Commentary

Cystic Fibrosis Transmembrane Conductance Regulator

Permeant Ions Find the Pore

David C. Dawson and Stephen S. Smith

From the Department of Physiology, University of Michigan Medical School, Ann Arbor, Michigan 48109

In the 8 yr since the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) was identified by means of positional cloning (Kerem et al., 1989; Riordan et al., 1989; Rommens et al., 1989), the Cl channel function of CFTR has been studied in a wide variety of expression systems. Macroscopic and single-channel currents have been measured and a large number of mutant constructs containing either single amino acid substitutions or major deletions have been examined. It is ironic, however, that despite this intense activity, there is little available in the published literature in the way of a systematic characterization of the conduction properties of this unique Cl channel. In this issue of The Journal of General Physiology, John Hanrahan and his colleagues make an important contribution to this question in the form of three papers in which they report experimental and modeling results pertaining to anion permeation. Although it seems unlikely that these papers will attract the same attention as the cloning of farm animals or new approaches to gene therapy, they contain information that is crucial to developing an understanding of the Cl permeation mechanism of CFTR; and the results extend the functional fingerprint of the channel in a way that will be useful to other investigators seeking to identify CFTR channels (e.g., in reconstituted systems).

The amino acid sequence of the CFTR protein and its predicted topology accurately foreshadowed the regulation of CFTR channel function by phosphorylation and ATP binding/hydrolysis (Kerem et al., 1989; Riordan et al., 1989; Rommens et al., 1989; Anderson and Welsh, 1992; Gadsby and Nairn, 1994), but the primary structure provided no hint as to the nature of the anion conduction path. There is no homology or internal symmetry to suggest how the 12 membrane-spanning segments might be arranged to form a pore, although the abundance of patient mutations provides clues as to specific residues that might be important for pore function (Anderson et al., 1991; Shreffler et al., 1993; Nasr et al., 1996). The difficulty in probing pore structure is compounded by the fact that CFTR, expressed predominantly in epithelial cells, does not appear to be a target for the sorts of highly specific toxins that have been so useful in deciphering the conduction properties of voltage-gated channels. Although several compounds have been advanced as blockers of the CFTR pore (Sheppard and Welsh, 1992; McCarty et al., 1993; Lindsell and Hanrahan, 1996), it nevertheless appears that the best probes of the pore are, in fact, permeant anions that must, by definition, enter, traverse, and interact with the conduction path. In this regard, the study of CFTR conduction is well endowed as this channel shares with other anion channels the property of permitting the permeation of a wide variety of inorganic and organic anions; e.g., Cl, Br, NO₃, I, SCN, formate, and acetate. It seems likely that important keys to understanding the conduction path are likely to emerge from studies like those presented in the three subject papers, in which behavior of permeating ions is used to draw inferences about the physical nature of the interaction of anions with the CFTR pore.

Previous studies of CFTR’s anion conduction properties suggested a pattern that is common to a number of anion channels. Relative halide permeabilities, determined from shifts in reversal potential, appear to be ordered more or less as would be predicted for a so-called “weak site” ion–channel interaction, so that permeability ratios are highly dependent on differences in anion–water interactions (Eisenman and Horn, 1983; Bormann et al., 1987; Anderson et al., 1991). Some ions, however, most notably SCN⁻, tend to stick tightly in anion channels such that ionic throughput is slowed and conductance is reduced. Substitution of external Cl⁻ by SCN⁻ tends to produce a result that seems at first to be anomalous. The reversal potential shifts to more negative values consistent with $P_{\text{SCN}}/P_{\text{Cl}}$ of between 3 and 4, but the conductance is reduced to ~15% of that seen with Cl⁻. This behavior is perfectly consistent, however, with that predicted for channels in which the conduction pathway contains binding sites with which anions associate transiently as they traverse the pore. It is useful (although not completely accurate) to think of permeability ratios determined from shifts in reversal potential as measuring how easy it is
for an ion to leave some or all of its waters of hydration and enter the pore. Conductance ratios, in contrast, measure relative rates of anion throughput. As a consequence, the latter are very sensitive to block by a sticky anion, whereas permeability ratios, derived from zero current measurements, are relatively insensitive to ion binding. In a previous report from the Hanrahan laboratory (Tabcharani et al., 1993), it was shown that SCN\textsuperscript{−} was apparently so sticky that at least two of these anions could bind in the channel simultaneously, giving rise to the so-called “anomalous mole fraction effect” in which interionic repulsion due to multiple anion occupancy increases the throughput of sticky ions.

