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Proteins, channels and crowded ions

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Abstract

Ion channels are proteins with a hole down their middle that control a vast range of biological function in health and disease. Selectivity is an important biological function determined by the open channel, which does not change conformation on the biological time scale. The challenge is to predict the function—the current of ions of different types and concentrations through a variety of channels—from structure, given fundamental physical laws. Walls of ion channels, like active sites of enzymes, often contain several fixed charges. Those fixed charges demand counter ions nearby, and the density of those counter ions is very high, greater than 5 molar, because of the tiny volumes of the channel's pore. Physical chemists can now calculate the free energy per mole of salt solutions (e.g. the activity coefficient) from infinite dilution to saturation, even in ionic melts. Such calculations of a model of the L-type calcium channel show that the large energies needed to crowd charges into the channel can account for the substantial selectivity and complex properties found experimentally. The properties of such crowded charge are likely to be an important determinant of the properties of proteins in general because channels are nearly enzymes.

Keywords: Physical; Selectivity; Saturation; Density

1. Introduction

Ion channels are proteins with holes down their middle of enormous biological importance [1–3] that perform many of their biological functions without changing structure. The physics of ion movement through channels, once they are open, is simple: only electrodiffusion, convection and heat flow occur in condensed phases under biological conditions, and electrodiffusion clearly dominates the properties of permeation through open channels in many cases. The challenge is to predict the current of ions of different types and concentrations through the many different types of channels using only the language of physics of condensed phases. The opportunity is the substantial experimental knowledge of channels: thousands of scientists (literally) measure the ions going through channels, one molecule at a time, every day.

Ions and channels are inseparable. Without ions, channels do not function. The fixed charge of the channel protein demands counter ions in a nearby ionic atmosphere: otherwise, impossible electrical forces are created, as shown in the first paragraphs of Feynman's text [4]. Cohn and Edsall recognized long ago that proteins bristle with charge (cf. [5]

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as cited on p. 73 of [6]) and thus with counter ions.

We argue that many of the properties of channels are produced by the crowding of counter ions near active sites, allowing their different sizes and charges to determine and modulate the free energy available for ion permeation. Similar energetics are likely to be important in protein function in general. After all, the phrase 'channels are enzymes' is a tautology as well as oxymoron [7]. Of course, some proteins (particularly enzymes) use additional sources of energy beyond those in the simplest physical models of ion channel selectivity.

The fixed charge that crowds ions is permanent in the sense that it arises from the nature of the chemical bonds of the protein, i.e. from the solution of Schrödinger's equation. Those bonds are built into proteins following the blueprint of their DNA. The permanent charge of proteins and the surrounding ionic atmosphere is under genetic, and thus evolutionary control. The modulation of the energy of crowded charge is a mechanism by which the genome and protein might control biological function.

The ionic atmosphere surrounding an active site can be viewed as a compressible plasma. The pun on 'plasma' is precise. The ions of the ionic atmosphere are non-cellular components of a biological fluid. The ionic atmosphere contains ions moving in a dielectric background quite like a dense gaseous plasma, whether the plasma is that of a real gas [8–10] or that of a gas of quasi-particles, like the holes and 'electrons' of a semiconductor [11–18].

The ionic atmosphere is compressible because its number and charge density is variable, although its conventional (gravitational) density is not. Large amounts of energy are involved in changing the number and charge density of the ionic atmosphere near active sites, even if the water and solution itself are (nearly) incompressible. A major result of modern physical chemistry is the fact that many properties of salt solutions arise from the variation in volume and charge density of solutes [19–36].

2. Physical chemistry of concentrated solutions

Many specific chemical properties of concentrated salt solutions (having nothing to do with proteins) are known now to arise from changes in the number density, and thus density of charge and excluded volume. According to modern physical chemistry [37–45], the diameter and charge of ions alone is enough to explain many complex properties of concentrated salt solutions without considering other specific chemical properties of the ionic species, e.g. its orbital structure. Specific chemical interactions with the solvent (produced by electron delocalization, for example) are not included in these modern successful treatments of concentrated salt solutions. Models that analyze the free energy of crowded spheres, treating the water as a uniform dielectric, with dielectric constant of the ionic solution (not of pure water), do remarkably well because the dominant effect in concentrated salt solutions comes from the electrostatic energy and entropy of crowded spheres. The centers of crowded spheres can only approach within 1 diameter of each other (more precisely, the sum of the radii of two perhaps unequal spheres) [46,47]. Debye–Hückel theory treats its central ion as a sphere but it treats other ions as points—even though they are identical to the central ion—so their centers of charge can approach within a radius, not diameter of each other. The electric field is strongest close to an ion so the errors in Debye–Hückel theory are large, particularly at high concentrations, where it is now customarily replaced by the Mean Spherical Approximation MSA [37–43,46–54].

