Anomalous Mole Fraction Effect, Electrostatics, and Binding in Ionic Channels

Wolfgang Nonner,* Duan P. Chen,# and Bob Eisenberg#
*Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33101-4819, and #Department of Molecular Biophysics and Physiology, Rush Medical College, Chicago, Illinois 60612 USA

ABSTRACT Ionic channels bathed in mixed solutions of two permeant electrolytes often conduct less current than channels bathed in pure solutions of either. For many years, this anomalous mole fraction effect (AMFE) has been thought to occur only in single-file pores containing two or more ions at a time. Most thinking about channels incorporates this view. We show here that the AMFE arises naturally, as an electrostatic consequence of localized ion specific binding, if the average current through a channel is described by a theory (Poisson-Nernst-Planck, PNP) that computes the average electric field from the average concentration of charges in and near the channel. The theory contains only those ion-ion interactions mediated by the mean field, and it does not enforce single filing. The AMFE is predicted by PNP over a wide range of mean concentrations of ions in the channel; for example, it is predicted when (on the average) less, or much less, than one ion is found in the channel's pore. In this treatment, the AMFE arises, in large measure, from a depletion layer produced near a region of ion-specific binding. The small excess concentration of ions in the binding region repels all nearby ions of like charge, thereby creating a depletion layer. The overall conductance of the channel arises in effect from resistors in series, one from the binding region, one from the depletion zone, and one from the unbinding region. The highest value resistor (which occurs in the depletion zone) limits the overall series conductance. Here the AMFE is not the result of single filing or multiple occupancy, and so previous views of permeation need to be revised: the presence of an AMFE does not imply that ions permeate single file through a multiply occupied pore.

INTRODUCTION

Single-file phenomena have been thought, for more than 40 years, to dominate the behavior of channels, at least since Hodgkin and Keynes measured the unidirectional tracer flux of potassium in squid axons (Hodgkin and Keynes, 1955). Their measurements from ensembles of K channels gave a ratio of unidirectional influx to efflux characteristic of single-file systems, quite different from the ratio in bulk solution (Jacquez, 1985; Hille, 1989, 1992). Measurements of unidirectional flux are difficult at best, and rarely made, and no one knows how to measure tracer flux through one channel. Thus, ever since channology became a molecular science—since individual channels could be studied in patch clamp (Neher and Sakmann, 1976; Sakmann and Neher, 1995) or reconstituted systems (Miller, 1986; Rudy and Iverson, 1992)—other signatures of single-file behavior have been sought and studied.

Most notably, the anomalous mole fraction effect (AMFE) has been defined from measurements of the conductance of the open channel, in mixed solutions of two permeant electrolytes: ionic channels bathed in such mixed solutions often (anomalously) conduct less current than channels bathed in a pure solution of either (Eisenman et al., 1986). The AMFE is measured with the same total concentration of ions in all of the mixtures, and on both sides of the channel, for example, in 300 mM mixtures of RbCl and NH_4Cl ranging from pure NH_4Cl (shown in the figures as mole fraction 0) through {200 mM NH_4Cl; 100 mM RbCl} (shown as mole fraction 0.33), to pure RbCl solutions. In ionic channels, the conductance in such experiments usually, if not always, varies nonlinearly from its value in pure NH_4Cl to pure RbCl. Indeed, in most ionic channels two species of permeant ion, when mixed, produce currents smaller than either species by itself: the conductance often shows a minimum, as the mole fraction of Rb varies from zero to one. Similar effects have been seen in crystalline channels (Wilmer et al., 1994), where they are called "mixed alkali effects," and analogous effects on activity coefficients are even found in bulk solution (Anderson and Wood, 1973; Chen, 1997; Robinson and Stokes, 1959, Ch. 15, provide entries into the vast literature).

When found in channels, such anomalous mole fraction effects are almost always explained by theories of a single-file channel occupied by two or more ions (Hagiwara et al., 1977; Ciani et al., 1978; Hille and Schwartz, 1978; Almers and McCleskey, 1984; Eisenman et al., 1986; Armstrong and Neyton, 1992; Heginbotham and MacKinnon, 1993; Yool and Schwarz, 1996). The usual image of a single-file channel is oversimplified, however, because of the difference in time scale between interatomic interactions in condensed phases and measurements of current in laboratory experiments. Single-file interactions of permeating ions occur on a time scale between that of interatomic collisions ($\sim 10^{-15}$ s) and that of correlated motions of water ($\sim 10^{-12}$

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Address reprint requests to Dr. Bob Eisenberg, Department of Molecular Biophysics and Physiology, Rush Medical College, 1750 West Harrison, Chicago, IL 60612. Tel.: 312-942-6467; Fax: 312-942-8711; E-mail: beisenbe@rush.edu.

