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Electrodiffusion in Ionic Channels of Biological Membranes

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Abstract

An important class of biological molecules - proteins called ionic channels conduct ions (like Na^+, K^+, Ca^{++} and Cl^-) through a narrow tunnel of fixed charge. Ionic channels are the main pathway by which substances move into cells and son are of great biological and medical importance: asubstantial fraction of all drugs used by physicians act on channels. Ionic channels can be modified by the powerful techniques of of molecular biology. Charged groups can be engineered (i.e. replaced one at a time) and the location of every atom can be determined. channels can be studied in the tradition of electrochemistry. If coupled to the Poisson equation, the drift diffusion equations (i.e. Nernst-Planck equations) form an adequate model of the current through 6 different channel proteins with quite different characteristics in 10 solutions over $\pm 150\, mV$. In this theory the channel is represented as a distribution of fixed charge, and the ion as a mobile charge with a diffusion coefficient. The theory predicts the electric field (i.e. potential profile) and resulting current produced by the fixed charge and other charges in the system. In this theory, the shape of the electric field is found to be a sensitive function of ionic conditions and the potential difference across the channel, in contrast to traditional theories that assume potential profiles (or rate constants) independent of experimental conditions. Traditional theories fail to fit data, probably for because they assume the shape of the electric field. The Poisson-Nernst-Planck (PNP) theory is nearly idemntical to the drift diffusion equations used to analyze the flow of quasi-particles in semiconductors, implying that - given appropriate geometry and profiles of fixed charge - ionic channels can perform many of the useful functions of transistors, acting as resistors, voltage amplifiers, current amplifiers, or logic elements. Channels form a useful system for electrochemistry since they are biologically and clinically important, they follow the simple rules of electrodiffusion, and they promise to be of considerable use in technology.

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An important class of biological molecules—proteins called ionic channels—conduct ions (like Na^+, K^+, Ca^{++}, and Cl^-), and thus current, through a narrow tunnel of fixed charge formed by the polar residues of the protein[54]. These proteins can be studied with the full power of molecular biology[1]; for example, they can be modified one atom at a time with the techniques of molecular genetics. Thus, these natural nanotubes are a natural 'hole in the wall' that can be controlled more precisely and easily than many physical nanostructures.

Ionic channels open and close ('gate') to give currents that are a random telegraph signal[51]. The properties of gating are complex and the structure(s) and mechanism(s) that produce gating are not known (see p. 479 of Hille[33]), but the flow of ions through open channels is much simpler, and obeys a combination of the Poisson and Nernst-Planck (*PNP*) equations as we shall see.

Channels are the main pathway by which substances move in and out of cells and so are of great biological and medical importance: they are responsible for signaling in the nervous system; for coordination of muscle contraction—including the coordination of cardiac muscle that allows the heart to function as a pump—and they are involved in transport in every cell and organ, for example, in the kidney, intestine and endocrine glands[54]. A substantial fraction of all drugs used by physicians act directly or indirectly on channels[52].

Channels are studied one molecule at a time in hundreds, if not thousands of laboratories every day[47,19-21], using Neher & Sakmann's patch clamp method[51,49,8] (for which they received the Nobel Prize). The concentrations of ions outside channels (that carry current through the channel) can be directly controlled[56] and the shape of current voltage (IV) relations can be manipulated. In this way, a wide range of IV behavior can be measured from the single profile of fixed charge of one type of channel

and so much can be inferred about that profile (if IV measurements are taken in many different (pairs of) concentrations of current carriers). For all these reasons, channels are a popular object for experimentation: thousands(!) of abstracts describing their properties are presented each year at the annual meeting of the Biophysical Society and hundreds of papers are published about them each year, chiefly in the Biophysical Journal and the Journal of Physiology (London).

Channels are also an appealing and important object for theoretical analysis and simulation. Open channels are probably the simplest protein structures of general biological importance. Unlike many other subjects of biophysical investigation, ionic channels are a general biological system with importance for every organ, tissue, and cell in an animal and plant. Indeed, they are probably just as important for subcellular organelles (e.g., mitochondria or the sarcoplasmic reticulum) as for cells. Ionic channels are well defined biophysical systems that can be investigated both with the techniques of molecular biology and of electrochemistry.

Ions play an important role in the function of all proteins—e.g., enzymes—and so a model that describes ionic movement in channels is likely to give important insight into protein function in general. Indeed, the closely related Poisson-Boltzmann theory [24,38] has been of considerable help already, even though it is a strictly equilibrium theory that does not permit flux at any time or location.

Validity of PNP. Five laboratories have shown that the *PNP* equations form an adequate model of IV relations of 6 different channel proteins in ~10 pairs of solutions (containing different concentrations of the charge carriers Na^+, K^+, Cl^- from some 30 mM to 3 M) in the range ±150 mV [27,55,17,14-16,46,45]. The IV relations are qualitatively different in different types of channels—some are linear, some sublinear and some superlinear—because different channel proteins have qualitatively different profiles of fixed charge arising from the different sequences of amino acids that form the lining of their pore.

We have studied the properties of two of these proteins (the bacterial channels porin and its mutant G–119D) whose structures are known: the location of every atom has been determined by x-ray diffraction[59,40,42,50,22] with an accuracy of ~0.1Å. The mutant has one extra negative charge. Measurements of *IV* relations from a single molecule of porin[55] allow the *PNP* model to estimate the additional charge as –0.97e, although this estimate will undoubtedly change as more work is done. (We hasten to add that no information about the proteins is used in the analysis except the length and diameter of the channel; parameters were not adjusted in any way.)