The MSA (or its analogs) represents solvation energies only in a primitive way as the interaction of finite charged spheres (ions) with each other and with a uniform dielectric (water). Nonetheless, the theories quite successfully predict the activity coefficients of ions over a range of concentrations. [37–42] The finite (i.e. excluded) volume of the ions distorts the electric field (compared to that of point particles) and accounts for much of the excess free energy; the finite volume of uncharged spheres accounts for much of the entropic component of the excess free energy of the solution. Specific quantum chemical effects exist, but are

small in comparison, fundamentally because changes in density of the excluded volume and disturbances in the electric field swamp the energetic effects of orbital delocalization. It seems likely then that crowding effects might be important in the concentrated ionic atmosphere near active sites of proteins, particularly because the concentration of charge density there is so large, accounting for a substantial fraction of all the atoms, and that charge density is variable.

3. Selectivity in channels

Selectivity can be explained by a simple model of the crowded charge in and near their selectivity filter, using the same principles that are used to understand concentrated salt solutions without proteins [55–58].

In this treatment, selectivity arises from the properties of the concentrated ion plasma near the active site, more than anything else. The complex properties of selectivity arise from changes in the density of the concentrated ion plasma near the active site. Changes in the density of permanent charge at the active site change the density of counter ions, whether the active site changes in volume, conformation, or a number of fixed charges. That change in density has large effects because the energy stored in the ionic atmosphere is so large. The permanent charge of the active site and strength of the electric field enforce severe crowding of mobile ions, allowing their specific chemical properties (that arise chiefly from their excluded volume and charge) to become important determinants of protein function. In this view, selectivity and other properties of proteins arise from the balance of electrical and excluded volume in places crowded with charge, e.g. the selectivity filter of channels and active sites of proteins. In this view, the protein modulates and uses the energies of ions in the dense plasma near the active site much as an automobile engine modulates and uses gasoline and air.

Indeed, the energetics of a compressible plasma is enough to explain rather complex chemical and biological properties of the channel without invoking specific chemical properties or a specific geometrical arrangement of atoms of the channel protein. In the MSA version of this idea, the energy of the protein is (nearly) ignored; the MSA model leaves out both specific chemical bond energies and energy stored in the protein away from the active site. The theory, nonetheless, is quite successful in dealing quantitatively with the phenomena of selectivity, using only the known properties of concentrated salt solutions and two physical parameters to describe the role of the protein, although these models will undoubtedly need successive extension.

This physical explanation of selectivity is quantitative and physically specific in contrast to verbal descriptions of selectivity. Physical models are specific and testable and thus can be systematically improved by the usual scientific process.

I think utilitarian engineering analysis is likely to be more productive than narratives of trajectories or traditional literary discussions of binding sites. What is needed in my view is engineering as usual, but now engineering on the (sub) nanoscale of proteins, relying mostly on the techniques of ‘reverse engineering’ (i.e. the solution of inverse problems [59]), trying to design systems rather than just describe them.

4. Calcium channels

We illustrate these general ideas by considering the selectivity of calcium channels [55–58,60].

The L-type calcium channel is one of the most selective channels known and has complex properties [61–63]. Nearly all the current through the channel is carried by Ca^{2+} ions under normal conditions, when Ca^{2+} is approximately 1% of the external cations and 0.001% of the internal cations. The functional group of the L-type calcium channel is formed (mostly) from four glutamates, made from 8 half charged carboxylate ions, the ‘selectivity oxygens’, to emphasize their function.

