Mechanisms of selectivity in biological ion channels

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ABSTRACT

Ca, K, and Na channels of biological membranes are proteins that pass ions through a molecular pore, thereby selecting particular ions of biological importance. We report here theoretical work suggesting that interactions among charged hard spheres can to a large degree account for the ionic selectivity of such channels.

Keywords: Ion Channels, Ionic Selectivity, MSA

Biophysicists have long reasoned that ionic selectivity occurs in special parts of biological ion channels, which they call 'selectivity filters', and recent studies of channel structure support this view. A small group of amino acids, located in spatially coordinated positions of the protein sequence, seems to form the selectivity filter in each of these structurally related ion channels.

In Ca channels, the selectivity filter involves four glutamate side chains (EEEE), whereas the selectivity filter of Na channels seems to be formed by aspartate, glutamate, lysine, and alanine side chains (DEKA) [1]. These charged side chains and their accompanying mobile counterions are confined in the small aqueous pore of the selectivity filter. In K channels, the filter does not seem to involve the side chains of the protein. Rather it is produced by the backbone of the protein, with negatively charged side chains buried in the protein at some distance from the pore lumen [2]. The backbone of the protein can be viewed as made of permanent dipoles. The local charge is sufficient to attract about two charges into the filter in the form of two K^+.

The density of ions in the selectivity filter of these channels is strikingly large. Consequently, both the charge density and the fraction of the space occupied by the ions are also large (tens of molar, i.e. $6 \times 10^{22} C/m^3$; for comparison, pure water is about 55 molar). Most thermodynamic properties of such charged liquids arise from the strong Coulombic and excluded volume interactions. We adopt the working hypothesis that selectivity in ion channels arises from the interactions of crowded spheres of charge. The channel protein sets the stage that allows these interactions: it provides the framework of the pore; it supports the mechanical forces necessary to maintain the required distribution of fixed charge; it provides the needed amount of induced charge by creating the appropriate dielectric coefficient.

Highly concentrated salt solutions are analyzed in a hierarchy of theories. We use the simplest of these theories – the mean spherical approximation (MSA) [3]– to see where improvements and extensions are needed.

We describe the EEEE locus of the Ca channel as the eight half-charged oxygen ions of the carboxylates that belong to the side chains of the four glutamate residues of the selectivity filter ([4], [5]). Each oxygen has diameter 0.28 nm. The model protein confines these oxygens into a small volume (e.g., a cylinder 0.5 nm in length and 0.8 nm in diameter) by a constant pressure (typically 400 MPa). Water is represented in the solvent primitive model (SPM) as molecular hard spheres in a continuum dielectric. The filter contents and the bath around the pore are treated as two bulk electrolyte solutions in equilibrium. In the real channel geometry anisotropies exist which we ignore at this stage; indeed the MSA screening radius under these conditions is < 0.1 nm, several fold smaller than the dimensions of the filter in the model.

The SPM/MSA predicts many of the selectivity properties of the Ca channel. The filter of the Ca channel accumulates Ca^{++} even if the surrounding solution contains 10^5 times more Na^+ than Ca^{++}. Theoretical curves of the accumulation of Ca^{++} approximate the first-order isotherm measured experimentally. This model of selectivity involves a simple mechanism: one divalent Ca^{++} screens the oxygen ions more effectively than two monovalent Na^+ because it delivers the same amount of charge in half the volume. The filter (in this model) nearly excludes water due to the confining pressure. Computations using different confining pressures show that low pressures produce less selectivity for Ca^{++} but steeper slopes of the Ca^{++} binding curves. The volume of the filter varies with the ionic densities and is balanced by a constant protein pressure.

SPM/MSA can also predict many of the properties of the Na channel if reasonable and testable assumptions are made about its structure. The Na channel selects for Na^+ in presence of an equal concentration of K^+ and 2 mM Ca^{++}. We explain the physiological selectivity for Na^+ over K^+ as an excluded volume effect that favors the smaller ion. In this view, the filter actually is not rigid; rather the groups of the DEKA lo-

cus compete with mobile ions for the space in the pore. The Na channel binds divalent Ca^{++} poorly (compared to monovalent Na^{+}) because it has a region of approximately zero net structural charge. If substantial net charge were present, the divalent would be preferred, as in the Ca channel. We imagine that the structure of the channel constrains the location of the single positive lysine group so it overlaps with just one of the carboxylate groups, making a region of zero average charge.

MSA can also predict many properties of the K channel when it is combined with a particulate description of the solvent and when information on channel structure is used. Physiologically, the most important properties of the K channel are its 100-fold selectivity over Na^{+} when equal concentrations of the two ions are present on either side of the membrane; physiological concentrations of Ca^{++} do not interfere with conduction of K^{+}. We roughly model the electrostatics of the K channel as two plates of a capacitor. One plate is formed by the ions in the pore; the other plate is formed from fixed charges buried in the protein. The polar peptide groups of the pore wall form the dielectric of the capacitor. Excluded-volume effects are introduced by allowing the oxygen atoms of the protein backbone to compete with mobile ions for the space in the pore, and these excluded volume effects prevent conduction of cations substantially larger than K^{+}. A suitable selectivity against Na^{+} can arise in this model if the polar groups of the wall cannot form optimal solvation arrangements around ions smaller than K^{+}, for instance, if there is a limiting diameter for optimal solvation ≈ 0.01 nm larger than the diameter of Na^{+}. This mechanism for the rejection of Na^{+} by K channels would predict that the channel makes a much stronger distinction between the divalent analogs of Na^{+} and K^{+}. Indeed, Ba^{++} binds to K channels at micromolar concentrations in the presence of 0.1 M K^{+}, whereas millimolar Ca^{++} does not.

In summary, it seems possible that channels exploit the physical properties of concentrated solutions in different ways to produce biological selectivity. Excluded volume and electrostatic free energies are biased by different structural constraints to produce channels selective for Ca, Na, or K. No special chemical interactions beyond those of charged spheres seem necessary to explain the physiological selectivity of these channels.

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