder with a length of 50 Å and a diameter of 5 Å (37, 38). The single-channel permeability can be estimated from Equation 6 by setting β equal to unity and using the value of D_{Cl} for diffusion in free solution ($D_{\text{Cl}} = 10^{-5} \text{ cm}^2/\text{s}$). This yields a value of $P_{\text{Cl}} = 3.9 \times 10^{-14} \text{ cm}^3/\text{s}$. Inserting this into Equation 7 and setting $[\text{Cl}]_{\text{b}} = 100 \text{ mM}$ yields a value of 14 pS for γ , the single-channel conductance at $V_{\text{m}} = 0$. Although this must be considered the crudest of approximations, it is remarkable that the value is within a factor of 3 of the actual value of 6 pS, suggesting that the description of ion translocation as a diffusional process provides a reasonable starting place for thinking about anion conduction in CFTR.

B. Selectivity in the Nernst-Planck Channel

The ability to selectively conduct a particular ion is perhaps the most important physiological feature of an ion channel. Selectivity can be defined by comparing permeability ratios or conductance ratios, and for the simple N-P channel, these two approaches yield identical results, i.e., for two permeant ions A and B

$$P_{\text{A}}/P_{\text{B}} = \gamma_{\text{A}}/\gamma_{\text{B}} = (\beta_{\text{A}}/\beta_{\text{B}})(D_{\text{A}}/D_{\text{B}}) \quad (8)$$

where the first term $\beta_{\text{A}}/\beta_{\text{B}}$ has been referred to as the “equilibrium selectivity” in as much as it would compare the ratio of the probabilities that the channel would be occupied by ion A or B under equilibrium conditions. The second term $D_{\text{A}}/D_{\text{B}}$ has been referred to as the “nonequilibrium selectivity” because in the N-P model it reflects the relative restriction to ion flow within the channel, due for example to steric constraints.

Eisenman and co-workers (39, 50, 162) developed a unified theory of equilibrium ion selectivity that provides a useful framework for thinking about permeability ratios. The theory focused on the underlying forces that are expected to determine the equilibrium distribution of an ion between an aqueous solution and the channel interior. Eisenman and co-workers (39, 50, 162) reasoned that such a distribution would be determined by the balance between the energies associated with ion-water and ion-channel interactions, respectively. To remove an ion from aqueous solution and place it inside a channel, the work required to overcome the highly favorable interaction of

the ion with surrounding, polar water molecules must be balanced by favorable interactions with the peptide backbone or amino acid side chains that line the channel interior. Eisenman and co-workers recognized that if both ion-water and ion-channel interactions were viewed as being dominated by electrostatic forces, then both would vary inversely with the radius of the ion. The smaller the ionic radius, the larger would be both the hydration energy and the energy of interaction with charged or polar moieties in the channel. In the original Eisenman model, the balance between this tug of war is decided by the apparent “field strength” of the portion of the channel interacting with the ion. If a channel exhibited a “high field strength” behavior, then the ion-channel energy would be the dominant influence on β , and smaller ions would have a greater tendency to partition into the channel so that the predicted selectivity sequence for a high field strength anion channel would be in the order of increasing ionic radius, i.e., $\text{F} > \text{Cl} > \text{Br} > \text{NO}_3 > \text{I}$.

In contrast, if the intrachannel environment is characterized by a “weak field strength,” then the energetics of the partitioning process will be dominated by hydration energies, i.e., the smaller the ion, the more likely that it will be retained in the aqueous solution, and the selectivity sequence will be in the order of decreasing ionic radius: $\text{I} > \text{NO}_3 > \text{Br} > \text{Cl} > \text{F}$.

For a weak field strength channel, the larger the ion, the more likely that it will escape its hydration shell and enter the channel. Variations in the field strength of the site produce five intermediate sequences. Although this simple equilibrium model ignored some of the complexities of the ion dehydration and channel solvation processes (see Ref. 43), for example, the effect of an “optimal fit” into a cavity within the protein which stabilizes the ion (7), it served to focus attention on the importance of the balance between ion-water and ion-channel interactions in determining selectivity. The fact that the permeability selectivity sequence for CFTR more closely resembles that for the weak field strength model foreshadowed the importance of anion dehydration as an important factor in determining the sequence of anion permeation through CFTR (6).

C. Rate Theory Model: Ion Binding

The simple N-P channel is a direct extension of the continuum model for the electrodiffusional movement of ions in a dilute aqueous solution, and it does not allow for any interactions between permeating ions. The predicted permeability is concentration independent, and conductance is predicted to be a linear function of ion concentration. Evidence obtained from a wide variety of channels, however, including CFTR, suggests that ions interact with biological channels in a way such as to prolong the dwell

time of ions in the channel over that which would be expected for an equivalent volume of aqueous solution. This situation is modeled by assuming that a permeating ion associates transiently with "binding sites" that form part of the conduction path. Models incorporating ion binding can account for concentration-dependent ion permeability and the saturable relation between single-channel conductance and ion concentration, and also observations that suggest that ion flows can be coupled (38, 67, 68). The single-channel conductance of CFTR is a saturable function of $[Cl]_o$ in symmetric solutions with a mean affinity constant ($K_{1/2}$) of ~ 38 mM (146). In addition, CFTR Cl conductance can be blocked by SCN (101), and in the presence of mixtures of SCN and Cl, CFTR conductance exhibits an anomalous dependence on the mole fraction of SCN (147). These observations provide strong evidence for the binding of permeant anions in the pore of CFTR.

The N-P theory can be modified to incorporate the effects of ion binding in the permeation path (33, 88, 89), but a conceptually simpler approach is based on the Theory of Absolute Reaction Rates or Eyring Rate Theory (51). In this formalism, ion movement is viewed as a series of hopping events. Diffusion in aqueous solution, for example, is envisioned as a series of hops of an ion from one point in a lattice of water molecules to another. Each point in the lattice of water molecules could be viewed as a binding site of sorts, and translocation involves a series of hops from one site to the next and so on. It is, therefore, straightforward to extend this formalism to an ion that hops from water into a channel, moves across the channel, and hops out on the other side.

Although elements of the rate theory approach to ion conduction have been questioned (89), this formalism has become a popular way of envisioning ion transport through pores for several reasons. First, because it describes ion movement as a series of hopping events driven by thermal energy and the electric field, rate theory lends itself naturally to describing ion translocation in a setting in which it is envisioned that ions move by hopping from one site to the next and can associate more or less strongly with each site. Second, because the formalism is basically that of reaction rate kinetics and compartmental analysis, it lends itself very well to thinking about permeability, which is best thought of in terms of the behavior of a labeled form of the ion (tracer) that is present in low molar abundance (36, 38). Most importantly, the rate theory model provides an intuitively useful framework for understanding the difference in the behavior of permeability and conductance in channels that bind ions. Here, we briefly outline the development of the simplest model for an ion channel, that in which the channel can accommodate only one ion at a time. More complex models can be envisioned (32, 95), but the simple model is intuitively accessible and contains sufficient complexity to introduce

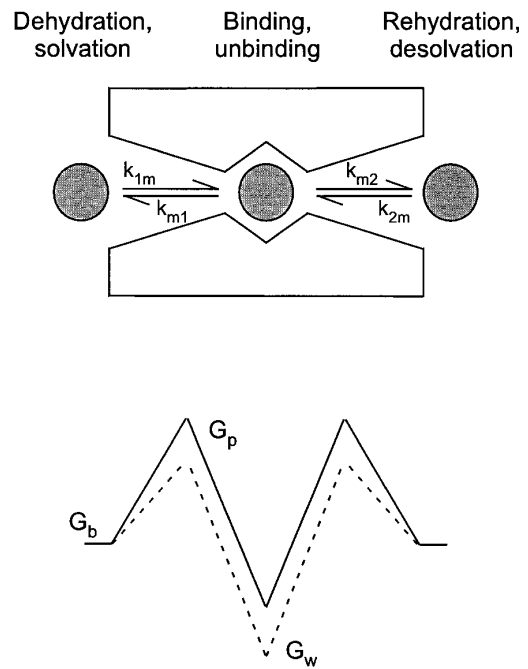


FIG. 3. Diagrammatic representation of a 2-barrier 1-site model for an ion channel that allows for a description of ion entry process as an energy barrier representing process of dehydrating ion and solvation by channel and accounts for binding of ions within channel by including an energy well. G_p , peak height; G_w , depth of energy well; G_b , energy in ion bath, defined as zero.

the most widely utilized generalizations that are derived from this approach. The derivations are presented here in a highly condensed form. The details can be found in Reference 38 and a variety of other sources (67, 85, 106, 160).

Rate theory models are most properly regarded as models for the process of ion conduction rather than as models for the structure of the channel. Accordingly, the process of ion translocation through a single-site, one ion channel is represented by the diagram shown in Figure 3 in which crossing the channel requires two hops. In the first, an ion located in the "capture volume" in the aqueous solution adjacent to the mouth of the channel hops into the channel and is able to interact with a "binding site." In the second hop, the ion leaves the binding site and exits the channel, entering the aqueous bath on the *trans*-side. The permeation process is represented as surmounting a series of two energy barriers, each of which depicts in a very general way the forces that impact the ion translocation events. As indicated in section III B, these are envisioned as representing the difference in the energies associated with ion-water and ion-channel interactions. For example, the height of the barrier to anion entry is presumably related in part to the fact that the ion must be at least partially dehydrated, but the height of this barrier would be diminished by favorable ion-channel interactions. The depth of the energy well representing the

site reflects the apparent affinity of the ion for some sort of binding site, and the depth of this well also contributes to the energy barrier to ion exit from the channel. Shown in the diagram is a symmetric two-barrier, one-site energy profile for a channel that can be occupied by only one ion at a time.

The properties of the channel are specified in four unidirectional rate coefficients, k_{1m} , k_{m1} , k_{m2} , and k_{2m} , the magnitudes of which depend on the height of the relevant energy barrier (Fig. 3). The relationship is assumed to be exponential and of the form $\exp(-\Delta G_p/RT)$, where ΔG_p is the peak barrier height. The preexponential term is taken to be the frequency of thermal vibration given by kT/h , where k is Boltzmann's constant, T is absolute temperature, and h is Planck's constant so that at 25°C, kT/h is equal to $\sim 6 \times 10^{12} \text{ s}^{-1}$. One criticism of the rate theory approach is based on the fact that this preexponential term is taken to be independent of the ion and its physical environment (21). The four rate coefficients are written as

$$k_{1m} = (kT/h) \exp(-\Delta G_{1p}/RT) \quad (9)$$

$$k_{m1} = (kT/h) \exp(-\Delta G_{mp}/RT) \quad (10)$$

$$k_{m2} = (kT/h) \exp(-\Delta G_{mp}/RT) \quad (11)$$

$$k_{2m} = (kT/h) \exp(-\Delta G_{2p}/RT) \quad (12)$$

where the terms in ΔG represent the height of the barrier that must be overcome in one hop, i.e., $\Delta G_{1p} = G_p - G_b$, $\Delta G_{mp} = G_p - G_w$, and $\Delta G_{2p} = G_p - G_b$. The preexponential factor is sometimes multiplied by a transmission coefficient, but the latter is usually set equal to unity (67).

The net fluxes of the ion into, and out of, the channel must be equal in the steady state so that if these are written as the difference between the relevant one-way hopping rates we obtain

$$J_{1m}^{\text{Cl}} - J_{m1}^{\text{Cl}} = J_{m2}^{\text{Cl}} - J_{2m}^{\text{Cl}} \quad (13)$$

where J_{1m} , J_{m1} , J_{m2} , and J_{2m} are the unidirectional fluxes into or out of the channel in molar units. Influx into the channel, for example, J_{1m}^{Cl} , is written as

$$J_{1m}^{\text{Cl}} = v[\text{Cl}]_1 k_{1m} (1 - f_o) \quad (14)$$

here v is the capture volume from which ions may enter the channel, $[\text{Cl}]_1$ is the Cl concentration within this volume, k_{1m} is the unidirectional rate coefficient for the entry process, and f_o is the probability that the channel is occupied. The product of v and $[\text{Cl}]_1$ is the number of moles of Cl ions in the capture volume so that multiplying by the entry rate coefficient in units of s^{-1} yields the flux in

units of mol/s. The term $(1 - f_o)$ is the probability that the channel is unoccupied, and multiplying by this factor applies the constraint that ions may only enter an empty channel.

Efflux from the channel, e.g., J_{m1}^{Cl} , is written as

$$J_{m1}^{\text{Cl}} = (1/N_A) k_{m1} f_o \quad (15)$$

where N_A is Avogadro's number and k_{m1} is the rate coefficient for Cl exit. Multiplying by f_o expresses the fact that ions may only exit an occupied channel.

Combining *Equations 13–15* and solving for f_o when $[\text{Cl}]_1 = [\text{Cl}]_2 = [\text{Cl}]_b$ yields

$$f_o = [\text{Cl}]_b / ([\text{Cl}]_b + K_{1/2}) \quad (16)$$

$$\text{where } K_{1/2} = (1/vN_A)(k_{m1} + k_{m2}) / (k_{1m} + k_{2m}). \quad (16a)$$

As expected for a single-site binding process, the occupancy of the channel saturates as $[\text{Cl}]_b$ is increased and the $K_{1/2}$ for the process is proportional to the ratio of the sum of the off rates and the sum of the on rates. Inserting expressions for the rate coefficients for a symmetric 2B1S channel yields

$$K_{1/2} = (1/v N_A) \exp(-\Delta G_w/RT) \quad (17)$$

where ΔG_w is the "depth" of the energy well measured with respect to the external bath. Note that because a value for the capture volume v is not easily specified, $(1/v N_A)$ is usually set at 1 M so that values of ΔG_w are referenced to a 1 M standard state (28), i.e.

$$K_{1/2} = \exp\left(\frac{-\Delta G_w + RT \ln C_b^*}{RT}\right) \quad (18)$$

where $C_b^* = 1 \text{ M}$.

The saturable occupancy of the 2B1S channel has important implications for the interpretation of permeability and conductance measurements. Permeability, as noted earlier, is best defined in terms of a one-way tracer flow measurement through a channel that is always open so that the permeability P_{Cl} can be defined as

$$P_{\text{Cl}} = \frac{J_{12}^{\text{Cl}}}{[\text{Cl}]_1} \quad (19)$$

where J_{12}^{Cl} is the unidirectional flow of Cl from side 1 to side 2 as would be determined in a tracer flow experiment, and is given by

$$J_{12}^{\text{Cl}} = \frac{J_{1m} J_{m2}}{J_{m1} + J_{m2}} \quad (20)$$

The one-way flow of Cl through the channel is equal to the rate of Cl entry (J_{im}) multiplied by the fraction of entering ions that exit on side 2. Substituting from *Equations 14–20* yields

$$P_{\text{Cl}} = v \cdot \frac{K_{1/2}}{K_{1/2} + [\text{Cl}]_{\text{b}}} \cdot \frac{k_{1\text{m}}k_{\text{m}2}}{k_{\text{m}1} + k_{\text{m}2}} \quad (21)$$

Here P_{Cl} is seen to be the product of two terms, the right most reflecting the intrinsic hopping rates into and out of the channel and the left most pertaining to the concentration-dependent loading of the channel. Clearly, as $[\text{Cl}]_{\text{b}}$ is increased, P_{Cl} declines. This is because a tracer ion moving from side 1 to side 2 must compete with unlabeled ions for the binding site in the channel. The maximum value for P_{Cl} defined in this way, denoted as P_{Cl}^0 , is found when $[\text{Cl}]_{\text{b}} = 0$ and is given by

$$P_{\text{Cl}}^0 = \frac{vk_{1\text{m}}k_{\text{m}2}}{k_{\text{m}1} + k_{\text{m}2}} \quad (22)$$

It is useful to think of P_{Cl}^0 as the “intrinsic” permeability of the channel, i.e., that which reflects only the rate coefficients for entry into and exit from an empty channel. Thus the permeability of a channel defined by a tracer flux measurement is equal to the intrinsic permeability multiplied by a factor that accounts for the fact that tracer may enter only unoccupied channels so that P_{Cl} will equal P_{Cl}^0 only in the limit $[\text{Cl}]_{\text{b}} = 0$.