In the first paper of this series, Tabcharani et al. (1997) examine the permeability ratios for Cl\textsuperscript{−}, Br\textsuperscript{−}, F\textsuperscript{−}, and I\textsuperscript{−}. The results are consistent with a “weak field strength” sequence indicative of a major role for anion-water interactions in determining how readily halides may enter the channel. This conclusion seems simple enough in itself, but reaching it required that the authors confront the rather puzzling behavior of I\textsuperscript{−}, which appears to be either more or less permeant than Cl\textsuperscript{−}, depending upon how the measurements are made. The authors conclude that it is possible to distinguish two states of the channel, one in which conduction is partially blocked by I\textsuperscript{−}, and another in which it is not. The conversion between these two apparent states exhibits an intriguing dependence on the polarity of the membrane potential and the flow of Cl\textsuperscript{−}. If I\textsuperscript{−} is present on one side of the membrane and Cl\textsuperscript{−} on the other, the rate of conversion is enhanced at potentials that drive Cl\textsuperscript{−} into and I\textsuperscript{−} out of the channel! Importantly, the conversion is associated with a dramatic change in \( P_I/P_C \) from 1.8 to <0.4, an observation that may be relevant to the range of values of \( P_I/P_C \) that have been reported for CFTR. Conversion was not seen at high ionic strength. Nor was it seen in R347D CFTR, a construct previously shown to lack the anomalous dependence of channel conductance on the mole fraction of SCN characteristic of wild-type CFTR (Tabcharani et al., 1993). Interestingly, however, the R347D construct was characterized by an intermediate value of \( P_I/P_C \) (~1.0) and exhibited a reduced conductance in the presence of I\textsuperscript{−} as expected if this ion binds more tightly than Cl\textsuperscript{−} in the pore of the mutant protein. The authors conclude that it may be possible for I\textsuperscript{−} to occupy the pore by binding to a site in such a way as to only partially obstruct Cl\textsuperscript{−} flow. Clearly, a mechanistic understanding of the behavior of I\textsuperscript{−} will be an important clue to pore structure.

In the second paper, Lindsell et al. (1997a) examine permeability ratios for a series of polyatomic anions in an attempt to estimate the size of the narrowest part of the pore. By comparing permeability ratios for nitrate, formate, pyruvate, propionate, gluconate, methane sulfonate, ethane sulfate, and acetate, they arrive at an estimated diameter of ~5.3 Å, larger than the unhydrated diameter of Cl\textsuperscript{−}, ~3.60 Å. The behavior of these ions is consistent with a Hofmeister series; i.e., one governed by hydration energy. Of particular interest in this paper is the behavior of a mutant CFTR, T338A, T339A, in which two threonines in TM6 were substituted with alanines. This construct exhibited markedly increased permeability ratios for iodide and nitrate and apparent permeation of propionate and pyruvate not seen in wild-type CFTR, leading the authors to speculate that these two residues may be near a “selectivity filter.” This result is of interest because, in another study in which 11 mutants were compared, permeability ratios were found to be relatively insensitive to point mutations in TM1, TM5, and TM6 (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, manuscript submitted for publication).

In the final paper, Lindsell et al. (1997b) present a simulation of some of the CFTR multi-ion behavior using a rate theory model. The model is derived primarily from experiments in which block of CFTR channels by gluconate applied to the cytoplasmic side was studied. A strong interaction between gluconate and the trans concentration of Cl\textsuperscript{−} is modeled by allowing the pore to be occupied simultaneously by gluconate and Cl\textsuperscript{−}. The IV curves can be described by models that allow for either two or three intrachannel, anion binding sites. The two-site model was also used to simulate the block of CFTR by SCN\textsuperscript{−}, but a three-site model was used to generate the anomalous mole fraction effect previously reported, as well as the properties of the mutant, R347D, in which the anomalous mole fraction effect is lost. It will be interesting to extend these first modeling efforts and determine the minimal requirements for single and multiple occupancy models that are necessary to simulate permeation properties. For example, can relief of gluconate block be simulated by a single-site model and will alternate placement of sites allow the two-site model to simulate anomalous mole fraction effects?

It is important to note the limitations of any modeling effort, and the authors are appropriately circumspect about their results. Rate theory models provide an approach to describing the process of ion conduction, which is, in a sense, removed from any direct consideration of structure. As such, it provides a very useful way of integrating an ever increasing database of conduction measurements, which will ultimately place some constraints on structure. It is interesting to note that in this case the authors, despite their conservative approach to interpretation, could not resist the temptation to assign the three binding sites in the model to three residues found in TM6, where mutations have been found to alter anion conduction.
The structural basis for anion conduction by CFTR remains largely a mystery, but research such as that presented here represents the sort of systematic approach to the biophysics of the permeation process that will be central to any understanding of the physical basis for anion entry and translocation. It will be of interest to compare conduction across anion channel types and separate out the general properties of an anion-selective pore from those that may be specific to an individual channel such as CFTR. A detailed analysis of the conduction mechanism will also establish an important standard that can be used to compare anion conduction by channels that are covalently modified (Akabas et al., 1994), that result from the expression of truncated CFTRs (Sheppard et al., 1994; Carroll et al., 1995), or that are observed when the channel, or parts of it, are reconstituted in planar bilayers (Oblatt-Montal et al., 1994; Ma et al., 1996). Where there is a pore, permeant ions will find it and their behavior will constitute its fingerprint.

REFERENCES

Akabas, M.H., C. Kaufmann, T.A. Cook, and P. Archdeacon. 1994. Amino acid residues lining the chloride channel of the cystic fibrosis transmembrane conductance regulator. J. Biol. Chem. 269: 14865–14868.