Here, we consider a model in which the selectivity of the channel arises from physical effects, involving no quantum mechanical delocalization [64–66]. This model does not depend on the precise location of individual atoms of the protein [67–72] but it does depend on some properties of the channel protein in an important way: the volume of the channel’s pore determines selectiv-

ity, as does the 'concentration' (i.e. number density and excluded volume) of its glutamates and (to a lesser extent) the mechanical properties of the protein, and its effective dielectric 'constant'.

Indeed, physical models are specific and falsifiable precisely because they depend on only a small number of measures of specific properties of the protein. The remarkable fact is that these few measures are all that are needed to predict the selectivity of the L-type calcium channel under a wide range of conditions. These measures are the only traces of the channel structure found in this model; yet the model is quantitatively successful in dealing with many of the properties of selectivity [55–58].

In the physical approach, the crystallographic structure of the active site is unimportant, except insofar as it determines these specific measures, i.e. properties of the protein, e.g. volume, charge density, excluded volume, effective dielectric constant, and elasticity. These properties are very important. Other properties of the protein are unimportant in the model and perhaps in reality. Of course, the selectivity of some types of channels for some ions undoubtedly will depend on orbital interactions and the specific location of ions and peptides.

A physical model of this sort can be written at many levels of resolution. Here, I consider only mean field models because of limitations of space, although physical models of ion channels at higher resolution are of considerable importance [73–76] and I spend a good fraction of my time trying to develop them and learning to average their trajectories in a mathematically defined way [77].

Molecular dynamics models are not considered here because regrettably they cannot yet estimate the macroscopic quantities and behaviors known to be important in channel function, e.g. concentration, electrical potential, ionic current, Ohm's law, and Fick's law. We adopt an engineering approach developed and used in computational electronics [12,14,18,78,79]. This engineering analysis of proteins uses only as much detail as needed to explain biological phenomena. The goal is to use as little atomic detail as possible, although of course some atomic detail is essential.

5. L-type Ca channel

In our treatment of the L-type calcium channel the protein is treated as 8 half charged oxygens in a pore of (to be determined) volume and effective dielectric constant. The ions and oxygens are described by the MSA using crystal radii (in the simplest version [56]) or radii that change in simple ways (in more complex versions).

The calculation of the properties of the L-type calcium channel can be done in different ways, even if one is concerned only with mean field theories. Nonner et al. [56] show that the main properties of the Z-type calcium channel—its binding and anomalous mole fraction effect—can be reproduced by MSA using only two adjustable parameters, the dielectric coefficient and volume of the selectivity filter, which are set to reasonable values (dielectric coefficient 63.5 and volume 0.375 nm^3) to fit one data point, and then not changed. Patterns of selectivity closely resemble those reported experimentally, although the affinity of Mg^{2+} is incorrectly predicted (by a factor of $10\times$ or so).

The physical origin of selectivity between two ions is predicted without ambiguity in the model, as well as the dependence of that selectivity on the properties of the channel protein, namely its charge, size of its charges, volume of its selectivity filter, and dielectric coefficient. But the physical basis involves a number of terms, most of which depend on the concentration of all other ions. Thus, the selectivity depends on the (1) electrical potential; (2) ideal chemical potential (i.e. concentration); (3) entropy of charged and (4) uncharged hard spheres; (5) energy of charged hard spheres. Each of these terms is different for each type of ion and most depend on concentration. Thus, to understand the selectivity between two particular situations of biological interest one must compare all the terms. This is not hard to do, since the equations are algebraic and explicit, and all terms are known without possibility of adjustment. The terms are of nearly the same size, however, and so the results must be computed. Their relative size cannot be evaluated by verbal discussion.

Qualitative analysis is possible, on the other hand, if a few terms dominate. Interestingly, the

most important biological property of L-type calcium channels—their selectivity for Ca^{2+} over Na^{+}—is dominated by two terms. This is not the only case in which evolution seems to have used a particular subset of all physical possibilities: see any text of physiology or biophysics. It seems that evolution often chooses the strategy of a sensible engineer, KISS—Keep it Simple Stupid—when it chooses the physics to create a biological function. Complexity often arises in the structure and diversity, not the physics of biological systems. One imagines that when a few terms dominate the physics of a system, genetic control is easier, and the resulting phenotype is more likely to follow simple reproducible rules that are robust and survive unforeseen changes in environment. Here the selectivity of the L-type calcium channel for Ca^{2+} vs. Na^{+} is dominated by only a few terms and so can be understood qualitatively.