The conductance of the 2B1S channel in the presence of symmetric Cl can be derived from the relation

$$(\gamma_{\text{Cl}})_0 = \frac{(zF)^2}{RT} P_{\text{Cl}} \cdot [\text{Cl}]_{\text{b}} \quad (23)$$

where $(\gamma_{\text{Cl}})_0$ is the conductance of the channel at $V_{\text{m}} = 0$, P_{Cl} is the permeability determined at $V_{\text{m}} = 0$ by a tracer flow measurement, and $[\text{Cl}]_{\text{b}}$ is the symmetric Cl concentration. Inserting the expression for P_{Cl} from *Equation 21* yields

$$(\gamma_{\text{Cl}})_0 = \frac{(zF)^2}{RT} vK_{1/2} \frac{[\text{Cl}]_{\text{b}}}{[\text{Cl}]_{\text{b}} + K_{1/2}} \left(\frac{k_{1\text{m}}k_{\text{m}2}}{k_{\text{m}1} + k_{\text{m}2}} \right) \quad (24)$$

which can be written as

$$(\gamma_{\text{Cl}})_0 = \frac{(zF)^2}{RT} K_{1/2} \frac{[\text{Cl}]_{\text{b}}}{[\text{Cl}]_{\text{b}} + K_{1/2}} P_{\text{Cl}}^0 \quad (25)$$

It can be seen that γ_{Cl} is proportional to the intrinsic channel permeability P_{Cl}^0 but is a saturable function of $[\text{Cl}]_{\text{b}}$. If $[\text{Cl}]_{\text{b}} \ll K_{1/2}$, then

$$(\gamma_{\text{Cl}})_0 = \frac{(zF)^2}{RT} P_{\text{Cl}}^0 \cdot [\text{Cl}]_{\text{b}} \quad (26)$$

At low concentrations of Cl, channel conductance is a linear function of $[\text{Cl}]_{\text{b}}$ as it is for the N-P channel (*Eq. 8*). This makes intuitive sense in as much as conductance, a measure of ionic throughput, requires not only that the channel be permeable to the ion, but also that the ion be present. If the intrinsic permeability (P_{Cl}^0) is constant, then for $[\text{Cl}]_{\text{b}} \ll K_{1/2}$, as the abundance of the permeant ion increases the current that can be driven by an applied voltage increases linearly, just as it would in the N-P channel in which ions do not influence each other. As $[\text{Cl}]_{\text{b}}$ becomes comparable to $K_{1/2}$, however, the effects of channel occupancy become apparent. Successive increments in $[\text{Cl}]_{\text{b}}$ produce smaller and smaller increases in γ as increasing channel occupancy reduces the probability that the channel will be available to an entering ion. When $[\text{Cl}]_{\text{b}} \gg K_{1/2}$, γ is independent of $[\text{Cl}]_{\text{b}}$, and the conductance approaches a maximum given by

$$(\gamma_{\text{Cl}})_{\text{max}} = \frac{(zF)^2}{RT} K_{1/2} P_{\text{Cl}}^0 \quad (27)$$

In this condition, the channel is occupied virtually 100% of the time, and throughput is limited by the rate of exit of ions from a loaded channel. This can be seen clearly if the expressions for $K_{1/2}$ and P_{Cl}^0 are inserted in *Equation 27*, i.e.

$$(\gamma_{\text{Cl}})_{\text{max}} = \frac{(zF)^2}{RT} \frac{1}{N_{\text{A}}} \frac{k_{\text{m}2}k_{1\text{m}}}{k_{1\text{m}} + k_{2\text{m}}} \quad (28)$$

$(\gamma_{\text{Cl}})_{\text{max}}$ is seen to be proportional to the intrinsic exit rate, $k_{\text{m}2}$, multiplied by the fraction of exiting ions that entered on the opposite side. For the symmetric case considered here, however, $k_{1\text{m}} = k_{2\text{m}}$ so that

$$(\gamma_{\text{Cl}})_{\text{max}} = \frac{(zF)^2}{RT} \frac{1}{N_{\text{A}}} \frac{k_{\text{m}2}}{2} \quad (29)$$

because in the symmetric case, regardless of the barrier heights, 50% of the exiting ions will have entered from the opposite side and, therefore, must represent conducted ions.

D. Influence of Ion Binding on Permeability and Conductance

Although the predicted dependence of P_{Cl} and γ_{Cl} on the energy barrier profile of a channel was derived above only for the simplest case, the results suggest some key features of the behavior of channels for which permeation

involves the transient binding of ions in the channel. Probably the most important of these relates to the interpretation of experiments in which the selectivity of the conduction path is probed in an anion substitution experiment in which the ratio of the permeability of a substitute anion to that of Cl is estimated, by measuring the change in E_{rev} , and the channel conductance is compared with and without the substitute ion. Here we develop the useful generalization that conductance and conductance ratios are very sensitive to ion binding, whereas permeability ratios are much less so.

The dependence of γ_{Cl} on ion binding is readily apparent from Equation 25, which shows that γ_{Cl} exhibits a saturable dependence on ion concentration, and Equation 27, which shows that $(\gamma_{\text{Cl}})_{\text{max}}$ is directly proportional to $K_{1/2}$ so that high-affinity binding (low $K_{1/2}$) implies reduced conductance. In the symmetric model, it is particularly apparent that the maximum value of the conductance is inversely proportional to the well-to-peak energy embedded in the term k_{m2} so that tighter binding (increased well depth) decreases the rate of ion exit from the channel. All of this makes intuitive sense from the perspective that conductance is a measure of throughput, of net ionic flow per unit voltage. Any ion that “sticks” in the channel will impede flow and reduce the maximum rate of throughput. A tightly binding, permeant ion will block or impede the flow of other ions that bind less tightly. From this perspective, highly conductive ions and “blocking” ions clearly represent two points on a continuum. For the purpose of illustration, we can examine some of the predictions of the admittedly over-simplified symmetric 2B1S model for CFTR with two equally spaced barriers and one well. The value of $K_{1/2}$ for the saturable dependence of γ on $[\text{Cl}]_{\text{b}}$ of 38 mM reported by Tabcharani et al. (146) would predict a well depth of about $-3.3RT$, with respect to a 1 M standard state. The well-to-peak energy that governs maximal conductance would be $\sim 14.5RT$ if $\gamma = 10$ pS (symmetric $[\text{Cl}]_{\text{b}} = 100$ mM), yielding a peak barrier height of $11.2RT$. The behavior of SCN is particularly interesting because it is highly permeant but also blocks the channel. A permeability ratio $P_{\text{SCN}}/P_{\text{Cl}}$ of 3.3 translates into a difference in the peak barrier height of $1.2RT$. The tighter binding of SCN would be compatible with a well depth of $-6RT$ for this ion (Fig. 4).

The absolute permeabilities measured for the 2B1S channel will also be sensitive to ion binding, as shown by Equation 21. The same effect that reduces maximum conductance will also cause tracer ions to experience more difficulty in finding an unoccupied channel as $[\text{Cl}]_{\text{b}}$ is raised. Permeability has a unique value as a comparative tool, however, in that it is possible, in principle, to compare the permeability of the channel with two different ions under identical conditions. The simplest way to envision such a comparison is in terms of a double-label, tracer flow experiment. For example, the one-way fluxes

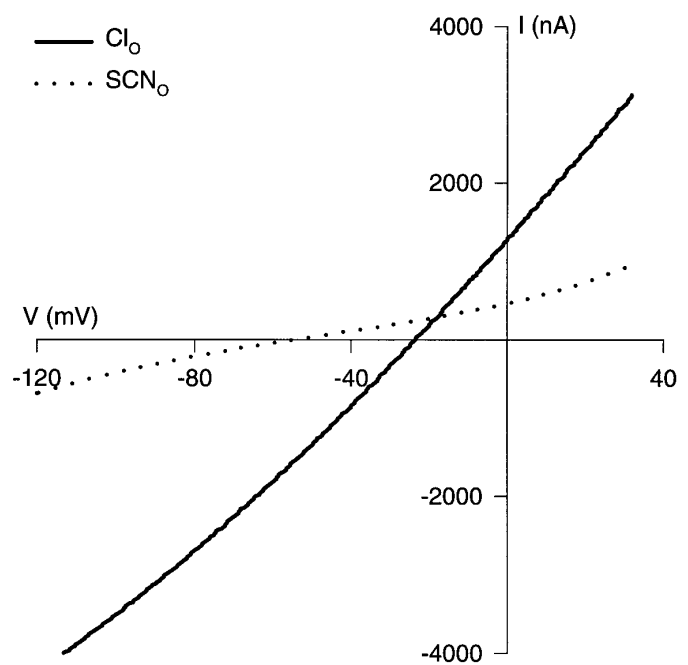


FIG. 4. Representative macroscopic current (I)-voltage (V) plots recorded from a *Xenopus* oocyte expressing wild-type human CFTR. Shown are plots in presence of 105 mM external Cl (Cl_o) and after replacing 98% of Cl_o with external SCN (SCN_o). Note that in presence of SCN reversal potential shifts to left as expected if $P_{\text{SCN}} > P_{\text{Cl}}$, but conductance is dramatically reduced.

of ^{36}Cl and labeled SCN could be determined simultaneously, in the presence of bathing solutions containing unlabeled Cl or SCN in any combination. The unidirectional flows of both tracer ions would be reduced by occupancy of the channel by Cl or SCN, but to an identical extent. That is to say, in the ratio $P_{\text{SCN}}/P_{\text{Cl}}$ (determined at $V_m = 0$), the term describing channel occupancy would be identical for either ion and would cancel out so that the measured ratio would be equal to the ratio of the intrinsic permeabilities of the channel to Cl and SCN.

This result is particularly important with regard to the use of E_{rev} values to estimate permeability ratios (see sect. II C). It is useful to think of E_{rev} shifts as measuring the ratios of intrinsic permeabilities (38). As shown in Equation 22 for the 2B1S model, the intrinsic permeability is given by the rate coefficient for ion entry into the channel multiplied by the fraction of entering ions that exit on the *trans*-side. This fraction is independent of well depth, however, because any change in depth will affect the probability of an ion leaving the channel in either direction equally. On the other hand, the intrinsic permeability is very sensitive to barrier height, in the simple 2B1S model, the barrier to entry into the channel. Thus the permeability ratios, estimated from shifts in E_{rev} , for a 2B1S channel will be relatively insensitive to well depth (ion binding) and highly sensitive to peak height (ease of entering the channel), as recognized by Bezanilla and

Armstrong (11). This prediction is consonant with the fact that the E_{rev} , because it is determined at zero current, is not dependent on the rate of ionic throughput. Channel conductance, on the other hand, measures the time-average rate of ion flow and is expected to be very sensitive to anion binding in the channel. Thus it is useful, although not completely accurate, to think of permeability ratios as comparing the ease with which anions leave the aqueous solution and enter the pore, whereas the relative conductance can report the tightness of binding to sites in the conduction pathway. The dichotomy between permeability ratios and conductance ratios for CFTR is well illustrated by the behavior of SCN. Figure 4 shows two macroscopic i - V relations recorded from a *Xenopus* oocyte expressing wild-type CFTR, one recorded in the presence of 105 mM $[\text{Cl}]_o$ and the other recorded after replacing 90% of the Cl by SCN. In the presence of SCN, the E_{rev} shifted to more negative values, indicating that $P_{\text{SCN}}/P_{\text{Cl}} > 1$, but the conductance measured at the reversal potential is dramatically reduced. This behavior is predicted for an anion that enters the channel more readily than Cl but sticks tightly once inside. As always, one must approach with caution any attempt to generalize such predictions to real channels, in particular those that can be occupied by more than one permeant ion (67, 68, 147), but they provide a useful guide or zeroth approximation.

E. Linking the Nernst-Planck and Rate Theory Models

The simplest N-P model, as presented here, represents a limiting case in which the interior of the channel is viewed as an equivalent volume of aqueous solution where ions move without interacting with the channel or each other. It is important to point out, however, that the physical parameters that characterize the permeability of the N-P channel can be readily interpreted within the framework of the simple rate theory description. The permeability of the N-P channel to an ion i is given by

$$P_i = \frac{A\beta_i D_i}{\Delta x} \quad (30)$$

where A is the channel area, β_i is the channel/bulk solution partition coefficient, D_i is the intracellular diffusion coefficient, and Δx is the length of the channel.

The partition coefficient for the simple 2B1S channel (Fig. 3) is a function of the well depth, $G_w - G_b$, i.e.

$$\beta_i = \exp\left[\frac{-(G_w - G_b)}{RT}\right] \quad (31)$$

The intramembrane diffusion coefficient D_i can be written as (12, 67)

$$D_i = \frac{kT}{h} \exp\left[\frac{-(G_p - G_w)}{RT}\right] \quad (32)$$

so that in the product $\beta_i D_i$, the term representing the well depth (G_w) drops out, i.e.

$$\beta_i D_i = \frac{kT}{h} \exp\left[\frac{-(G_p - G_b)}{RT}\right] \quad (33)$$

Thus, for any permeability ratio, say P_j/P_i , we obtain the result that

$$\frac{P_j}{P_i} = \exp\left[\frac{-(G_p^j - G_b^j)}{RT}\right] \quad (34)$$

permeability ratios are not sensitive to ion binding. But what then are the properties of a free solution or N-P channel expressed in the language of rate theory? The hallmark of the simplest N-P channel is short residence time for the ion within the channel. The channel is never seen by an entering ion as being occupied, and there is no saturation of channel conductance as the concentration of permeant ions in the bath is raised. The free solution 2B1S channel, therefore, is one characterized by a shallow or no energy well. For example, in relation to Figure 3, if $G_w = G_b$ so that the well depth is zero, the $K_{1/2}$ for the dependence of γ on concentration is 1,000 mM. If the concentrations of permeant ions in the bathing solution are significantly less than the $K_{1/2}$, then the conductance exhibits a simple linear dependence on the permeability given by *Equations 7 and 26*.

Finally, we note that there is perhaps a subtle deception effected by the tendency, within the rate theory formalism, to view well depth and peak height as independent parameters. This seems unlikely to be the case in general, and one might expect, for example, that channel modifications that reduced the binding of ions by reducing the ion-channel interaction energy might also influence the peak height, which is expected to depend in large part on the balance between ion-water and ion-channel interactions. This effect is, in fact, seen in CFTR mutations like G314E, which destabilizes relative anion binding, but also reduces single-channel conductance rather than increasing single-channel conductance as would be expected if only the well depth were affected (Mansoura and Dawson, unpublished data).

TABLE 1. Representative values for permeability and conductance ratios for anion-selective channels

Channel	P_s/P_{Cl}							g_s/g_{Cl}						
	SCN	ClO ₄	I	NO ₃	Br	HCOO	F	SCN	ClO ₄	I	NO ₃	Br	HCOO	F
CFTR (96, 146)			2.0	1.4	1.2	0.18	<0.06							
CFTR (6)			0.6		1.1		0.30			0.29		1.26		0.15
CFTR (101)	3.4	0.3	0.4	1.4	1.2	0.39		0.14	0.20	0.18	0.75	0.64	0.52	
CIC-1 (52)	0.9		0.3	0.5	0.6									
CIC-3 (52)	4.5		2.1	2.3	1.4									
GABAR (13)	7.3		2.8	2.1	1.5	0.50	0.02	0.75		0.82		0.88		
GABAR (54)	4.3		2.4	2.7	1.4	0.55	0.09	0.38		0.57	0.58	0.63	0.31	0.49
GlyR (13)	7.0		1.8	1.9	1.4	0.33	0.03	0.54		0.65		0.74		
GlyR (54)	4.3		2.3	2.8	1.5	0.60	0.09	0.41		0.61	0.65	0.71	0.30	0.05
T84-ORCC (63)	2.1	1.7	1.7	1.7	1.4	0.76	0.40	1.29	1.20	0.99	1.56	0.99	0.53	0.41

Reference numbers are given in parentheses. P_s , permeability of substituted ion; P_{Cl} , Cl permeability; g_s , conductance of substituted ion; g_{Cl} , Cl conductance.

IV. DESIGNING AN ANION CHANNEL: ORIGINS OF ANION SELECTIVITY AND ANION BINDING

A. Model Systems and the Role of Hydration Energy

Studies of anion conduction by CFTR and several other anion channels have established two important properties of the anion conduction path. First, compared with cation channels, anion channels tend to be rather nonselective. Second, certain anions bind within the pore such that they act as blockers of the channel (13, 58, 101, 146, 147, 157). These two characteristics have been derived from a comparison of permeability ratios and conductance ratios for permeant ions, the former reflecting primarily the relative ease with which an anion enters the channel and the latter reporting the tightness of anion binding. Representative values for permeability and conductance ratios for several anion channels are compiled in Table 1. Although it is not possible to specify exactly how these properties arise, studies of CFTR as well as other anion channels, viewed in light of the known properties of anions and water, provide the basis for some provocative speculation.