Anderson, M.P., R.J. Gregory, S. Thompson, D.W. Souza, S. Paul, R.C. Mulligan, A.E. Smith, and M.J. Welsh. 1991. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. Science (Wash. DC). 253:202–205.

Anderson, M.P., and M.J. Welsh. 1992. Regulation by ATP and ADP of CFTR chloride channels that contain mutant nucleotide-binding domains [published erratum appears in Science (Wash. DC). 1992. 258:1719]. Science (Wash. DC). 257:1701–1704.

Bormann, J., O.P. Hamill, and B. Sakmann. 1987. Mechanism of anion permeation through channels gated by glycine and gamma-aminobutyric acid in mouse cultured spinal neurones. J. Physiol. (Camb.). 385:243–286.

Carroll, T.P., M.M. Morales, S.B. Fulmer, S.S. Allen, T.R. Flotte, G.R. Cutting, and W.B. Guggino. 1995. Alternate translation initiation codons can create functional forms of cystic fibrosis transmembrane conductance regulator. J. Biol. Chem. 270:11941–11946.

Eisenman, G., and R. Horn. 1983. Ionic selectivity revisited: the role of kinetic and equilibrium processes in ion permeation through channels. J. Membr. Biol. 76:197–225.

Gadsby, D.C., and A.C. Nairn. 1994. Regulation of CFTR channel gating. Trends Biochem. Sci. 19:513–518.

Kerem, B., J.M. Rommens, J.A. Buchanan, D. Markiewicz, T.K. Cox, A. Chakravarti, M. Buchwald, and L.C. Tsui. 1989. Identification of the cystic fibrosis gene: genetic analysis. Science (Wash. DC). 245:1073–1080.

Lindsell, P., J.A. Tabcharani, J.M. Rommens, Y-X. Hou, X-B. Chang, L-C. Tsui, J.R. Riordan, and J.W. Hanrahan. 1997a. Permeability of wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels to polyatomic anions. J. Gen. Physiol. 110:355–364.

Lindsell, P., J.A. Tabcharani, and J.W. Hanrahan. 1997b. Multi-ion mechanism for ion permeation and block in the cystic fibrosis transmembrane conductance regulator chloride channel. J. Gen. Physiol. 110:365–377.

Lindsell, P., and J.W. Hanrahan. 1996. 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. (Camb.). 496:687–693.

Ma, J., J.E. Tasch, T. Tao, J. Zhao, J. Xie, M.L. Drumm, and P.B. Davis. 1996. Phosphorylation-dependent block of cystic fibrosis transmembrane conductance regulator chloride channel by exogenous R domain protein. J. Biol. Chem. 271:7351–7356.

McGarty, N.A., S. McDonough, B.N. Cohen, J.R. Riordan, N. Davidson, and H.A. Lester. 1993. Voltage-dependent block of the cystic fibrosis transmembrane conductance regulator Cl– channel by two closely related arylaminobenzoates. J. Gen. Physiol. 102:1–23.

Nasr, S.Z., T.V. Strong, M.K. Mansoura, D.C. Dawson, and F.S. Collins. 1996. A novel missense mutation (G314R) in a cystic fibrosis patient with hepatic failure. Hum. Mutat. 7:151–154.

Oblatt-Montal, M., G.L. Reddy, T. Iwamoto, J.M. Tomich, and M. Montal. 1994. 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.

Riordan, J.R., J.M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenksi, S. Lok, N. Plavsic, J.L. Chou, et al. 1989. Identification of the cystic fibrosis gene: cloning and characterisation of complementary DNA [published erratum appears in Science (Wash. DC). 1989. 245:1437]. Science (Wash. DC). 245: 1066–1073.

Rommens, J.M., M.C. Iannuzzi, B. Kerem, M.L. Drumm, G. Melner, M. Dean, R. Rozmahel, J.L. Cole, D. Kennedy, N. Hidaka et al. 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science (Wash. DC). 245:1059–1065.

Sheppard, D.N., L.S. Ostergaard, D.P. Rich, and M.J. Welsh. 1994. The amino-terminal portion of CFTR forms a regulated Cl– channel. Cell. 76:1091–1098.

Sheppard, D.N., D.P. Rich, L.S. Ostergaard, R.J. Gregory, A.E. Smith, and M.J. Welsh. 1993. Mutations in CFTR associated with mild-disease-form Cl– channels with altered pore properties [see comments]. Nature (Lond.). 362:160–164.

Sheppard, D.N., and M.J. Welsh. 1992. Effect of ATP-sensitive K+ channel regulators on cystic fibrosis transmembrane conductance regulator chloride currents. J. Gen. Physiol. 100:573–591.

Tabcharani, J.A., J.M. Rommens, Y-X. Hou, X-B. Chang, L-C. Tsui, J.R. Riordan, and J.W. Hanrahan. 1993. Multi-ion pore behaviour in the CFTR chloride channel. Nature (Lond.). 366:79–82.

Tabcharani, J.A., P. Lindsell, and J.W. Hanrahan. 1997. Halide permeation in wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels. J. Gen. Physiol. 110: 341–354.