McK channel structure. Recently, Doyle, et al,[25] have reported the structure of a bacterial K^+ channel. We hope others will join us in calling this the McK channel in recognition of the scientific significance of the MacKinnon contribution, and the general name recognition of the Mac and Mc prefix.

The structure of the McK channel is striking because it contains three elements, which seem likely to produce three of the more complex permeation properties of channels, not found so clearly in the bacterial porin channels.

The narrow pore formed by polar amino acids of the McK protein seems ideally suited to provide selectivity between ions, and our preliminary analysis (in collaboration with Lesser Blum and Luigi Catacuzenno) suggests that the *MSA* can account for the selectivity observed (see p. 1301 of Nonner and Eisenberg[46]). The *MSA* describes (among other things) the effect of the finite volume of ions on the free energy of ions in bulk solution; it can also be used to calculate the mobility (i.e., conductance) of ions in the baths. It is obvious that this treatment of 'selectivity' in free solution should be extended to the channel's pore. The difficulty is that the parameters of the *MSA* appropriate for the channel environment are not known; nor is it clear that the relative size of different terms in the formulas of *MSA* is the same in the open space of bulk solution and the confined volume of pores. Nonetheless, it seems clear that a treatment based on the *MSA*, which is

the most successful theory of selectivity in bulk solution, should be tried. It is certainly preferable to the alternative of biologists trying to create their own theory of selectivity independent of the work of physical chemists in bulk solution.

The narrow pore of the McK channel empties into a roughly spherical central cavity which then joins another pore, this formed by ***nonpolar*** amino acids. The nonpolar pore was not expected: most workers have thought all pores would be lined with hydrophilic amino acids.

We suggest that a nonpolar pore is an ideal structure to allow modulation of channel function (e.g., open channel current). Nearby charges, whether in the bath or in neighboring proteins—or even in specialized receptor structures of the McK channel itself—would have large effects on the potential profile within the nonpolar pore. The nonpolar lining would have low fixed charge, and that fixed charge would not move much in an electric field, i.e., it would have a low dielectric constant. The lining of the nonpolar pore would provide little dielectric shielding and little permanent charge to swamp the effect of charged structures outside the pore. (This is in marked contrast to the situation in a polar lined pore. There, the fixed charge is large—as is the dielectric constant—and so charges outside the pore would be both shielded and swamped, thus having little effect on the potential or permeation in the pore lumen.)

For these reasons, we propose that the ***nonpolar pore is the main site of channel modulation.***

α-helices as gating particles. The spherical cavity of the McK channel is formed (in large measure) by the ends of four α helices, which link the central cavity to the external solution. These α helices seem ideally placed to be push rods, that move slightly in response to the electric potential difference between the two ends of the channel (i.e., the *trans*membrane potential) while being reasonably independent of the local electrical potential inside the cavity or pore itself. Thus, these α helices seem to have the properties

expected of the 'delayed rectifier', the voltage dependent system that open and control channels. Cole[35,18] and then Hodgkin, Huxley, and Katz[34] recognized that gating in voltage sensitive channels was surprisingly independent of current flow through the channel, and that is only possible if gating is reasonably independent of the potential profile in the channel's pore (because current flow through the channel is strongly influenced by the potential profile in the pore). Hodgkin and Huxley[36] later showed that gating of K^+ channels could be described by the conjoint effect of four more or less independent and identical gating particles each with substantial effective charge. It has always been unclear how gating particles could manage to avoid strong interactions if they had a large formal charge. But the structure of the McK channel shows how these interactions could be minimized, if not avoided.

If each α helix of the McK channel were a gating particle, carrying (more or less) the charge of a α helical dipole[58,2,37,53], or carrying mostly induced charge, they would behave more or less independently because of the special structure of this channel. The helices in the McK channel are separated and shielded from one another by the selectivity filter with its highly polar wall, and by the ion filled pore of the selectivity filter.

All this is well and good, but we recognize that the McK channel, unlike the more widely studied eurkaryotic K^+ channels, is not a typical voltage gated delayed rectified and so our speculations are premature, at least until the structure of voltage gated K^+ channels are reported; indeed, the speculations may then prove to be incorrect. Nonetheless, we suggest that ***the α-helices will prove to be the gating particles of the delayed rectifier:*** vestigial structures abound in biology and the α-helices of the McK channel may prove to be vestigial remnants of the gating particles of more traditional delayed rectifiers. The α-helical gates in the McK channel may be unable to respond typically to membrane potential because they are pinned down by regressive mutations that produce excessive friction or electrostatic charge.

where the resolution is thought to be important. The potential profile from *PNP* provides an automatic self-consistent link between the potential profile in the highly resolved region and the charge distribution and boundary conditions in the macroscopic domain. These methods are described in some detail in references[32,41,43,57,48] and on the web site[23].

Of course, ionic solutions and proteins are not semiconductors, but the mathematical methods used to solve charge transport problems in semiconductors should be tried in ionic solutions and proteins, at least in my opinion, particularly in view of the tremendous success semiconductor workers have had in predicting macroscopic nonequilibrium properties using simulations in atomic detail, computed on the femtosecond time scale. Such properties cannot be predicted in ionic solutions or proteins with standard methods.

Conclusion. Ionic channels are so important biologically, but so well defined physically, that they are an ideal object of biophysical investigation. Nothing is likely to be simpler physically than a hole in the wall. Few systems are more important biologically than ionic channels. Few are a more promising object of electrochemical investigation, at least in my view.

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