One approach to identifying the critical design criteria for an anion channel is to examine the behavior of model systems for which the structure is more or less established. One candidate for such a comparison is the channel formed by the antibiotic gramicidin (43, 90). It has been proposed that the channel is formed from a dimer consisting of two helical peptides joined at the amino-terminal ends. Each helix consists of 15 alternating L- and D-amino acids, containing 6.3 peptide units per turn. This arrangement dictates that the lining of the channel is formed by the carbon, oxygen, nitrogen, and hydrogen atoms of the polypeptide backbone, whereas the side chains face the surrounding lipid. The channel contains

no charged groups, but it is, nevertheless, highly cation selective. Another interesting model is the channel(s) that is formed when designed, amphiphilic peptides are incorporated into a lipid bilayer. Lear et al. (86) synthesized 21-residue peptides composed of leucine and serine, (LSSLLSL)₃, that, when incorporated into a lipid bilayer, gave rise to cation-selective channels, again in the absence of any charge on the peptide. Although anions and cations are predicted to interact differently with a peptide structure because of the difference in their net charge, Levitt (90) called attention to the fact that the cation selectivity of gramicidin could arise in part from the fact that more work is required to dehydrate an anion than a cation of comparable size (12, 16, 25, 102, 103). Cox et al. (30) compared the free energy required to transfer anions and cations from water to dimethylformamide, a compound which might be viewed as a model peptide. They found that the transfer of univalent cations was favored over that of anions by anywhere from 2 to 13 kcal/mol, although this difference reflects, in part, stronger interactions of cations with the aprotic solvent (35, 100). Levitt (90) proposed that anion selectivity might require either the placement of positive charges in the channel or that the effective diameter of the channel be sufficiently large that the anion could migrate through the channel while retaining some of its waters of hydration. A similar conclusion was reached by Dorman et al. (43) who used a Monte Carlo approach to simulate permeation energetics in the gramicidin channel. They considered interaction between anions and cations and the peptide backbone of the channel as well as ion-water interactions and concluded that the lack of anion permeation was primarily attributable to the increased energy required to remove the anion from water so that anion permeation might require a larger cross section so as to accommodate the more highly hydrated anions. This perspective seems to fit, in a general way, the findings for a number of anion channels that are, as a group, relatively nonselective and in which perme-

ability ratios, which measure the ability of an anion to enter the channel, fall in the order predicted on the basis of hydration energy, i.e., a “weak field strength” site sequence according to Eisenman and Horn (50) (Table 1).

There is evidence that positive charges may be important for anion selectivity. The structure of the transmembrane segments presumed to line the pores of the GABA_A receptor and the glycine receptor (GlyR) both contain arginines near the cytoplasmic and extracellular ends that have been implicated in anion/cation discrimination. When Langosch et al. (83) incorporated the presumed pore-lining GlyR M2 peptides into planar bilayers, they observed multiple unitary conductance events, some anion selective but some cation selective. A modified peptide, however, in which the terminal arginines were replaced by glutamic acids led to the formation of cation-selective channels. Reddy et al. (121) reported that peptides modeled after the M2 segment of GlyR, when incorporated into planar bilayers formed at the tip of a patch pipette, gave rise to channel events that exhibited distinct anion selectivity ($P_{Cl}/P_K = 5.6$). In contrast, an identical peptide in which the flanking arginines were replaced with glutamic acids produced cation-selective channel events ($P_K/P_{Cl} = 5.3$). Furthermore, a tethered tetramer of M2 GlyR peptides also formed anion-selective channels ($P_{Cl}/P_K = 9.0$). Oblatt-Montal et al. (111) assayed the channel-forming abilities of peptides modeled after the first six membrane-spanning segments of CFTR. Only two, transmembrane segment (TM) 2 and TM6, gave rise to channel-like activity. Both of these peptides contain arginines and/or lysines near their ends.

The importance of the arginine residues in GlyR was also apparent in a mutant form, R271Q, associated with the inherited disease hyperekplexia, which exhibited a diminished single-channel conductance (84). Arginine has also been implicated in anion selectivity in the glutamate receptor channel. The so-called “Q/R site” in the glutamine R2 subunit was identified as being important for calcium permeation and also block by polyamines (18, 19). It was also found, however, that the Q to R substitution increased the relative anion permeability such that, in the presence of high external calcium, the ratio P_{Cl}/P_{Cs} was 1.4, i.e., with regard to these two similarly sized ions, the channel behaved as if it was anion selective.

There is evidence that the membrane-spanning peptides that appear to be important for the conduction path of anion-selective channels can induce anion permeation in cell membranes. Wallace et al. (154) reported that if apical membranes of Madin-Darby canine kidney monolayers were exposed to a GlyR M2 peptide modified to contain four lysine residues at the carboxy terminus, Cl secretion was induced. Recently, Lencer et al. (87) reported that a class of compounds called cryptidins, which are related to neutrophil defensins, induced Cl secretion in monolayers of T84 cells. The most active peptide, cryptidin 3, contains eight arginines and three lysines.

The importance of the structural context in which charges that line the pore may act to influence permeability and conductance was highlighted by the experiments of Galzi et al. (57), who attempted to design anion-selective variants of a GABA receptor (GABAR)/GlyR homolog, the acetylcholine receptor. In the acetylcholine receptor (AChR) M2 segment, glutamates at position 237 are thought to contribute to a cytoplasmic ring of negative charge, whereas the corresponding position in the GABAR and GlyR M2 receptor is occupied by an alanine. The adjacent residue (238) is an arginine in GABAR and GlyR M2 and a lysine in the AChR M2. Replacing glutamate-237 with alanine resulted in an anion-selective channel, but only if an additional neutral residue (proline or alanine) was inserted between positions 236 and 237 to bring the length of the M2 segment or the MI-M2 linker into register with that of GABAR and GlyR. This important result strongly suggests that although anion to cation selectivity is likely to be charge dependent, the structural context in which potential permeant ions experience these charges can be critical. The results obtained by Mansoura et al. (101) in studies of mutant CFTR suggest that a similar caution will apply to the results of charge substitution experiments in the CFTR Cl channel.

B. Anion Binding

Collins (25–27) has called attention to the roles of ion-water and water-water interactions in the binding of ions to proteins. The tightness of binding of water to individual ions can be compared by comparing the entropy of pure water with that of the water near an ion. For small ions like F^- or Li^+ , the difference between the entropy of bulk water and that of water near an ion is negative, because the tightly held water molecules are less mobile than those in bulk water. Small ions that strongly immobilize water are classified as kosmotropes. Ions for which this entropy difference is positive are surrounded by more loosely bound water and are called chaotropes. The latter group tends to absorb to weakly hydrated surfaces, driven by the strong water-water interactions that tend to exclude the ion. Thus chaotropic ions are predicted to be “sticky” because they can be pushed out of water onto weakly hydrated surfaces even in the absence of any strong ion-surface interaction. If values of the entropy difference between bulk and bound water for univalent cations and anions are plotted versus ionic radius, it is apparent that the isentropic point occurs at a larger radius for anions, another reflection of the fact that anions begin to immobilize water at a lower charge density than do cations (130). On this scale, Cl is modestly chaotropic and other halides more so as the increasing radius weakens the ion-water interaction. This implies that ions like SCN, that are weakly hydrated, may be more likely to stick inside ion channels.

In their simulation of the gramicidin channel, Dorman et al. (43) discovered an anion/cation difference that could be important for anion binding. In modeling the interaction of ions with the peptide backbone of gramicidin, they found that the interaction energy for cations was more or less position independent, whereas that for anions was characterized by peaks and wells. This result, which is independent of any side chain that might line a pore like that of CFTR, suggests that the intrinsic nature of the anion-peptide backbone interaction might lead to the appearance of energy wells or binding sites. It is also noteworthy that the interaction of the backbone with anions was predicted to be more favorable than that with cations because of the influence of the peptide dipole.

V. POTENTIAL PROBES OF THE PORE

Generally, Cl channels do not appear to be the target for the sort of highly specific blockers that have been so useful in deciphering the structure of voltage-dependent Na and K channels, but in the interval since the identification of CFTR, it has become apparent that there are several classes of compounds that may provide useful probes of the CFTR protein. These include the arylamino benzoates [e.g., diphenylamine-2-carboxylic acid (DPC) and flufenamic acid], the sulfonylureas (e.g., glibenclamide), disulfonic stilbenes [DIDS, 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS)], and certain permeant, polyatomic anions (e.g., SCN and the pseudohalides). In addition, polar thiol reagents have been used as probes of the pore of CFTR constructs in which cysteine residues have been inserted into suspected pore-lining TM.

A. Arylamino Benzoates

The arylamino benzoates were developed by Greger and co-workers (46) as blockers of epithelial Cl channels, and they showed that DPC as well as related compounds blocked Cl conductance in renal epithelial cells. The mechanism of DPC blockade of CFTR was first investigated by McCarty et al. (104). They studied macroscopic as well as single-channel CFTR currents using human CFTR and an oocyte expression system. In a macroscopic setting, they found that after exposing an oocyte to 1 mM DPC, partial block of CFTR currents ensued immediately, but a steady-state condition was not reached for 7–10 min. In the steady state, the attenuation of the macroscopic current exhibited a distinctive voltage dependence with slight or no block evident at holding potentials positive to reversal voltage and clear attenuation at potentials negative to the reversal voltage. Single-channel recordings provided evidence for an open-channel block mechanism. Importantly, the voltage dependence of the kinetics was virtually independent of the side of the membrane from

which DPC was applied. This suggested a model in which DPC could reach its binding site within the channel by entering the channel from either side (as well as by crossing the lipid portion of the membrane), but in which it was more difficult for the blocker to access the site from the extracellular than from the cytoplasmic side.

B. Sulfonylureas

Sheppard and Welsh (139) showed that the sulfonylureas, glibenclamide and tolbutamide, both inhibited macroscopic cAMP-activated currents in NIH 3T3 and HeLa cells expressing CFTR. Venglarik et al. (153) used patches excised from CFTR-expressing mouse L cells to study the mechanism of tolbutamide block. The compound behaved as a relatively “fast” blocker in that exposure of the cytoplasmic side of the patches to 200 μ M tolbutamide caused an apparent reduction in single-channel amplitude and a distinct increase in open-channel noise. The action of tolbutamide was quantified by analyzing the drug-induced fluctuations in current. At a concentration of 1 mM, tolbutamide induced a clearly discernible high-frequency Lorentzian (corner frequency = 650 Hz), and the concentration dependence of the corner frequency was in accord with the prediction of a simple three-state ($C \leftrightarrow O \leftrightarrow B$) model in which the drug interacts with the open state of the channel.

Schultz et al. (134) analyzed blockade of single CFTR channels by glibenclamide. In the absence of the drug, single channels in detached patches from CFTR transfected L cells (at 32–37°C) exhibited gating behavior consisting of bursts containing short-lived closures. Exposure to glibenclamide on the cytosolic side produced a concentration-dependent reduction in the burst duration consistent with a $C \leftrightarrow O \leftrightarrow B$ mechanism. The apparent affinity was 14 μ M, a result of an apparent higher affinity of the bound state than that for tolbutamide. Fluctuation analysis of channel activity from multi-channel patches revealed that glibenclamide induced an additional Lorentzian component in the power density spectrum in the range of 3–40 Hz. The sulfonylureas are potentially of great interest as CFTR ligands. They are known to interact with the sulfonylurea receptor, also a member of the ATP binding cassette superfamily of proteins (134), and interest in the effect of these compounds on insulin secretion stimulated the exploration of literally thousands of related compounds that can be assayed for potential CFTR specificity. At present, the nature and location of the binding site has not been explored in detail, but the kinetic behavior is consistent with mechanisms that involve occlusion of the channel due to binding in the pore.

C. Disulfonic Stilbenes

One of the signature features of CFTR is that extracellular application of disulfonic stilbenes (DIDS or DNDS)

is without effect. Linsdell and Hanrahan (93), however, showed that both DIDS and DNDS blocked macroscopic CFTR-mediated Cl currents in detached patches from baby hamster kidney cells when present on the cytoplasmic side. In both cases, the block exhibited a voltage dependence consistent with a blocking mechanism in which these divalent anions entered the pore from the cytoplasmic side. The apparent affinity of the DIDS was about twice that of DNDS. Block by DNDS was nearly abolished in the R347D CFTR construct, whereas that by DIDS was reduced but still readily apparent. This result is of interest because it suggests a possible similarity between CFTR and certain outwardly rectifying channels studied by Bridges et al. (15), although in the latter, DNDS block occurs with much higher affinity ($2\text{--}3\ \mu\text{M}$) and the block by DIDS is irreversible.

D. Intracellular Anions and Osmolytes

Overholt and co-workers (112, 113) first called attention to the fact that large anions such as glutamate, which are expected to be impermeant (or modestly permeant), can block CFTR from the cytoplasmic side. In guinea pig cardiac myocytes, as well as T84 cells and transfected HEK 293 cells expressing human CFTR, they found that the shape of the whole cell i - V relation was linear in the presence of symmetric 150 mM $[\text{Cl}]_o$. Reduction of $[\text{Cl}]_i$ by substitution of glutamate or sucrose produced outwardly rectifying currents that could not be attributed to the Cl concentration gradient, as predicted by the Goldman-Hodgkin-Katz equation. Rather, the rectification appeared to be attributable to block, in particular by the intracellular substitute anion glutamate. The concentration and voltage dependence of the block was consistent with a pore containing a single binding site for which Cl and glutamate could compete, which could be accessed more readily from the cytoplasmic side of the pore. Sucrose substitution for $[\text{Cl}]_i$ reduced but did not eliminate rectification. The authors suggested that under physiological conditions intracellular anions could influence the magnitude of inward current (outward Cl flow).

Linsdell and Hanrahan (92) reported similar findings for CFTR expressed in Chinese hamster ovary cells. They studied single channels in detached patches and obtained direct evidence for a voltage-dependent, flickery block of CFTR by glutamate or gluconate that was only seen when ions were present on the cytoplasmic side of the patch. The magnitude of the voltage dependence was consistent with the ion experiencing from 30 to 60% of the transmembrane potential as it accessed the binding site. The magnitude of the blocking effect at negative potentials was enhanced when $[\text{Cl}]_o$ was reduced as expected if the two ions compete for a site in the pore. Interestingly, three uncharged, intracellular osmolytes, sucrose, sorbitol, and

urea, also appeared to produce blockade of CFTR which, although modest, was discernible as a flicker in the single-channel record.

E. Permeant Ions

Permeant anions must, by definition, enter, interact with, and traverse the CFTR pore, and this characteristic makes them potentially very useful probes of the pore interior. The CFTR, and anion channels in general, tend to be relatively nonselective; a large number of inorganic and organic anions that differ widely in their size and shape appear to permeate (13, 63, 67, 101, 146). The differences in physical properties across such a large panel of anions should make it possible to infer what properties (e.g., size, hydration energy, polarizability) are most important for permeation. For example, Tabcharani et al. (146) and Linsdell et al. (96) recently examined halide permeation in CFTR and found that the sequence of permeability ratios was $P_I > P_{\text{Br}} > P_{\text{Cl}} > P_{\text{F}}$, consistent with that predicted on the basis of the ease of dehydration of the anions. This sequence was only observed, however, if special attention was paid to the behavior of the iodide ion which, as described in section *VE4*, appears to interact strongly with CFTR in a way that leads to a modification of its own permeability ratio ($P_I/P_{\text{Cl}} = 2$ to $P_I/P_{\text{Cl}} = 0.4$). It seems likely that understanding the behavior of anions like I and SCN, which bind tightly inside the channel, will be central to deciphering the structure of the conduction path.

1. SCN as a probe of Cl channels

Tabcharani et al. (147) provided evidence for tight binding of the polyatomic anion SCN in the CFTR pore. From 1 to 10 mM SCN on the cytoplasmic side of detached patches blocked Cl currents, and this effect was abolished when R347 in TM6 was substituted with aspartic acid (R347D). Equimolar, symmetric substitution of SCN for Cl produced an anomalous mole fraction effect (147), i.e., at low concentrations of SCN, single-channel conductance was reduced, reaching a minimum at $\sim 15\ \text{mM}$, but further increases in the mole fraction of SCN ($X_{\text{SCN}} = [\text{SCN}] / ([\text{SCN}] + [\text{Cl}])$) actually increased conductance. This behavior is consistent with a model in which the CFTR pore can be occupied by at least two SCN ions at one time. The anomalous mole fraction effect was abolished in the R347D construct, and if a histidine was substituted for R347 (R347H), the blocking effect of SCN ($X_{\text{SCN}} = 0.075$) was greatly enhanced at pH 5.5, suggesting that the presence of the positive charge is important for the high-affinity SCN binding. The single-channel conductance of R347D was reduced to $\sim 50\%$ of wild-type CFTR and was not greatly different in Cl or SCN. Linsdell et al. (95) used rate theory models to simulate the anomalous mole

fraction effect seen with SCN and its absence in R347D CFTR. Although efforts to model the permeation process are at an early stage, it seems clear that realistic models will have to account for the effects of multi-ion occupancy of the pore.