6. Selectivity of the L-type Ca channel

The selectivity between Ca^{2+} and Na^{+} arises in a simple way. The four glutamates of the channel demand the presence of four mobile positive charges nearby. If only Ca^{2+} is present, two Ca^{2+} provide the four charges. If only Na^{+} is present, four Na^{+} provide the four charges, but the four Na^{+} are twice as crowded as two Ca^{2+}. The four Na^{+} occupy twice the space of the two Ca^{2+} (because Ca^{2+} has the same diameter as Na^{+}). The free energy necessary to pack the extra two charged spheres into the channel accounts for $\sim$60% of selectivity. Approximately 35% of the selectivity comes from the different electrical potential found in the channel when Ca^{2+} is present. The electrical potential is different because Ca^{2+} provides better screening (of the negative charge of the glutamates). The double valence of Ca^{2+} allows two charges to approach within one ion diameter of the glutamate oxygen where Na^{+} allows only one. Five percent of the selectivity comes from other effects, e.g. entropy resulting from specific arrangements of the ions and glutamates.

It is important to emphasize how different this view of selectivity is from the traditional view. Traditional models of binding more or less ignore the electrical term altogether, as can be easily verified by noting the absence of Coulomb's law in their derivation or the absence of a dielectric constant (or permittivity parameter) in their output equations. Physical models predict electrical potentials of hundreds of millivolts—i.e. of the order of $4\ k_BT/e$—and those potentials vary more or less linearly with the logarithm of Ca^{2+} concentration under standard conditions. Thus, a substantial fraction of selectivity in a physical model comes from an effect ignored in most traditional treatments of binding and selectivity, the electrical energy needed to bind an ion to a charged site [80].

Traditional models of binding and selectivity (and of enzyme kinetics, for that matter [81,82]) also assume rate constants and binding constants independent of concentration of the binding species. This seems unlikely on physical grounds, because the binding of a charged group to a charged site will inevitably change the electrical potential, according to Gauss' law of electrostatics (i.e. Maxwell's equations) [80]. The change in potential will be large because the binding sites are so small and the 'capacitance to infinity' is so small (i.e. the self-energy is so large), as was pointed out sometime ago [80,83]. The existence of such effects is the basis of single charge devices [84,85] studied experimentally in hundreds of laboratories every day, and the effects are likely to be larger in proteins than in single charge devices because the binding sites are smaller. One can use other language, to describe the same physics. The change in potential with concentration is an example of shielding. Shielding determines many properties of channels [60,77,80,86–104] as it does many properties of ionic solutions [19,105–107], plasmas of a real gas [8–10], or plasmas of quasiparticles—the holes and 'electrons' in a semiconductor [11–18].

When shielding is important, the binding constant changes many fold as conditions vary. Thus, many of the qualitative features of binding are produced by a variation of binding constant not permitted in traditional theories of chemical kinetics.

Of course, the MSA treatment of binding has significant limitations. Perhaps most importantly, it is a theory of a spatially homogeneous solution

but has been applied to a strikingly inhomogeneous system. Henderson and colleagues [25,57,58] have addressed some of these issues, as has Hansen in a different context [32,33,108] and all agree that more precise simulations and theories confirm the effects found in the MSA model.

Of course, there are other limitations in the MSA theory. (1) The theory needs to be extended to non-equilibrium by embedding in PNP or something similar. (2) The theory needs to be extended to a range of pH. The original theory was computed at basic pH because the physical parameters of un-ionized species are not available in the physical chemistry literature. (When available, the variable ionization can be easily incorporated into the numerical scheme [60]). (3) The treatment of water should be more realistic. (4) The treatment of the protein should have higher resolution. (5) The macroscopic parameters, crucial to selectivity—volume of the pore, diffusion coefficient, and dielectric coefficient—need to be understood in atomic detail.