Students of anion conduction have long been attracted to the anomalous behavior of SCN. This polyatomic anion behaves in E_{rev} assays as if $P_{SCN}/P_{Cl} > 1$, but also acts as a channel blocker (see Fig. 4). The general similarity in the behavior of SCN across anion channels suggests that sites to which SCN binds with relatively high affinity could be a general feature of these proteins. Nearly 40 years ago, Hutter and Padsha (71) found that as little as 10% SCN in the external bath increased the membrane resistance of frog skeletal muscle by 70%. Harris (64) reported that the efflux of ^{36}Cl from the same muscle fell by nearly 70% if $\sim 10\%$ of $[Cl]_o$ was replaced by SCN, despite the fact that the membrane potential was apparently not altered. Takeuchi and Takeuchi (148) reported that GABA-induced conductance in the crayfish neuromuscular junction was decreased by $\sim 40\%$ if 25% of $[Cl]_o$ was replaced by SCN. In an elasmobranch muscle in which the resting Cl conductance can be more than sevenfold higher than the resting K conductance, Hagiwara and Takahashi (62) reported that replacing $\sim 10\%$ of $[Cl]_o$ with SCN reduced membrane conductance by $\sim 75\%$. In a later study of frog muscle, Vaughan (152) reported that at extracellular pH 5, the addition of 5 mM SCN to a solution containing 23 mM Cl reduced currents by $\sim 25\%$. In some, but not all, of these studies (62, 71, 148, 152), substituting SCN for extracellular Cl produced an anomalous mole fraction effect, i.e., the conductance passed through a minimum and then increased as X_{SCN} was raised. In all of these studies, however, it was found that the apparent permeability for SCN exceeded that of Cl. Recently, it was reported that SCN blocks an I-selective channel in thyroid cells (58).

More recent studies of single Cl-selective channels are also consistent with models in which SCN binds tightly in Cl channels. Bormann et al. (13) studied single-channel currents through glycine and GABA receptor channels and found that P_{SCN}/P_{Cl} was ~ 7 , but conductance ratios ranged from 0.54 to 0.75. As little as 2% SCN produced channel blockade. White and Miller (157) found that SCN blocked the Cl channel, now known as ClC-O, reconstituted from *Torpedo electroplax*. SCN block was voltage dependent and competitive with Cl, but SCN currents could not be detected. In an apical, outward rectifying channel from T84 cells, Halm and Frizzell (63) found that $P_{SCN}/P_{Cl} = 2$, but single-channel conductance was reduced when $X_{SCN} = 0.2$ (63). Single-channel studies also supported the notion of anomalous mole fraction behavior of SCN, suggesting that there are multiple binding sites for SCN and that two of these linear, polyatomic anions can reside in the channel at one time (13, 63, 147).

It is of interest in this regard that there is evidence for tight binding of SCN to another well-characterized membrane transport protein, the anion exchanger of the human red blood cell. Dalmark and Wieth (31) found that replacing nearly all of the external Cl with SCN reduced ^{36}Cl efflux by 93%. Dissing et al. (42) measured the H₂DIDS-sensitive efflux of ^{35}SCN from human red blood cells and found that SCN had the highest apparent affinity for the transporter of any inorganic ion and that the rate of SCN equilibrium exchange was >20 times less than that for Cl. These observations are consistent with a model in which SCN interacts strongly with an anion binding site on the exchanger and slows the conformational changes that are the basis for the anion exchange process.

2. Anion binding to proteins: a role for arginines?

Anion binding appears to be a common feature of anion-selective channels, including CFTR, and results obtained by Tabcharani et al. (147) strongly suggest that arginine-347 in TM6 is important for tight binding of SCN in the pore. A survey of the literature reveals that there is abundant evidence that arginine residues may be critical components of anion binding sites in a variety of proteins. Arginine may be well suited to be involved in anion binding. Its positive charge is located at the end of a long, flexible side chain, and the guanidinium group contains five hydrogen bond donors in a planar array (74, 125). This results in the guanidinium moiety being the most highly hydrated of the amino acid side chains (120, 159) so that the strength of any "binding" interaction between the side chain and a permeant ion would depend on the net free energy change associated with the change in ion-water, side chain-water, ion-side chain, and water-water interactions. The halides, particularly Cl, are strong hydrogen bond acceptors that might compete with water for interaction with arginines (73), a principle that has been exploited in the design of anion-sensitive electrodes (41, 47).

Anion binding to soluble proteins like serum albumin (75, 76, 109, 110, 114, 132, 133) and hemoglobin (23, 77, 78, 127) is well documented. Anion binding to albumin has been particularly well studied and modeled on the basis of at least two classes of sites of differing affinity (109, 110). SCN binds more tightly than Cl, and several studies associated the binding of SCN and other anions with arginine residues. Pande and McMenamy (114) used chemically modified bovine albumin to infer that SCN binding was primarily to arginine residues and that the positive charge was important for the binding site. Jonas and Weber (75) reached a similar conclusion in a study of the binding of the hydrophobic anion 1-anilino-naphthalene-8-sulfonate to albumin. This conclusion was strengthened by the results of Norne and co-workers (109, 110) who used NMR methods to study anion binding to human

serum albumin. The results were consistent with two classes of sites, both of which interacted strongly with SCN as well as two other pseudohalide anions, Pt(CN)₄ and Au(CN)₂. A model for the anion-site interaction was consistent with Cl binding to arginines. Arginines were also implicated by Fairbrother et al. (53) in their NMR study of SO₃ and PO₄ binding to yeast phosphoglycerate kinase. In a NMR study of horse liver alcohol dehydrogenase, Bull et al. (17) used Au(CN)₂ and Pt(CN)₄ as probes of Cl binding sites and concluded that the quadruple coupling constant estimated from a simple electrostatic model was consistent with a Cl ion interacting with an arginine group.

The exact nature of the anion binding reaction is not known for any protein, but it has been noted that the selectivity of binding tends to follow the so-called lyotropic or Hofmeister series (34, 156), i.e., Au(CN)₂ > SCN > NO₃ > Br > Cl.

Tight binding ions, like SCN, tend to be large and highly polarizable ("soft") (44). The large size results in reduced strength of interactions with water, and the net result of water-water, water-anion, water-guanidinium, and anion-guanidinium interactions may, therefore, favor binding (34).

Arginine residues have also been proposed to function in the binding of Cl to hemoglobin and in the anion transport protein halorhodopsin. Chiancone et al. (23) used ³⁵Cl-NMR to implicate an arginine and a histidine in Cl binding to hemoglobin. A more dramatic effect is seen in the mutant hemoglobin Rothschild (HbR), in which a tryptophan in the β -subunit is replaced by an arginine (77, 78, 127). In this mutant, Cl alters oxygen and CO binding, and the basis for effect could be localized to a mutation-generated, anion binding site in which Cl acts as a counterion for the mutant arginine residue in HbR.

The light-driven anion pump *Halorhodopsin* couples light absorption to Cl transport, and two arginines have been implicated as possible Cl binding sites (14, 155). If one of these, R108, is removed (R108Q), Cl transport is completely inactivated, but transport is partially restored by exposing the protein to guanidinium (129). The authors suggest that external guanidinium binds near residue 108 and restores the Cl binding by mimicking the hydrogen bonding interaction of the original arginine.

With regard to CFTR, the circumstantial evidence favoring a possible role for arginine residues in anion binding must be interpreted with caution for a number of reasons. First, it has been demonstrated (101) that the CFTR mutations (e.g., G314Q or G314E) result in CFTR channels that exhibit markedly reduced anion binding, despite the fact that a full complement of naturally occurring arginines is retained. Second, it has been pointed out by Dorman et al. (43) and Linsdell et al. (97) that

the peptide backbone may interact strongly with anions. Finally, arginine residues within membrane-spanning segments may serve an important role in maintaining the architecture of the pore by forming salt bridges with aspartic or glutamic acid residues.

3. Other polyatomic anions

Linsdell et al. (96) used a series of polyatomic anions to size the CFTR pore. A comparison of nitrate, formate, pyruvate, propionate, gluconate, methanesulfonate, ethanesulfonate, and acetate permeation predicted an apparent pore size of 5.3 Å, a value that is close to that estimated for the GABAR and GlyR channels (13, 54) and for an outwardly rectifying channel from T84 cells (63). A double mutation in TM6 (TT338:339AA) appeared to increase the apparent size of the CFTR pore. Smith et al. (143) investigated interactions with CFTR of a large family of polyatomic anions related to SCN and known as pseudohalides, including compounds such as OCN, N(CN)₂, Au(CN)₂, C(CN)₃, and Pt(CN)₄. Many of these compounds interacted strongly with CFTR, exhibiting not only permeation but also tight binding in the pore and blockade of Cl currents. The pseudohalides span a wide range of size and shape, and their hydration energies are expected to span a correspondingly large range. Smith et al. (143) found that the permeability ratios measured for these compounds in CFTR expressed in *Xenopus* oocytes ranged from 1 to 8 and were highly correlated with the output of an anion-selective electrode, the selectivity of which is dominated by hydration energy. If we take permeability ratios derived from shifts in E_{rev} , because they are primarily determined by the peak height of the energy barriers to permeation, to measure the ease of entry into the pore, then the behavior of the pseudohalides is consistent with the hypothesis that those anions that are most easily dehydrated enter the CFTR pore most readily. The binding of the pseudohalides in the pore, as measured by their ability to block Cl flow, was also correlated with ease of dehydration, consistent with the suggestion of Collins (25) that ions that exhibit weak interactions with water can be "pushed" on to the surface of proteins by water-water interactions. This effect could augment the attractive force due to any hydrogen bond interaction between the pseudohalide and amino acid residues, like arginine, so that the polyatomic anion could displace water interacting with the side chain.

4. Iodide permeation

In any survey of the permeation of monatomic and polyatomic anions through CFTR, iodide stands out as exhibiting some unique, or perhaps exaggerated, properties. The ion initially attracted attention because P_I/P_{Cl} (derived from E_{rev} values) is sensitive to amino acid substitution in the TM. This was first shown by Anderson et

al. (6), who focused on basic residues in TM 1, 6, and 10 that they reasoned might be important for anion selectivity. They compared the effects of substituting acidic for basic residues on $P_{\text{Na}}/P_{\text{Cl}}$ as well as on the relative anion permeability and conductance seen with substitution of Br, I, or F for external Cl in the whole cell patch-clamp configuration. The substitutions examined were K95D (TM1), K335E (TM6), R347E (TM6), and R1030E (TM10). None of these substitutions altered $P_{\text{Na}}/P_{\text{Cl}}$, which was of the order of 1:10, but two of the substitutions (K95D and K335E) altered the sequence of relative anion permeabilities by increasing the ratio for iodide, $P_{\text{I}}/P_{\text{Cl}}$. In the wild-type channel, the sequence was $\text{Br} > \text{Cl} > \text{I} > \text{F}$, and $P_{\text{I}}/P_{\text{Cl}}$ was ~ 0.6 . In the two mutant channels, this ratio was increased to ~ 1.4 , such that the selectivity sequence was $\text{I} > \text{Br} > \text{Cl} > \text{F}$. These substitutions, particularly K335E, also increased the apparent conductance ratio. The R347E substitution did not alter either the sequence of permeabilities or conductance, although there was the suggestion of an increased relative permeability for I. Overall, the changes in permeability were not remarkable except for that of iodide. Mansoura et al. (101) obtained similar results using human CFTR expressed in *Xenopus* oocytes. The $P_{\text{I}}/P_{\text{Cl}}$ ratio was 0.44 for wild-type CFTR and increased to 1.10 in K335E CFTR and 0.65 in K335D CFTR. These changes in $P_{\text{I}}/P_{\text{Cl}}$ are even more provocative when viewed from the perspective of the role of hydration energy in anion permeation. Smith et al. (143) compared permeability ratios across a panel of monatomic and polyatomic anions with relative ease of dehydration estimated by means of an anion-sensitive electrode. The correlation was striking, with all anions following the lyotropic or Hofmeister series, except for iodide. The general pattern was for the permeability ratio, $P_{\text{S}}/P_{\text{Cl}}$, to increase with increasing ease of dehydration, but $P_{\text{I}}/P_{\text{Cl}}$ was lower by a factor of 6 from that predicted by its relative hydration energy. The ratio $P_{\text{I}}/P_{\text{Cl}}$ was increased in K335E CFTR but was nevertheless substantially less than that predicted by the general pattern (101).

Tabcharani et al. (146) conducted a detailed study of iodide permeation in CHO cells and reported complex behavior, which they interpreted in terms of a model that allowed for two states, one in which iodide was able to permeate relatively freely and another in which iodide bound to the channel in such a way as to block its own permeation, but only partially block the movement of Cl. The conversion between the free permeation and bound states was relatively slow (1–2 min) and under bi-ionic conditions was favored by membrane potentials that would tend to drive Cl into the channel and iodide out of the channel. The behavior was eliminated by bathing the channel in solutions of high ionic strength or symmetric iodide. Importantly, the two states were characterized by quite different values of $P_{\text{I}}/P_{\text{Cl}}$, 1.8 in the unblocked state and >0.4 in the blocked state. Thus, in the unblocked

state, the permeability sequence was $P_{\text{I}} > P_{\text{Br}} > P_{\text{Cl}} > P_{\text{F}}$, i.e., that expected for a weak site channel, whereas in the blocked state, the sequence was $P_{\text{Br}} > P_{\text{Cl}} > P_{\text{I}} > P_{\text{F}}$ similar to that reported by Anderson et al. (6). This observation is particularly interesting in light of the disparity in the values reported for $P_{\text{I}}/P_{\text{Cl}}$, i.e., 0.4–0.6 (6, 101) or 1.0–2.0 (59, 145, 146). This behavior is consistent with the notion that iodide can reside in the channel without completely blocking anion permeation.

Finkelstein and Cass (55) called attention to the fact that the electrical behavior of planar lipid membranes bathed by iodide-containing solutions may be strongly influenced by the presence of polyiodides such as I_3 and I_5 generated by I^- combining with molecular iodine (I_2), but their results suggested that the formation of the polyiodides can be effectively eliminated in the presence of a reducing agent like thiosulfate ion. Tabcharani et al. (146), however, reported that the anomalous behavior of iodide persisted in the presence of 20 mM thiosulfate, and Smith and Dawson (unpublished data) did not find any effect of thiosulfate on the behavior of wild-type CFTR expressed in *Xenopus* oocytes in the presence of external iodide.

F. Cysteine Accessibility

A natural extension of the idea of using blockers or permeant ions that bind in the pore to study pore properties is that of actually engineering “new” binding sites at locations predicted to be within the pore, and then determining whether these sites are accessible to molecules that are predicted to enter the pore. The now widely used method known as “cysteine scanning” offers this tantalizing possibility. The approach relies on the introduction of cysteine residues into various locations and the use of polar thiol reagents as probes. Those most commonly employed are metals like Hg, Cd, or Ag or derivatives of methane thiosulfonate (MTS) first introduced by Kenyon and Bruce (79). The cysteine-scanning method provides several advantages over using mutagenesis to substitute residues of varying properties. First, the experiments can usually be designed so that channel properties can be directly compared before and after the modifying reaction. Second, the variety of thiol reagents available permits the investigator to vary the size and charge of the attached moiety. Finally, the covalent and essentially irreversible nature of the reaction facilitates testing the accessibility of residues in different conformational states of the channel.

Akabas et al. (3) synthesized three charged MTS derivatives by adding to MTS the negatively charged ethylsulfonate (MTSES⁻) or the positively charged ethylammonium (MTSEA⁻) or ethyltrimethylammonium (MTSET⁻). These reagents react with the SH group of cysteines to form mixed disulfides such that the charged

portion of the molecule is added in the form R-S-SCH₂CH₂X where X represents SO₃⁻ for MTSES⁻, NH₃⁻ for MTSEA⁻, and N(CH₃)₃⁻ for MTSET⁻. The strategy is based on the presumption that the desired reaction will take place only in a polar environment or “water accessible surface” because 1) the reaction requires ionization of the SH moiety and 2) the charged form of the reagent would be most likely to access the channel via a polar route. Ideally, therefore, the probe is most likely to react with cysteines that are exposed in the “lining” of the pore, which is reasonably presumed to be a water-filled space. The bulk and charge of the added group creates the reasonable expectation that such a reaction would modify the conduction properties of the pore. Akabas et al. (3) used this approach to scan TM1 of CFTR by determining the accessibility of cysteines substituted individually for nine consecutive residues. The macroscopic conductance due to the activation of wild-type CFTR in *Xenopus* oocytes was unaffected by the external application of either MTSES⁻ or MTSEA⁻, suggesting that none of the 17 cysteines in the protein was accessible from the outside in this condition. In three TM1 mutants, G91C, K95C, Q98C, all of which fall on the same face of a predicted TM1 α -helix, the conductance was irreversibly altered by either MTSES⁻ or MTSEA⁻.

Akabas and Cheung (2) examined residues in TM6 and reported that of 24 cysteine-substituted constructs, 9 exhibited inhibition of CFTR conductance when exposed to either MTSES⁻ or MTSEA⁻. The cationic reagent also produced inhibition in one construct (I331C) in which the anionic MTSES⁻ did not. Three residues, R334C, R347C, and R352C, were also inhibited by the larger cation MTSET⁻. One construct, I344C, exhibited an increase in conductance when exposed to MTSEA⁻. The pattern of apparent accessibility was not compatible with simple α -helical secondary structure; although seven of the residues deemed accessible lay on one side of a predicted helix, two (Q353 and K335) lay on the opposite side.

In a subsequent study, Cheung and Akabas (22) showed that the rates of reaction of MTSES⁻ and MTSET⁻ with cysteines engineered into TM6 were voltage dependent in a manner expected if the access process required electrodiffusional movement through a portion of the membrane field, and they used the voltage dependence of the rate of inhibition to calculate the apparent “electrical distance” of the TM6 cysteines from the outside of the channel. This calculation, based on the approach of Woodhull (161), measures the fraction of the total membrane potential required to account for the effect of V_m on the access rate. For the first five accessible residues, (L)333C, (R)334C, (K)335C, (F)337C, and (S)341C, the voltage dependence of the on rate for either MTSES⁻ or MTSET⁻ was either modest or nonexistent, yielding a fractional electrical distance of between 2 and 20% that did not show any striking correlation with the physical location of the

residue predicted on the basis of an α -helix. Thus, for at least one-half of the predicted physical distance, the rates were largely voltage independent. There was, however, a striking change in behavior with residue (T)351C. The “on” rates for this construct behaved as if the blocking reagents were seeing ~75% of the membrane voltage (for MTSES⁻; ~50% for MTSET⁻). Interestingly, the apparent distance for (R)352C appeared to be less than that for 351, but actually increased for (Q)353C, leading the authors to speculate that the cytoplasmic end of TM6 may loop up into the channel such that R352 is more extracellular than T351. The authors also compared the selectivity of the access process by comparing the access rate of MTSES⁻ and MTSET⁻ to the ratio of the reaction rates for these two compounds in solution with β -mercaptoethanol. This value is ~0.08, presumably due to the repulsion of the negatively charged MTSET⁻ and the ionized thiol group, R-S⁻. Compared in this way, the initial half of the predicted TM exhibited no selectivity, but at (T)351C, the selectivity jumped up to ~15. It was reduced to ~2 in the (R)352C construct and increased to 25 in the (Q)353C construct. The authors conclude that the “selectivity filter” is close to the cytoplasmic end of the pore and that R352 may play a role in determining charge selectivity for CFTR.

The results obtained from cysteine scanning studies are provocative, but there are several important caveats with regard to their interpretation. Any interpretation of the results in terms of pore structure relies on the assumption that the only water-accessible surface at which engineered cysteines can react with the charged MTS reagents in the pore interior. The potential flaw in this assumption was elegantly demonstrated by Horn and co-workers (163) who showed that cysteine residues engineered into the S4 segment of the voltage-dependent Na channel were accessible from either the extracellular or the cytoplasmic side and that the reaction resulted in profound gating effects without altering conduction. Thus this segment apparently exists in a water-filled crevice or “gating pore” that is distinct from the permeation path. Although there appears to be evidence that MTS reagents interact with cysteine-substituted CFTR, the mechanism(s) by which these interactions lead to changes in function has not been investigated. If the action of these compounds is confined to the pore, then the fact that significant conduction remains after the irreversible reaction has taken place, must be taken as evidence that the conduction pathway is not occluded, but instead only modified so that the time-average ion transit time is reduced. The deposition of nonoccluding, charged moieties in the channel lumen might be predicted to alter selectivity and, perhaps, to produce rectification in the i - V relation due to accumulation or depletion of permeant ions. In addition, the changes in gating (137) and sensitivity to activation by forskolin/IBMX (101) produced by mutations in the trans-

membrane segments raises the possibility that attaching the MTS moiety to engineered cysteines could alter macroscopic currents via an effect on gating or activation. This latter possibility could be important with regard to the action of at least one of the compounds in question, MTSEA⁻, which has been shown to cross cell membranes readily (70). Another concern, as pointed out by Cheung and Akabas (22), is that the effect of the cysteine substitution may, itself, cause a profound change in protein conformation that could influence the accessibility of a particular residue to MTS reagents. Another possibility is that an engineered cysteine may form a disulfide linkage within the protein leading to dramatic structural changes (81, 82). It will be of interest to see the results of studies in which the properties of the cysteine-substituted and the thiol-modified channels, which retain 50% or more of their conductance, are studied in detail.

VI. STRUCTURE AND FUNCTION OF THE CONDUCTION PATH

A. Mutagenesis: Predictions and Pitfalls

Site-directed mutagenesis has been widely employed as a means of obtaining information about the functional significance of amino acid residues in a protein. In the case of ion channels, this approach is utilized in conjunction with what is generally minimal information about the structure of the folded protein so that inferences about structure must be derived from changes in function. This so-called "structure-function" strategy would appear to have the potential for producing penetrating insights into the inner workings of ion channels, because one can combine the ability to alter protein structure one amino acid at a time with the ability to measure with exquisite precision the functional properties associated with ion conduction and channel gating. The ability to resolve the properties of a single ion channel with high resolution in real time would seem to maximize the potential for obtaining useful information. The rush of enthusiasm that one initially feels for this process is rapidly tempered, however, by the sobering realization that mutation-induced changes in function often provide little direct insight into structural features of the folded proteins. One issue is the sphere of influence of the mutations. The aim of a "site-directed" approach is to implicate specific regions of a protein in specific aspects of function, but if a change at *site A* results in a conformational change at *site B*, which is distant from *site A*, then the spatial information is lost. Even if the amino acid substitutions may be judged to be very local in their effects, a detailed interpretation of the functional consequences may require detailed information about the relation of *residue A* to nearby residues in the

peptide backbone that can only come from a three-dimensional structure.

All of this reminds one of Pogo's problem of being "surrounded by insurmountable opportunity." It is far from obvious that one can ever really defeat the inherent complexity of protein structure using this type of analysis alone. In the absence of structures for channel proteins, however, it has been possible to draw useful inferences about specific structural features of channel proteins using this approach. Noteworthy among these are inferences derived concerning the location and functional significance of reentrant loops for the pore properties of cation-selective channels (98, 99). Success in any such venture is contingent on developing a mutagenesis strategy that recognizes the inherent problems of interpretation.

A prudent mutagenesis strategy would include some of the following precautions: 1) a comprehensive set of functional assays should be employed to characterize mutants and to examine the trends in changes across a panel of substitutions at a single site. A comprehensive set of assays permits some assessment of the specificity of the functional change. For example, does a substitution alter both conduction and gating? The more specific the functional change, the less likely that an amino acid substitution has effected some cataclysmic, global change in protein conformation. 2) Comparing effects across a panel of mutations permits some inference about which property of the residue (size, polarity, charge) is critical to the observed change and also provides some control for the effects of certain substitutions on protein expression. It is important to compare mutants at the site of interest with other mutations at distant sites, again to judge the specificity of the effect. 3) It is important to develop some model for the structure or the process to use in interpreting the data. The process of ion conduction, for example, can be modeled using rate theory or continuum approaches. The resulting energy barrier profiles are not equivalent to structural information, but they do provide a framework for systematizing and comparing the results of different mutants for consistency. Similarly, even the most primitive structural model, such as that provided by the channels formed by designed peptides or gramicidin, provides at least some basis for placing results in a zeroth-order structural context.

B. Predictions for the Pore

As a prelude to a survey of the effects on CFTR conduction properties of amino acid substitutions in the transmembrane segments, it is useful to recapitulate some general expectations for anion-selective pores that emerge from the material considered in the preceding sections. The design considerations articulated by Levitt (90) and Dorman et al. (43), along with observations on

a wide variety of anion selective channels, lead us to expect that anion-selective permeation is strongly influenced by anion-water interactions and that discretely placed positively charged amino acids, particularly arginines, may be used to encourage anion permeation and chase away cations. The relative lack of selectivity of anion channels may be related to the difficulty in dehydrating Cl, the principal anion of physiological interest. Finally, although the basis for interaction between anions and the pore is not well understood, there seems ample reason to speculate that the same arginines that may be placed so as to encourage anion entry into the pore may contribute in some way to binding sites, particularly for nonphysiological anions such as SCN that, when the net result of ion-water, ion-site, site-water, and water-water interactions are considered, may be more likely than Cl to stick to the "walls" of the channel.

Thus we might envision the following zeroth-order picture of an anion conduction path. A pore could be formed by an antiparallel array of transmembrane segments. Bormann et al. (13), for example, envisioned the pore of the GABAR channel as being lined by portions of five helices packed so as to provide a channel lumen of ~ 5.8 Å in diameter. Depending on the prevailing ionic conditions, we would expect to find that the channel was occupied by anions and water molecules. The water permeability of CFTR demonstrated by Hasegawa et al. (65) is consonant with this picture. To enter the channel, an anion must displace water, and the entry process might be imagined in terms of the sort of thermodynamic cycle utilized by Dorman et al. (43) to model permeation in the gramicidin pore, i.e., one that takes into account anion-water, anion-channel, water-channel, and water-water interactions. Once inside the pore, the anion would compete with water and other anions for interactions with the peptide backbone or the pore-lining side chains like the guanidinium groups of arginines, that are arrayed in a specific structural context that favors the binding of anions like SCN.

It is instructive to compare the selectivity properties of CFTR with those of the bacterial K channel, for which the permeation mechanism has been clarified by visualizing the three-dimensional structure of the selectivity filter (45). In the K channel, a plot of permeability ratios is a nonmonotonic function of the inverse of ionic radius, i.e., $\text{Cs} > \text{Rb}, \text{K} \gg \text{Na}, \text{Li}$. [The inverse of the ionic radius is a natural choice for such a plot because the energy of ion interaction with a polarizable medium varies with the inverse of ionic radius (12, 124).] The permeabilities of the two larger cations, Cs and Rb, are less than or equal to K, whereas those of the two smaller, Na and Li, are orders of magnitude less. Evidence from the crystal structure, viewed in light of studies of the K selectivity of axonal membranes (11), antibiotics (24, 40, 49), and more recent mutagenesis (66) strongly suggest that, within the

channel, the inner sphere waters that coordinate K in aqueous solution are replaced by the carbonyl oxygens of the peptide backbone of a segment of the so-called "p loop." With regard to selectivity, the structure confirms earlier suggestions that the key to selecting K, over the smaller Na, is the optimal fit (7) provided by the geometry of the tetrahedral cage of oxygen ligands. The strong interaction of the K ion with these oxygens compensates for the energy required to strip off water molecules, perhaps in a stepwise fashion (24, 32, 49), as the ion enters the pore. Thus the reduced barrier to entry into the pore is a reflection of the stabilization of the ion within the channel. Cations larger than K have smaller hydration energies but also weaker interaction with channel and, hence, lower permeability. Ions smaller than K have either the same or smaller interactions with the channel but larger hydration energies so that they are prevented from entering.

The pattern of halide selectivity of CFTR differs phenomenologically from the pattern of cation selectivity of the K channel in several important ways. First, if the permeability ratios P_s/P_{Cl} are expressed as the difference in barrier height in units of $\Delta G/RT$, this energy is seen to vary as a monotonic function of $1/r$, i.e., the larger the anion, the more readily it enters the channel compared with Cl. The larger the ion, the smaller is the difference between the ion-water and ion-channel interaction energies (91). The ability of anions to act as blockers of Cl flow increases with increasing size, consistent with the notion that larger, more weakly hydrated anions tend to stick more tightly in the channel than Cl. This stickiness impedes flow of the anion except at relatively high symmetric concentrations (147). Thus we see that the K channel and the anion channel, CFTR, operate on different principles. In the former, the cation of physiological importance, K, is the most highly permeant and also interacts most strongly with the channel. The potentially negative effect on conduction of the strong cation-channel interaction (required to overcome the energy of dehydration) is mitigated by repulsive effects that enhance K mobility within the selectivity filter. In the CFTR pore, the ion of physiological importance is one of the least permeant and also one of the least tightly bound. Larger anions that enter the channel more readily than Cl are, however, retarded in their transit due to binding within the channel.

C. Sensing Changes in Pore Architecture

In its simplest form, the object of any structure-function analysis of the conduction path of CFTR is to determine which amino acids are important for maintaining the unique functional properties of the pore. The use of multiple functional assays to test for possible changes in pore structure naturally raises the question of whether any one of these may be more sensitive than others to

changes in pore architecture brought about by mutations. If so, this assay or functional property might be chosen as the most useful screening tool with which to select particular mutants for detailed analysis. Mansoura et al. (101) addressed this question by comparing the impact of a limited set of mutations in TM1, TM5, and TM6 on four properties: permeability ratios derived from E_{rev} values, conductance ratios seen with substitute anions, shape of the macroscopic i - V curve, and the sensitivity of mutants to dose-dependent activation by IBMX in the presence of forskolin. This survey of 11 CFTR mutations at three sites suggested that ion binding by CFTR, as measured by the influence of substitute ions on CFTR conductance, was much more sensitive than either permeability ratios or i - V shape to changes in pore structure. Because of its relatively tight binding in the CFTR pore, SCN proved to be the most valuable probe. Mutations in TM5 and TM6 dramatically reduced the binding of SCN relative to Cl, whereas the same mutations produce little or no effect on permeability ratios. Permeability ratios turned out to be the property that tended to be the least affected by mutations generally. This result is in accord with the notion that anion permeability ratios, which to a first approximation measure how easy it is for an anion to enter the channel, are determined by the difference between the energy required to dehydrate the anion and some stabilization energy that is the consequence of a general interaction of the anion with the pore that is not highly dependent on the details of channel structure. The more dramatic effect of mutations on anion binding suggests that permeant anions can interact with the channel sufficiently strongly to impede conduction rates but that the "tight binding" is dependent on some element of pore conformation that is not critical to promote the entry of anions into the pore. The impact of mutations that influenced anion binding on channel structure was underscored by the finding that the dose-dependent activation of TM5 and TM6 mutants by IBMX in the presence of forskolin was moderately to severely impaired, as if TM 5 and 6 are also intimately involved in the process of opening the channel.

D. Structural Elements That Are Important for Pore Properties

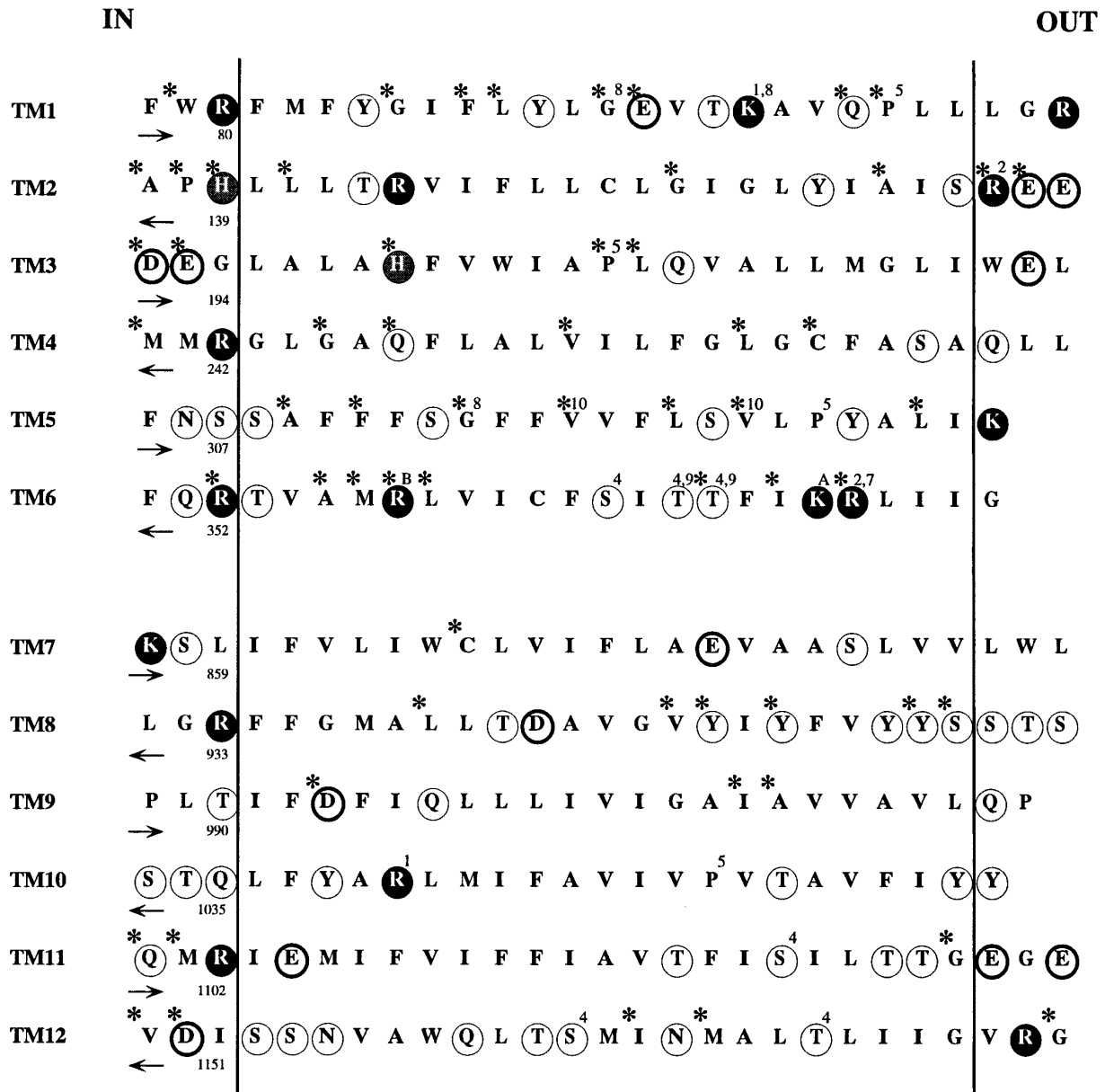
Figure 5 shows the TM of CFTR represented as an antiparallel, linear alignment of the amino acids predicted to lie within or near TM 1–12. Structure-function studies of CFTR have focused on the TM, there being thus far no compelling evidence for a role for reentrant loops in forming the anion-selective pore (29, 140). Indicated in Figure 5 are residues that have been substituted and which of these are sites of patient mutations. Also shown is the polarity and charge of each residue. The diagram is clearly not a structure, but it does provide a concise

summary, not only of the sorts of structural elements that are available within the TM to construct a pore, but also of the activity that has been directed at perturbing these elements and noting functional consequences. We will use this diagram to provide an overview of the results of pore-related structure-function studies which, although at an early stage, provide some tantalizing glimpses of possible major players in pore structure and also suggest that the architecture of CFTR may reflect some of the design considerations discussed above. We discuss the TM in their apparent order of importance, with the caveat that, at this juncture, only a few residues and TM have been systematically investigated.