The SPM is a step towards improving the theory of selectivity [55]. The properties of water and mechanical properties of the protein are included by representing the solvent molecules as uncharged spheres in a uniform dielectric. That treatment removes most of the difficulty in dealing with Mg^{2+} although it still has too low resolution to deal with all blocking effects of interest experimentally. The SPM shows how binding produces mechanical forces and how mechanical properties of the protein modify selectivity. These mechanical forces are large and inescapable; they may be the forces that drive conformational changes. Interestingly, the theory shows that all the properties of selectivity (in the SPM model) are functions of just one variable, the free energy per mole of the selectivity oxygens of the channel protein. It is tantalizing that this is exactly the variable controlled by the protein and its genome. The 'activity' of the selectivity oxygens is the actual phenotype of the genome, not the location of the atoms (in the SPM model and perhaps in reality).

Despite the evident limitations, what is striking is that simple physical models like the MSA and SPM can deal quantitatively with a large range of selectivity phenomena. The physical effects in these models are more or less confined to the concentrated ionic plasma in the selectivity filter. The properties of that plasma are calculated using known results from the physical chemistry of concentrated salt solutions.

In view of the success of these simple models, I believe that understanding of binding and selectivity should start with known physical properties of concentrated ionic solutions and add in the complexities brought by the protein, one by one. This approach might lead to progress in other areas, e.g. the study of drug docking and protein folding.

7. New work

Work is well underway extending this approach. Recently, Density Functional Theory (of statistical not quantum mechanics [19,24,27]) has been applied [34] using the approach of Rosenfeld [20–23,28,30,31,35,51,109–119] to extend our work to explicitly inhomogeneous systems. The theory computes easily enough in complex situations and reproduces the MSA quantitatively when applied to systems where both are valid. DFT has been combined with a coupled treatment of flux and electrostatics using the Poisson and Nernst–Planck equations, called PNP in the biophysical literature [60,77,80,86–104] and Poisson Drift Diffusion in the (enormous) literature of computational physics, where the integrated method was born [12,78,79,120,121]. These equations are solved essentially as the semiconductor community learned to do many years ago [11–15,17,122–131]. The tricks they use hold much promise for biophysics and physical chemistry in my view, where coupled solutions of Poisson and transport equations are difficult to find.

The selectivity of other types of channels has been analyzed. A Monte Carlo simulation of the DEKA locus of Na^+ channels has been performed with satisfactory results (Nonner and Henderson, personal communication) and the recent structure of the CP channel [132] has been used to calculate the selectivity of anion channels (Nonner and Gillespie, personal communication). A mean field theory of single filing has been used to build a physical theory of the K channel with some

success (Nonner and Gillespie, personal communication).

8. Summary and historical note

Writing on this proud occasion of Dr Edsall's 100th birthday, I note that we are stumbling along in the direction implied by Cohn and Edsall [5], a long time ago. They recognized that proteins bristle with charge (cf. p. 73 of [6]). Here, we consider the counter ions of that charge. We focus attention on the permanent charges of the active site of the protein, discovering that protein function can be driven by the large energies stored in the (non-ideal) plasma of crowded charge surrounding the active site. Protein function can be controlled by a few properties of the active site, its volume and permanent charge, its local dielectric constant, and its mechanical strength. Specific arrangements of atoms are not needed to account for the strikingly complex specificity of active sites of channels.

This view of protein function is in the engineering tradition, engineering as usual, but here of proteins. The engineering approach seeks control of a system by building a specific physical model, a device equation so simple that it demands to be falsified and then extended. The engineering approach builds a succession of device models and equations of increasing accuracy, as new structures and energy sources are discovered.

The sequence of engineering models of protein function will yield more understanding and control, with enormous consequences for science, technology, medicine, and life. All depend importantly on proteins, as John Edsall was the first to show me, thereby enriching my life, for which I am forever grateful.

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The stamp of the Edsall approach should be evident throughout this paper and the work it reports. That work has been done in a collaboration led by Wolfgang Nonner. It is a personal and professional pleasure to interact with Wolfgang every day. Dirk Gillespie, Doug Henderson, and many others have made important contributions. Funding provided generously by DARPA has been indispensable and we thank the several program managers who have supported us, particularly, Shaun Jones, Mildred Donlon, Anantha Krishnan, and Sri Kumar (in chronological order), and the other program managers and their bosses who made that support possible.

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