If the TM diagram in Figure 5 is viewed as a web site, then it is apparent that there have been a lot of hits on TM6, so that this TM emerges as the most studied and, at this stage therefore, the TM with the most clearly demonstrable importance to CFTR pore properties. Tabcharani et al. (147) demonstrated that TM6 is important for anion binding by CFTR. As discussed in section III D, block of Cl currents by SCN and the anomalous mole fraction effect, both indicative of tight binding of SCN in the CFTR pore, were altered by substitution for basic residues in TM6, K335, and R347. The results obtained with R347D were the most dramatic. This substitution abolished the anomalous mole fraction effect and SCN block, both results indicative of a reduced affinity (relative to Cl) for SCN binding in the pore. The pH-dependent behavior of an R347H construct suggested that a positive charge at this locus was important for high-affinity SCN binding. In confirmation of these results, Smith and Dawson (unpublished data) found that blockade of CFTR by external SCN was abolished in R347E and also in R347Q CFTR, confirming the importance of the positive charge at this site. In a subsequent study, Linsdell and Hanrahan (93) showed that block by DIDS and DNDS was attenuated in the R347D CFTR.

Tabcharani et al. (147) found that the anomalous mole fraction effect of SCN was reduced but not eliminated in a K335D construct. Mansoura et al. (101) confirmed that SCN binding was reduced but not eliminated in K335D and E CFTR and showed that simply deleting the charge (K335A) was without effect. K335 and R347 had been implicated by Anderson et al. (6) in CFTR anion selectivity, but this study focused primarily on permeability ratios so that, as discussed above, the changes were less dramatic than those seen with SCN binding. R352 in TM6 was implicated by Cheung and Akabas (2, 22) in cysteine-scanning studies, and Smith and Dawson (unpublished data) showed that SCN block is also markedly reduced in this construct.

McCarty et al. (104) used DPC block of CFTR to assay the effects of mutations in TM6 and TM12. Substituting an alanine for serine-341 in TM6 virtually abolished the voltage-dependent block by DPC seen at negative volt-



●● Basic residues; ○ Acidic residues; ○ Polar residues; * Patient mutations

References:

1= Anderson, MP et al. Science 253:202-5, 1991.	6= Linsdell and Hanrahan. J Physiol 496:687-93, 1996.
2= Sheppard, DN et al. Nature 362:160-4, 1993.	7= Hipper, A et al. FEBS 374:312-6, 1995.
3= Tabcharani, JA et al. Nature 366:79-82, 1993.	8= Mansoura, MK et al. Biophys J 74:1320-34, 1998.
4= McDonough, S et al. Neuron 13:623-34, 1994.	9= Linsdell, P et al. J Gen Physiol 110:355-64, 1997.
5= Sheppard, DN et al. JBC 271:14995-01, 1996.	10= Smith, SS et al Biophys J 72(2):A365, 1997

A= refs 1,3,4,7,8 B= refs 1,2,3,6,7

FIG. 5. Antiparallel alignment of 12 predicted membrane-spanning segments of CFTR showing basic residues, acidic residues, polar and nonpolar residues, and patient mutations as indicated. Also indicated are residues that have been mutated in structure-function studies of CFTR (6, 69, 93, 96, 101, 105, 137, 138, 142, 147).

ages, whereas substitution of the polar threonine produced an intermediate effect. The single-channel conductance of S341A was reduced to ~ 1 pS, and the macroscopic *i-V* relation became slightly inwardly rectifying, suggesting to the authors that this polar amino acid may be pore-lining and also constitute a binding site for DPC, via hydrogen bonding with the -OH group. An analogous substitution in TM12 (S1141A) did not alter anion conduction or DPC block but, if residues surrounding S1141 were altered so as to match those surrounding S341 and then combined with the S341A mutation, binding was restored, as if the binding site had been "moved to TM12." The authors predicted that residues in TM6 and TM12 lying closer to the extracellular end of the pore might interact with the other phenyl ring of DPC. One of these was K335 (TM6) that was implicated by Anderson et al. (6), Tabcharani et al. (147) and Mansoura et al. (101) as contributing to pore properties. The K335E construct was identical to wild type with regard to apparent affinity for DPC but did display slight inward rectification. Substituting the phenylalanine (K335F) led to a modest decrease in DPC binding and did not alter the *i-V* relation. In TM12, substituting a phenylalanine for T1134 increased the apparent affinity for DPC, as expected if DPC binding was stabilized by introducing the more hydrophobic residue. This substitution reduced the single-channel conductance by $\sim 30\%$.

The effects of site-directed mutations in TM6 on anion permeation and block strongly suggest that this TM plays an important role in the formation of the anion-selective pore of CFTR. Its four basic residues (K355, R334, R347, R352) recall the design criteria for anion selectivity (see sect. iv), and in view of the roles of arginines in anion binding in other proteins (see sect. vE2), it is tempting to suggest that this TM might actually line the pore as suggested by the voltage-dependent accessibility of some TM6 residues to MTS reagents (see sect. vF). Linsdell and Hanrahan (92, 93) suggested that the cytoplasmic face of the CFTR pore may contain a relatively wide "vestibule" that permits the entry not only of SCN but also of larger pore-occluding molecules such as DPC, DIDS, and organic anions (see sect. v).

By the criterion of anion binding, TM5, although less studied than TM6, emerges as a potentially important structural element in the CFTR pore. Mansoura et al. (101) studied the consequences of substitutions for G314, also the site of two patient mutations, and found that SCN block of CFTR was abolished in G314E and G314Q CFTR, moderately impaired in G314A CFTR, and unaffected in G314D CFTR. In the aggregate, these results suggest that glycine-314 in TM5 is important for anion binding by CFTR and that the presence, per se, of arginine residues such as R347 is not sufficient to produce anion binding. If anion-arginine interactions are responsible for anion binding, then presumably the structure of TM5 is an important determinant of the conformational features of the pore

that promote the strong interaction with the linear, polyatomic SCN anion. This result argues against the notion that anion binding might be a generalized feature of bundles of α -helices containing arginines, although Dorman et al. (43), in their analysis of a model for ion permeation in the gramicidin channel, suggested that anion interactions with the peptide backbone of this model system might produce energy minima that would behave as binding sites. One speculation is that TM5 and TM6 are both part of the "lining" of the pore such that the charged residues in TM6 and the exact conformation of TM5 are both strong determinants of the types of interaction that a permeating anion may experience with the pore wall. This tentative assignment of roles in pore formation to TM5 and TM6 is supported by the fact that these TM are also the sites of the largest number of patient mutations. Finally, the data of Mansoura et al. (101) and Smith and Dawson (unpublished data) indicate that both may be important for the conformational changes that gate the pore.

Transmembrane segment 1 has been the subject of several studies that have not produced consonant results. As many as three basic residues (as well as one acidic residue) lie in or near TM1, and mutating one of them, K95, to aspartic acid (K95D) increased P_i/P_{Cl} (6). A patient mutation, P99L, was associated with reduced single-channel conductance (138). In addition, Akabas et al. (3) identified three TM1 residues, G91, K95, and Q98, as accessible to MTS reagents in the respective cysteine-substituted constructs. A deletion construct, however, lacking TM1 was reported by Carroll et al. (20) to exhibit conduction and gating properties similar to wild-type CFTR. In addition, Mansoura et al. (101) found that neutral (G91A), acidic (G91E), and basic (G91R) substitutions in this TM had no effect on SCN binding or the sensitivity of the constructs to activation by IBMX, although the shape of the *i-V* plot was altered for G91R.

Transmembrane segment 2 has been implicated in the conduction properties of CFTR directly and indirectly. Sheppard et al. (137) reported that R117H CFTR exhibited a slightly reduced single-channel conductance but produced a substantial reduction in open probability. Oblatt-Montal et al. (111) used planar bilayers to assay the propensity of individual peptides modeled after CFTR TM to form channels. Transmembrane segment 2 and TM6 sequences formed anion-selective channels in bilayers, whereas peptides based on TM1, TM3, TM4, and TM5 did not. Mixtures of the TM2 and TM6 peptides produced channels of intermediate single-channel conductance, suggesting a heterologomeric structure. The latter result is not unexpected in view of the design criteria reviewed in section iv; both TM2 and TM6 peptides contain basic residues at or near the ends. The positive results obtained using this approach with TM2 and TM6 are intriguing, but the negative results obtained with other TM are more

difficult to interpret and do not negate a role for these TM in forming the pore of the intact protein.

Evidence for involvement of the remaining nine TM is minimal. Four (TM4, TM7, TM8, and TM9) have not been studied individually in intact CFTR, although reports on the behavior of truncated CFTR might provoke some speculation (albeit risky) about their role. Carroll et al. (20) reported that expression of a CFTR construct lacking TM1-TM4 gave rise to cAMP-activated Cl⁻-selective channel activity, although single-channel conductance was reduced by ~30%. Sheppard et al. (136) reported that a D836X CFTR comprising only the first six TM, NBF1, and the R domain gave rise to channels activated by ATP and protein kinase A that exhibited a single-channel conductance similar to wild-type CFTR and were thought to function as homodimers. This result as well as those from other large deletion constructs (135) are intriguing, but the properties of these proteins have not yet been studied in a detail sufficient to determine if the reported channel activity is a reflection of a protein conformation that is directly related to the functional unit of intact CFTR or if, instead, such activity is the result of some novel conformation that can only be attained in the truncated proteins.

In TM11, a serine to alanine substitution (S1118A) did not affect block by DPC or the shape of the *i*-*V* plot (105). In TM10 (6), substitutions of an arginine by a glutamic and (R1030E) did not alter the anion selectivity sequence, although P_{I}/P_{Cl} appeared to increase. Anion binding, per se, was not investigated, but the apparent block by iodide was not altered.

VII. FUTURE DIRECTIONS AND ROADS YET TO BE TRAVELED

The results reviewed in section VI might well call to mind the fable of the blind men attempting to describe an elephant. Although some effort has been devoted to tinkering with the transmembrane segments of CFTR, there is as yet no clear choice for a structural model of the pore. On the other hand, the results of amino acid substitutions, as well as the behavior of wild-type CFTR, suggest some interesting correlations between the anion-conducting properties of this channel and the behavior of other channels and even nonchannel proteins that exhibit specific interactions with anions. Taken together, these results suggest that, although it is not apparent to us yet, there may be some underlying unity to the design of anion-conducting pores and anion-binding sites that may emerge from increased structural information and careful comparisons of anion-protein interactions in a range of physical settings. It seems likely that an increased focus on the physical nature of the interactions of anions with proteins and water, together with structural information that may emerge from covalent modification of CFTR, will begin

to provide meaningful insights into the structure of the pore.

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REFERENCES

1. ABRAHAM, E. H., P. OKUNIEFF, S. SCALA, P. VOS, M. J. S. OOSTERVELD, A. Y. CHEN, B. SHRIVASTAV, AND G. GUIDOTTI. Cystic fibrosis transmembrane conductance regulator and adenosine triphosphate. *Science* 275: 1324–1325, 1997.
2. AKABAS, M. H., AND M. CHEUNG. Identification of channel-lining residues in the M6 membrane-spanning segment of CFTR and the position of the anion-selectivity filter (Abstract). *Biophys. J.* 70: A71, 1996.
3. AKABAS, M. H., C. KAUFMANN, T. A. COOK, AND P. ARCHDEACON. Amino acid residues lining the chloride channel of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* 269: 14865–14868, 1994.
4. ANDERSEN, O. S., AND R. E. D. KOEPE. Molecular determinants of channel function. *Physiol. Rev.* 72, Suppl.: S89–S158, 1992.
5. ANDERSON, M. P., H. A. BERGER, D. P. RICH, R. J. GREGORY, A. E. SMITH, AND M. J. WELSH. Nucleoside triphosphates are required to open the CFTR chloride channel. *Cell* 67: 775–784, 1991.
6. ANDERSON, M. P., R. J. GREGORY, S. THOMPSON, D. W. SOUZA, S. PAUL, R. C. MULLIGAN, A. E. SMITH, AND M. J. WELSH. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science* 253: 202–205, 1991.
7. ARMSTRONG, C. M. Reflections on selectivity. In: *Membrane Transport: People and Ideas*. Bethesda, MD: Am. Physiol. Soc., 1989, p. 261–273.
8. ARSHADI, M., R. YAMDAGNI, AND P. KEBARLE. Hydration of the halide negative ions in the gas phase. II. Comparison of hydration energies for the alkali positive and halide negative ions. *J. Phys. Chem.* 74: 1475–1482, 1970.
9. BAUKROWITZ, T., T. C. HWANG, A. C. NAIRN, AND D. C. GADSBY. Coupling of CFTR Cl⁻ channel gating to an ATP hydrolysis cycle. *Neuron* 12: 473–482, 1994.
10. BEAR, C. E., C. H. LI, N. KARTNER, R. J. BRIDGES, T. J. JENSEN, M. RAMJEESINGH, AND J. R. RIORDAN. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell* 68: 809–818, 1992.
11. BEZANILLA, F., AND C. M. ARMSTRONG. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* 60: 588–608, 1972.
12. BOCKRIS, J. O. M., AND A. K. N. REDDY. *Modern Electrochemistry*. New York: Plenum, 1970.
13. BORMANN, J., O. P. HAMILL, AND B. SAKMANN. Mechanism of anion permeation through channels gated by glycine and gamma-aminobutyric acid in mouse cultured spinal neurones. *J. Physiol. (Lond.)* 385: 243–286, 1987.
14. BRAIMAN, M. S., T. J. WALTER, AND D. M. BRIERCHECK. Infrared spectroscopic detection of light-induced change in chloride-arginine interaction in halorhodopsin. *Biochemistry* 33: 1629–1635, 1994.
15. BRIDGES, R. J., R. T. WORRELL, R. A. FRIZZELL, AND D. J. BENOS. Stilbene disulfonate blockade of colonic secretory Cl⁻ channels in planar lipid bilayers. *Am. J. Physiol.* 256 (Cell Physiol. 25): C902–C912, 1989.
16. BUCKINGHAM, A. D. A theory of ion-solvent interaction. *Faraday Discuss. Chem. Soc.* 24: 151–157, 1957.
17. BULL, T. E., B. LINDMAN, R. EINARSSON, AND M. ZEPPEZAUER.

- Binding of Au(CN)₂⁻ and Pt(CN)₄²⁻ to horse liver alcohol dehydrogenase. A ³⁵Cl NMR relaxation study. *Biochim. Biophys. Acta* 377: 1–8, 1975.
18. BURNASHEV, N., R. SCHOEPFER, H. MONYER, J. P. RUPPERSBERG, W. GUNTHER, P. H. SEEBURG, AND B. SAKMANN. Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. *Science* 257: 1415–1419, 1992.
 19. BURNASHEV, N., A. VILLARROEL, AND B. SAKMANN. Dimensions and ion selectivity of recombinant AMPA and kainate receptor channels and their dependence on Q/R site residues. *J. Physiol. (Lond.)* 496: 165–173, 1996.
 20. CARROLL, T. P., M. M. MORALES, S. B. FULMER, S. S. ALLEN, T. R. FLOTTE, G. R. CUTTING, AND W. B. GUGGINO. Alternate translation initiation codons can create functional forms of cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* 270: 11941–11946, 1995.
 21. CHEN, D., L. XU, A. TRIPATHY, G. MEISSNER, AND B. EISENBERG. Permeation through the calcium release channel of cardiac muscle. *Biophys. J.* 73: 1337–1354, 1997.
 22. CHEUNG, M., AND M. H. AKABAS. Locating the anion-selectivity filter of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. *J. Gen. Physiol.* 109: 289–299, 1997.
 23. CHIANCONE, E., J. E. NORNE, S. FORSEN, J. BONAVENTURA, M. BRUNORI, E. ANTONINI, AND J. WYMAN. Identification of chloride-binding sites in hemoglobin by nuclear-magnetic-resonance quadrupole-relaxation studies of hemoglobin digests. *Eur. J. Biochem.* 55: 385–390, 1975.
 24. CHOCK, P. B., F. EGGERS, M. EIGEN, AND R. WINKLER. Relaxation studies on complex formation of macrocyclic and open chain antibiotics with monovalent cations. *Biophys. Chem.* 6: 239–251, 1977.
 25. COLLINS, K. D. Sticky ions in biological systems. *Proc. Natl. Acad. Sci. USA* 92: 5553–5557, 1995.
 26. COLLINS, K. D. Charge density-dependent strength of hydration and biological structure. *Biophys. J.* 72: 65–76, 1997.
 27. COLLINS, K. D., AND M. W. WASHABAUGH. The Hofmeister effect and the behaviour of water at interfaces. *Q. Rev. Biophys.* 18: 323–422, 1985.
 28. CORONADO, R., R. L. ROSENBERG, AND C. MILLER. Ionic selectivity, saturation, and block in a K⁺-selective channel from sarcoplasmic reticulum. *J. Gen. Physiol.* 76: 425–446, 1980.
 29. COTTEN, J. F., L. S. OSTEDGAARD, M. R. CARSON, AND M. J. WELSH. Effect of cystic fibrosis-associated mutations in the fourth intracellular loop of cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* 271: 21279–21284, 1996.
 30. COX, B. G., G. R. HEDWIG, A. J. PARKER, AND D. W. WATTS. Solvation of ions. XIX. Thermodynamic properties for transfer of single ions between protic and aprotic solvents. *Aust. J. Chem.* 27: 477–501, 1974.
 31. DALMARK, M., AND J. O. WIETH. Temperature dependence of chloride, bromide, iodide, thiocyanate and salicylate transport in human red cells. *J. Physiol. (Lond.)* 224: 583–610, 1972.
 32. DANG, T. X., AND E. W. MCCLESKEY. Ion channel selectivity through stepwise changes in binding affinity. *J. Gen. Physiol.* 111: 185–193, 1998.
 33. DANI, J. A., AND D. G. LEVITT. Diffusion and kinetic approaches to describe permeation in ionic channels. *J. Theor. Biol.* 146: 289–301, 1990.
 34. DANI, J. A., J. A. SANCHEZ, AND B. HILLE. Lyotropic anions. Na channel gating and Ca electrode response. *J. Gen. Physiol.* 81: 255–281, 1983.
 35. DAVIDSON, W. R., AND P. KEBARLE. Ionic solvation by aprotic solvents. Gas phase solvation of the alkali ions by acetonitrile. *J. Am. Chem. Soc.* 98: 6125–6133, 1976.
 36. DAWSON, D. C. Thermodynamic aspects of radiotracer flow. In: *Biological Transport of Radiotracers*, edited by L. G. Colombetti. Boca Raton, FL: CRC, 1982, p. 79–95.
 37. DAWSON, D. C. Principles of membrane transport. In: *Handbook of Physiology. The Gastrointestinal System. Intestinal Absorption and Secretion*. Bethesda, MD: Am. Physiol. Soc., 1991, sect. 6, vol. IV, chapt. 1, p. 1–44.
 38. DAWSON, D. C. Permeability and conductance in ion channels: a primer. In: *Molecular Biology of Membrane Transport Disorders*, edited by T. F. Andreoli, J. F. Hoffman, D. D. Fanestil, and S. G. Schultz. New York: Plenum, 1996, p. 87–109.
 39. DIAMOND, J. M., AND E. M. WRIGHT. Biological membranes: the physical basis of ion and nonelectrolyte selectivity. *Annu. Rev. Physiol.* 31: 581–646, 1969.
 40. DIEBLER, H., M. EIGEN, G. ILGENFRITZ, G. MAAB, AND R. WINKLER. Kinetics and mechanism of reactions of main group metal ions with biological carriers. *Pure Appl. Chem.* 20: 93–115, 1969.
 41. DIETRICH, B. Design of anion receptors: applications. *Pure Appl. Chem.* 65: 1457–1464, 1993.
 42. DISSING, S., L. ROMANO, AND H. PASSOW. The kinetics of anion equilibrium exchange across the red blood cell membrane as measured by means of ³⁵S thiocyanate. *J. Membr. Biol.* 62: 219–229, 1981.
 43. DORMAN, V., M. B. PARTENSKII, AND P. C. JORDAN. A semi-microscopic Monte Carlo study of permeation energetics in a gramicidin-like channel: the origin of cation selectivity. *Biophys. J.* 70: 121–134, 1996.
 44. DOUGLAS, B. E., D. H. MCDANIEL, AND J. J. ALEXANDER. *Concepts and Models of Inorganic Chemistry*. New York: Wiley, 1994.
 45. DOYLE, D. A., J. M. CABRAL, R. A. PFUETZNER, A. KUO, J. M. GULBIS, S. L. COHEN, B. T. CHAIT, AND R. MACKINNON. The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity. *Science* 280: 69–77, 1998.
 46. DREINHOFER, J., H. GOGELIN, AND R. GREGER. Blocking kinetics of Cl⁻ channels in colonic carcinoma cells (HT29) as revealed by 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB). *Biochim. Biophys. Acta* 946: 135–142, 1988.
 47. ECHAVARRÉN, A., A. GALÁN, J. DE MENDOZA, AND A. SALMERÓN. Anion-receptor molecules: synthesis of a chiral and functionalized binding subunit, a bicyclic guanidinium group derived from L- or D-asparagine. *Helv. Chim. Acta* 71: 685–693, 1988.
 48. EGAN, M., T. FLOTTE, S. AFIONE, R. SOLOW, P. L. ZEITLIN, B. J. CARTER, AND W. B. GUGGINO. Defective regulation of outwardly rectifying Cl⁻ channels by protein kinase A corrected by insertion of CFTR. *Nature* 358: 581–584, 1992.
 49. EIGEN, M., AND R. WINKLER. Carriers and specificity in membranes. II. Characteristics of carriers. Alkali ion carriers: specificity, architecture, and mechanisms: an essay. *Neurosci. Res. Prog. Bull.* 9: 330–338, 1971.
 50. EISENMAN, G., AND R. HORN. Ionic selectivity revisited: the role of kinetic and equilibrium processes in ion permeation through channels. *J. Membr. Biol.* 76: 197–225, 1983.
 51. EYRING, H., R. LUMRY, AND J. W. WOODBURY. Some applications of modern rate theory to physiological systems. *Rec. Chem. Prog.* 10: 100–114, 1949.
 52. FAHLKE, C., H. T. YU, C. L. BECK, T. H. RHODES, AND A. L. GEORGE, JR. Pore-forming segments in voltage-gated chloride channels. *Nature* 390: 529–532, 1997.
 53. FAIRBROTHER, W. J., H. C. GRAHAM, AND R. J. WILLIAMS. An NMR study of anion binding to yeast phosphoglycerate kinase. *Eur. J. Biochem.* 190: 161–169, 1990.
 54. FATIMA-SHAD, K., AND P. H. BARRY. Anion permeation in GABA- and glycine-gated channels of mammalian cultured hippocampal neurons. *Proc. R. Soc. Lond. B Biol. Sci.* 253: 69–75, 1993.
 55. FINKELSTEIN, A., AND A. CASS. Permeability and electrical properties of thin lipid membranes. *J. Gen. Physiol.* 52, Suppl.: 145–173, 1968.
 56. GABRIEL, S. E., L. L. CLARKE, R. C. BOUCHER, AND M. J. STUTTS. CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship. *Nature* 363: 263–268, 1993.
 57. GALZI, J. L., A. DEVILLERS-THIERY, N. HUSSY, S. BERTRAND, J. P. CHANGEUX, AND D. BERTRAND. Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. *Nature* 359: 500–505, 1992.
 58. GOLSTEIN, P., M. ABRAMOW, J. E. DUMONT, AND R. BEAUWENS. The iodide channel of the thyroid: a plasma membrane vesicle study. *Am. J. Physiol.* 263 (Cell Physiol. 32): C590–C597, 1992.
 59. GRAY, M. A., A. HARRIS, L. COLEMAN, J. R. GREENWELL, AND B. E. ARGENT. Two types of chloride channel on duct cells cultured from human fetal pancreas. *Am. J. Physiol.* 259 (Cell Physiol. 28): C752–C761, 1989.
 60. GRYGORCZYK, R., AND J. W. HANRAHAN. CFTR-independent ATP

- release from epithelial cells triggered by mechanical stimuli. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C1058–C1066, 1997.
61. GUGGINO, W. B. Outwardly rectifying chloride channels and CF: a divorce and remarriage. *J. Bioenerg. Biomembr.* 25: 27–35, 1993.
 62. HAGIWARA, S., AND K. TAKAHASHI. Mechanism of anion permeation through the muscle fibre membrane of an elasmobranch fish, *Taeniura lymma*. *J. Physiol. (Lond.)* 238: 109–127, 1974.
 63. HALM, D. R., AND R. A. FRIZZELL. Anion permeation in an apical membrane chloride channel of a secretory epithelial cell. *J. Gen. Physiol.* 99: 339–366, 1992.
 64. HARRIS, E. J. Anion interaction in frog muscle. *J. Physiol. (Lond.)* 141: 351–365, 1958.
 65. HASEGAWA, H., W. SKACH, O. BAKER, M. C. CALAYAG, V. LINGAPPA, AND A. S. VERKMAN. A multifunctional aqueous channel formed by CFTR. *Science* 258: 1477–1479, 1992.
 66. HEGINBOTHAM, L., Z. LU, T. ABRAMSON, AND R. MACKINNON. Mutations in the K⁺ channel signature sequence. *Biophys. J.* 66: 1061–1067, 1994.
 67. HILLE, B. *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer, 1992.
 68. HILLE, B., AND W. SCHWARZ. Potassium channels as multi-ion single-file pores. *J. Gen. Physiol.* 72: 409–442, 1978.
 69. HIPPER, A., M. MALL, R. GREGER, AND K. KUNZELMANN. Mutations in the putative pore-forming domain of CFTR do not change anion selectivity of the cAMP activated Cl⁻ conductance. *FEBS Lett.* 374: 312–316, 1995.
 70. HOLMGREN, M., Y. LIU, Y. XU, AND G. YELLEN. On the use of thiol-modifying agents to determine channel topology. *Neuropharmacology* 35: 797–804, 1996.
 71. HUTTER, O. F., AND S. M. PADSHA. Effect of nitrate and other anions on the membrane resistance of frog skeletal muscle. *J. Physiol. (Lond.)* 146: 117–132, 1959.
 72. HWANG, T. C., G. NAGEL, A. C. NAIRN, AND D. C. GADSBY. Regulation of the gating of cystic fibrosis transmembrane conductance regulator Cl channels by phosphorylation and ATP hydrolysis. *Proc. Natl. Acad. Sci. USA* 91: 4698–4702, 1994.
 73. JEFFERY, G. A., AND W. SAENGER. Halides and halogen atoms as hydrogen-bond acceptors. In: *Hydrogen Bonding in Biological Structures*. New York: Springer-Verlag, 1991, p. 161–163.
 74. JEFFREY, G. A., AND W. SAENGER. *Hydrogen Bonding in Biological Structures*. Berlin: Springer-Verlag, 1991.
 75. JONAS, A., AND G. WEBER. Presence of arginine residues at the strong, hydrophobic anion binding sites of bovine serum albumin. *Biochemistry* 10: 1335–1339, 1971.
 76. JONAS, A., AND G. WEBER. Strong binding of hydrophobic anions by bovine serum albumin peptides covalently linked to lysozyme. *Biochemistry* 10: 4492–4496, 1971.
 77. KAVANAUGH, J. S., P. H. ROGERS, D. A. CASE, AND A. ARNONE. High-resolution X-ray study of deoxyhemoglobin Rothschild 37 beta Trp-Arg: a mutation that creates an intersubunit chloride-binding site. *Biochemistry* 31: 4111–4121, 1992.
 78. KELLY, R. M., H. L. HUI, AND R. W. NOBLE. Chloride acts as a novel negative heterotropic effector of hemoglobin Rothschild (beta 37 Trp→Arg) in solution. *Biochemistry* 33: 4363–4367, 1994.
 79. KENYON, G. L., AND T. W. BRUCE. Novel sulfhydryl reagents. *Methods Enzymol.* 47: 407–430, 1977.
 80. KEREM, B., J. M. ROMMENS, J. A. BUCHANAN, D. MARKIEWICZ, T. K. COX, A. CHAKRAVARTI, M. BUCHWALD, AND L. C. TSUI. Identification of the cystic fibrosis gene: genetic analysis. *Science* 245: 1073–1080, 1989.
 81. KIRSCH, G. E., J. A. DREWE, M. TAGLIALATELA, R. H. JOHO, M. DEBIASI, H. A. HARTMAN, AND A. M. BROWN. A single nonpolar residue in the deep pore of related K⁺ channels acts as a K⁺:Rb⁺ conductance switch. *Biophys. J.* 62: 136–144, 1992.
 82. KROVETZ, H. S., H. M. A. VAN DONGEN, AND A. M. J. VAN DONGEN. Atomic distance estimates from disulfides and high-affinity metal-binding sites in a K⁺ channel pore. *Biophys. J.* 72: 117–126, 1997.
 83. LANGOSCH, D., K. HARTUNG, E. GRELL, E. BAMBERG, AND H. BETZ. Ion channel formation by synthetic transmembrane segments of the inhibitory glycine receptor—a model study. *Biochim. Biophys. Acta* 1063: 36–44, 1991.
 84. LANGOSCH, D., B. LAUBE, N. RUNDSTROM, V. SCHMIEDEN, J. BORMANN, AND H. BETZ. Decreased agonist affinity and chloride conductance of mutant glycine receptors associated with human hereditary hyperekplexia. *EMBO J.* 13: 4223–4228, 1994.
 85. LÄUGER, P. Ion transport through pores: a rate-theory analysis. *Biochim. Biophys. Acta* 311: 423–441, 1973.
 86. LEAR, J. D., Z. R. WASSERMAN, AND W. F. DEGRADO. Synthetic amphiphilic peptide models for protein ion channels. *Science* 240: 1177–1181, 1988.
 87. LENCER, W. I., G. CHEUNG, G. R. STROHMEIER, M. G. CURRIE, A. J. OUELLETTE, M. E. SELSTED, AND J. L. MADARA. Induction of epithelial chloride secretion by channel-forming cryptins 2 and 3. *Proc. Natl. Acad. Sci. USA* 94: 8585–8589, 1997.
 88. LEVITT, D. G. Comparison of Nernst-Planck and reaction-rate models for multiply occupied channels. *Biophys. J.* 37: 575–587, 1982.
 89. LEVITT, D. G. Interpretation of biological ion channel flux data: reaction-rate versus continuum theory. *Annu. Rev. Biophys. Chem.* 15: 29–57, 1986.
 90. LEVITT, D. G. Solvation effects on the transport of ions across cell membranes. In: *The Chemical Physics of Solvation. Part C: Solvation Phenomena in Specific Physical, Chemical, and Biological Systems*, edited by R. R. Dogonadze, E. Kálman, A. A. Kornyshev, and J. Ulstrup. New York: Elsevier, 1988, p. 741–750.
 91. LEWIS, C. A., AND C. F. STEVENS. Acetylcholine receptor channel ionic selectivity: ions experience an aqueous environment. *Proc. Natl. Acad. Sci. USA* 80: 6110–6113, 1983.
 92. LINSDELL, P., AND J. W. HANRAHAN. Flickery block of single CFTR chloride channels by intracellular anions and osmolytes. *Am. J. Physiol.* 271 (*Cell Physiol.* 40): C628–C634, 1996.
 93. LINSDELL, P., AND J. W. HANRAHAN. Disulphonic stilbene block of cystic fibrosis transmembrane conductance regulator Cl⁻ channels expressed in a mammalian cell line and its regulation by a critical pore residue. *J. Physiol. (Lond.)* 496: 687–693, 1996.
 94. LINSDELL, P., AND J. W. HANRAHAN. Adenosine triphosphate-dependent asymmetry of anion permeation in the cystic fibrosis transmembrane conductance regulator chloride channel. *J. Gen. Physiol.* 111: 1–14, 1998.
 95. LINSDELL, P., J. A. TABCHARANI, AND J. W. HANRAHAN. Multi-ion mechanism for ion permeation and block in the cystic fibrosis transmembrane conductance regulator chloride channel. *J. Gen. Physiol.* 110: 365–377, 1997.
 96. LINSDELL, P., J. A. TABCHARANI, J. M. ROMMENS, Y.-X. HOU, X.-B. CHANG, L.-C. TSUI, J. R. RIORDAN, AND J. W. HANRAHAN. Permeability of wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels to polyatomic anions. *J. Gen. Physiol.* 110: 355–364, 1997.
 97. LINSDELL, P., S.-X. ZHENG, AND J. W. HANRAHAN. Possible contribution of the peptide backbone to CFTR anion selectivity (Abstract). *Pediatr. Pulmonol. Suppl.* 14: 214, 1997.
 98. MACKINNON, R. Pore loops: an emerging theme in ion channel structure. *Neuron* 14: 889–892, 1995.
 99. MACKINNON, R., AND G. YELLEN. Mutations affecting TEA blockade and ion permeation in voltage-activated K⁺ channels. *Science* 250: 276–279, 1990.
 100. MAGNERA, T. F., G. CALDWELL, J. SUNNER, S. IKUTA, AND P. KEBARLE. Solvation of halide anions in dimethyl sulfoxide. Factors involved in enhanced reactivity of negative ions in dipolar aprotic solvents. *J. Am. Chem. Soc.* 106: 6140–6146, 1984.
 101. MANSOURA, M. K., S. S. SMITH, A. D. CHOI, N. W. RICHARDS, T. V. STRONG, M. L. DRUMM, F. S. COLLINS, AND D. C. DAWSON. CFTR: anion binding as a probe of the pore. *Biophys. J.* 74: 1320–1332, 1998.
 102. MARCUS, Y. A simple empirical model describing the thermodynamics of hydration of ions of widely varying charges, sizes, and shapes. *Biophys. Chem.* 51: 111–127, 1994.
 103. MARCUS, Y. *Ion Properties*. New York: Dekker, 1997.
 104. MCCARTY, N. A., S. McDONOUGH, B. N. COHEN, J. R. RIORDAN, N. DAVIDSON, AND H. A. LESTER. Voltage-dependent block of the cystic fibrosis transmembrane conductance regulator Cl⁻ channel by two closely related arylaminobenzoates. *J. Gen. Physiol.* 102: 1–23, 1993.
 105. McDONOUGH, S., N. DAVIDSON, H. A. LESTER, AND N. A. MCCARTY. Novel pore-lining residues in CFTR that govern permeation and open-channel block. *Neuron* 13: 623–634, 1994.

106. MODCZYDLOWSKI, E. Single-channel enzymology. In: *Ion Channel Reconstitution*, edited by C. Miller. New York: Plenum, 1986, p. 75–113.
107. NASR, S. Z., T. V. STRONG, M. K. MANSOURA, D. C. DAWSON, AND F. S. COLLINS. Novel missense mutation (G314R) in a cystic fibrosis patient with hepatic failure. *Hum. Mutat.* 7: 151–154, 1996.
108. NEYTON, J., AND C. MILLER. Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high-conductance Ca^{2+} -activated K^+ channel. *J. Gen. Physiol.* 92: 569–586, 1988.
109. NORNE, J. E., S. G. HJALMARSSON, B. LINDMAN, AND M. ZEPPEZAUER. Anion binding properties of human serum albumin from halide ion quadrupole relaxation. *Biochemistry* 14: 3401–3408, 1975.
110. NORNE, J. E., H. LILJA, B. LINDMAN, R. EINARSSON, AND M. ZEPPEZAUER. $Pt(CN)_2-4$ and $Au(CN)_2-$: potential general probes for anion-binding sites of proteins. ^{35}Cl and ^{81}Br nuclear-magnetic-resonance studies. *Eur. J. Biochem.* 59: 463–473, 1975.
111. OBLATT-MONTAL, M., G. L. REDDY, T. IWAMOTO, J. M. TOMICH, AND M. MONTAL. Identification of an ion channel-forming motif in the primary structure of CFTR, the cystic fibrosis chloride channel. *Proc. Natl. Acad. Sci. USA* 91: 1495–1499, 1994.
112. OVERHOLT, J. L., M. E. HOBERT, AND R. D. HARVEY. On the mechanism of rectification of the isoproterenol-activated chloride current in guinea-pig ventricular myocytes. *J. Gen. Physiol.* 102: 871–895, 1993.
113. OVERHOLT, J. L., A. SAULINO, M. L. DRUMM, AND R. D. HARVEY. Rectification of whole cell cystic fibrosis transmembrane conductance regulator chloride current. *Am. J. Physiol.* 268 (Cell Physiol. 37): C636–C646, 1995.
114. PANDE, C. S., AND R. H. MCMENAMY. Thiocyanate binding with modified bovine plasma albumins. *Arch. Biochem. Biophys.* 136: 260–267, 1970.
115. PARTENSKII, M. B., V. DORMAN, AND P. C. JORDAN. Influence of a channel-forming peptide on energy barriers to ion permeation, viewed from a continuum dielectric perspective. *Biophys. J.* 67: 1429–1438, 1994.
116. QUINTON, P. M. Chloride impermeability in cystic fibrosis. *Nature* 301: 421–422, 1983.
117. QUINTON, P. M. Missing Cl conductance in cystic fibrosis. *Am. J. Physiol.* 251 (Cell Physiol. 20): C649–C652, 1986.
118. QUINTON, P. M. Defective epithelial ion transport in cystic fibrosis. *Clin. Chem.* 35: 726–730, 1989.
119. QUINTON, P. M. Cystic fibrosis: a disease in electrolyte transport. *FASEB J.* 4: 2709–2717, 1990.
120. RADZICKA, A., AND R. WOLFENDEN. Comparing the polarities of the amino acids: side-chain distribution coefficients between the vapor phase, cyclohexane, 1-octanol, and neutral aqueous solution. *Biochemistry* 27: 1664–1670, 1988.
121. REDDY, G. L., T. IWAMOTO, J. M. TOMICH, AND M. MONTAL. Synthetic peptides and four-helix bundle proteins as model systems for the pore-forming structure of channel proteins. II. Transmembrane segment M2 of the brain glycine receptor is a plausible candidate for the pore-lining structure. *J. Biol. Chem.* 268: 14608–14615, 1993.
122. REDDY, M. M., P. M. QUINTON, C. HAWS, J. J. WINE, R. GRYGORCZYK, J. A. TABCHARANI, J. W. HANRAHAN, K. L. GUNDERSON, AND R. R. KOPITO. Failure of the cystic fibrosis transmembrane conductance regulator to conduct ATP. *Science* 271: 1876–1879, 1996.
123. REISIN, I. L., A. G. PRAT, E. H. ABRAHAM, J. F. AMARA, R. J. GREGORY, D. A. AUSIELLO, AND H. F. CANTIELLO. The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J. Biol. Chem.* 269: 20584–20591, 1994.
124. REUTER, H., AND C. F. STEVENS. Ion conductance and ion selectivity of potassium channels in snail neurones. *J. Membr. Biol.* 57: 103–118, 1980.
125. RICHARDSON, J. S., AND D. C. RICHARDSON. Principles and patterns of protein conformation. In: *Prediction of Protein Structure and the Principles of Protein Conformation*, edited by G. D. Fasman. New York: Plenum, 1989, p. 1–99.
126. RIORDAN, J. R., J. M. ROMMENS, B. KEREM, N. ALON, R. ROZMAHEL, Z. GRZELCZAK, J. ZIELENSKI, S. LOK, N. PLAVSIC, J. L. CHOU, M. L. DRUMM, M. C. IANNUZZI, F. C. COLLINS, AND L.-C. TSUI. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245: 1066–1073, 1989.
127. RIVETTI, C., A. MOZZARELLI, G. L. ROSSI, L. D. KWIATKOWSKI, A. M. WIERZBA, AND R. W. NOBLE. Effect of chloride on oxygen binding to crystals of hemoglobin Rothschild (beta 37 Trp→Arg) in the T quaternary structure. *Biochemistry* 32: 6411–6418, 1993.
128. ROMMENS, J. M., M. C. IANNUZZI, B. KEREM, M. L. DRUMM, G. MELMER, M. DEAN, R. ROZMAHEL, J. L. COLE, D. KENNEDY, N. HIDAKA, M. ZSIGA, M. BUCHWALD, J. R. RIORDAN, L.-C. TSUI, AND F. C. COLLINS. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 245: 1059–1065, 1989.
129. RUDIGER, M., U. HAUPTS, K. GERWERT, AND D. OESTERHELT. Chemical reconstitution of a chloride pump inactivated by a single point mutation. *EMBO J.* 14: 1599–1606, 1995.
130. SAMOILOV, O. Y. Residence times of ionic hydration. In: *Water and Aqueous Solutions: Structure, Thermodynamics, and Transport Processes*, edited by R. A. Horne. Burlington, MA: Wiley-Interscience, 1972, p. 597–612.
131. SANCHO, M., M. B. PARTENSKII, V. DORMAN, AND P. C. JORDAN. Extended dipolar chain model for ion channels: electrostriction effects and the translocational energy barrier. *Biophys. J.* 68: 427–433, 1995.
132. SCATCHARD, G., J. S. COLEMAN, AND A. L. SHEN. Physical chemistry of protein solutions. Part VII: the binding of some small anions to serum albumin. *J. Am. Chem. Soc.* 79: 12–20, 1956.
133. SCATCHARD, G., AND W. T. YAP. The physical chemistry of protein solutions. Part XII: the effects of temperature and hydroxide ion on the binding of small anions to human serum albumin. *J. Am. Chem. Soc.* 86: 3434–3438, 1964.
134. SCHULTZ, B. D., A. D. DEROOS, C. J. VENGLARIK, A. K. SINGH, R. A. FRIZZELL, AND R. J. BRIDGES. Glibenclamide blockade of CFTR chloride channels. *Am. J. Physiol.* 271 (Lung Cell. Mol. Physiol. 15): L192–L200, 1996.
135. SCHWIEBERT, E. M., M. E. EGAN, T. H. HWANG, S. B. FULMER, S. S. ALLEN, G. R. CUTTING, AND W. B. GUGGINO. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell* 81: 1063–1073, 1995.
136. SHEPPARD, D. N., L. S. OSTEDGAARD, D. P. RICH, AND M. J. WELSH. The amino-terminal portion of CFTR forms a regulated Cl^- channel. *Cell* 76: 1091–1098, 1994.
137. SHEPPARD, D. N., D. P. RICH, L. S. OSTEDGAARD, R. J. GREGORY, A. E. SMITH, AND M. J. WELSH. Mutations in CFTR associated with mild-disease-form Cl^- channels with altered pore properties. *Nature* 362: 160–164, 1993.
138. SHEPPARD, D. N., S. M. TRAVIS, H. ISHIHARA, AND M. J. WELSH. Contribution of proline residues in the membrane-spanning domains of cystic fibrosis transmembrane conductance regulator to chloride channel function. *J. Biol. Chem.* 271: 14995–15001, 1996.
139. SHEPPARD, D. N., AND M. J. WELSH. Effect of ATP-sensitive K^+ channel regulators on cystic fibrosis transmembrane conductance regulator chloride currents. *J. Gen. Physiol.* 100: 573–591, 1992.
140. SIEBERT, F. S., P. LINSDELL, T. W. LOO, J. W. HANRAHAN, D. M. CLARKE, AND J. S. RIORDAN. Disease-associated mutations in the fourth cytoplasmic loop of cystic fibrosis transmembrane conductance regulator compromise biosynthetic processing and chloride channel activity. *J. Biol. Chem.* 271: 15139–15145, 1996.
141. SMIT, L. S., D. J. WILKINSON, M. K. MANSOURA, F. S. COLLINS, AND D. C. DAWSON. Functional roles of the nucleotide-binding folds in the activation of the cystic fibrosis transmembrane conductance regulator. *Proc. Natl. Acad. Sci. USA* 90: 9963–9967, 1993.
142. SMITH, S. S., M. K. MANSOURA, J. A. SCHAEFER, C. R. COOKE, Z. SHARIAT-MADAR, F. SUN, AND D. C. DAWSON. The fifth putative transmembrane helix of CFTR contributes to the pore architecture (Abstract). *Biophys. J.* 72: A365, 1997.
143. SMITH, S. S., E. D. STEINLE, V. PECORARO, M. E. MEYERHOFF, AND D. C. DAWSON. CFTR: probing the pore with permeant ions. *Pediatr. Pulmon.* 1997.
144. STUTTS, M. J., C. M. CANESSA, J. C. OLSEN, M. HAMRICK, J. A. COHN, B. C. ROSSIER, AND R. C. BOUCHER. CFTR as a cAMP-dependent regulator of sodium channels. *Science* 269: 847–850, 1995.
145. TABCHARANI, J. A., X. B. CHANG, J. R. RIORDAN, AND J. W. HANRA-

- HAN. The cystic fibrosis transmembrane conductance regulator chloride channel. Iodide block and permeation. *Biophys. J.* 62: 1–4, 1992.
146. TABCHARANI, J. A., P. LINSDELL, AND J. W. HANRAHAN. Halide permeation in wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels. *J. Gen. Physiol.* 110: 341–354, 1997.
147. TABCHARANI, J. A., J. M. ROMMENS, Y. X. HOU, X. B. CHANG, L. C. TSUI, J. R. RIORDAN, AND J. W. HANRAHAN. Multi-ion pore behaviour in the CFTR chloride channel. *Nature* 366: 79–82, 1993.
148. TAKEUCHI, A., AND N. TAKEUCHI. Anion interaction at the inhibitory post-synaptic membrane of the crayfish neuromuscular junction. *J. Physiol. (Lond.)* 212: 337–351, 1971.
149. USSING, H. H. The distinction by means of tracers between active transport and diffusion. *Acta Physiol. Scand.* 19: 43–56, 1949.
150. USSING, H. H. Some aspects of the application of tracers in permeability studies. *Adv. Enzymol.* 13: 21–65, 1952.
151. USSING, H. H. Interpretation of tracer fluxes. In: *Membrane Transport in Biology*, edited by G. Giebisch, D. C. Testeson, and H. H. Ussing. Berlin: Springer-Verlag, 1978, p. 115–140.
152. VAUGHAN, P. C. Chloride-thiocyanate interactions in frog muscle anion-conducting channels at pH 5. *Pflügers Arch.* 410: 153–158, 1987.
153. VENGLARIK, C. J., B. D. SCHULTZ, A. D. DEROOS, A. K. SINGH, AND R. J. BRIDGES. Tolbutamide causes open channel blockade of cystic fibrosis transmembrane conductance regulator Cl⁻ channels. *Biophys. J.* 70: 2696–2703, 1996.
154. WALLACE, D. P., J. M. TOMICH, T. IWAMOTO, K. HENDERSON, J. J. GRANTHAM, AND L. P. SULLIVAN. A synthetic peptide derived from glycine-gated Cl⁻ channel induces transepithelial Cl⁻ and fluid secretion. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C1672–C1679, 1997.
155. WALTER, T. J., AND M. S. BRAIMAN. Anion-protein interactions during halorhodopsin pumping: halide binding at the protonated Schiff base. *Biochemistry* 33: 1724–1733, 1994.
156. WASHABAUGH, M. W., AND K. D. COLLINS. The systematic characterization by aqueous column chromatography of solutes which affect protein stability. *J. Biol. Chem.* 261: 12477–12485, 1986.
157. WHITE, M. M., AND C. MILLER. Probes of the conduction process of a voltage-gated Cl⁻ channel from *Torpedo electroplax*. *J. Gen. Physiol.* 78: 1–18, 1981.
158. WILKINSON, D. J., M. K. MANSOURA, P. Y. WATSON, L. S. SMIT, F. S. COLLINS, AND D. C. DAWSON. CFTR: the nucleotide binding folds regulate the accessibility and stability of the activated state. *J. Gen. Physiol.* 107: 103–119, 1996.
159. WOLFENDEN, R., L. ANDERSSON, P. M. CULLIS, AND C. C. SOUTHGATE. Affinities of amino acid side chains for solvent water. *Biochemistry* 20: 849–855, 1981.
160. WOODBURY, J. W. Eyring rate theory model of the current-voltage relationships of ion channels in excitable membranes. In: *Chemical Dynamics: Papers in Honor of Henry Eyring*, edited by J. Hirschfelder. New York: Wiley, 1971, p. 601–617.
161. WOODHULL, A. M. Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* 61: 687–708, 1973.
162. WRIGHT, E. M., AND J. M. DIAMOND. Anion selectivity in biological systems. *Physiol. Rev.* 57: 109–156, 1977.
163. YANG, N., A. L. GEORGE, JR., AND R. HORN. Molecular basis of charge movement in voltage-gated sodium channels. *Neuron* 16: 113–122, 1996.