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s; table 1, p. 19 in Brooks et al., 1988), whereas the permeation time of an ion (i.e., its mean first passage time through a pore) is $\sim 10^{-8}$ s (see Barcilon et al., 1993, figures 4 and 5; Eisenberg et al., 1995, equations 5.24–5.27 and Section VII), and biological behavior and measurements of current start around 5×10^{-6} s (Hodgkin and Huxley, 1952; Sakmann and Neher, 1995). Measurements of flux are much slower, taking seconds at their fastest. Thus the relation of the single-file interactions of atoms and permeation, as studied in the laboratory, is not obvious. Many complex trajectories might occur in the time between an atomic collision and a single experimental measurement of flux or current. For example, trajectories might involve correlated motions of ions in pairs (or in clusters of ions) in which the ions enter, leave, interchange positions, reenter, and then move through the selectivity filter of channels.

Measurements of the AMFE are the historical source, more than flux measurements or anything else, of the present-day image of channels as single-file systems containing multiple ions. This view depends, however, on the model and theory used to interpret the data. Up to now, the AMFE has been interpreted with a transition state theory that assumes large potential barriers independent of the concentration of ions in the baths. But potential barriers arise from fixed (i.e., permanent) structural charge on the channel protein, and so seem certain to vary if the concentration of ions (that shield the fixed charge of the channel protein) is varied. (This point is discussed at length in earlier papers on PNP (e.g., Eisenberg, 1996) and is, in fact, nothing more than a restatement of the commonplace knowledge of electrochemistry and Debye-Hückel/Gouy-Chapman/Poisson-Boltzmann theory that ions in solutions have a strong shielding effect on fixed charge. Thus potential profiles created by structural fixed charge are expected to vary strongly as the concentration of shielding ions is varied.) The barrier theory also used an incorrect form and value of the prefactor (Chen et al., 1997b). Use of the correct prefactor makes it difficult, if not impossible, for a theory with high barriers to predict the levels of current actually recorded from most single channels. It seems time for another approach to the AMFE.

Mean-field theories that ignore the particulate properties of proteins and ions—and the single-file properties of channels—have recently proved quite successful in explaining the function of open channels (Chen and Eisenberg, 1993a; Chen et al., 1995, 1997a,b; Eisenberg, 1996b; Tang et al., 1997) and other proteins (Warshel and Russell, 1984; Davis and McCammon, 1990; Honig and Nichols, 1995). These mean-field theories work so well, in all likelihood, because of the large density of structural (i.e., fixed or permanent) charge (Chen et al., 1997b) that forms the lining of the pore of channels and that makes the surface of most proteins hydrophilic. Blum and his colleagues have shown (in related, but not identical physical systems; Blum et al., 1996; Bratko et al., 1991) that high densities of surface charge create (average) electric fields that dominate the properties of these systems, overwhelming most consequences of the particulate nature of matter.

Here we show that the anomalous mole fraction effect, widely found in ionic channels, occurs in a simple self-consistent electrostatic model of the open channel with ion specific binding, even though the theory does not enforce multiple occupancy (defined in Eq. 2) or single filing. Multiple occupancy can occur, but it does not have to, and in fact is not present in the calculations we present. The temporal and spatial average of the concentration of ions in the channel (i.e., the average probability of an ion being in the channel) is less than one in the calculations presented here. Similarly, single filing can occur, but need not: the repulsion of the average charge in a mean-field theory may well produce single-file motion of particles, although the theory used here does not make this a priori assumption.

If binding is localized, the AMFE is seen in our calculations. The physical interactions that produce the AMFE involve the electrostatic interactions captured by self-consistent theories of the average electric field. Binding of one type of cation in a region tends to exclude other cations from that region. The binding also repels nearby cations (of any type), creating a depletion zone with low concentration and conductance that therefore dominates the resistance of the whole channel. Variations in the size of the depletion zone produce the AMFE, as described in the text.

We use a self-consistent theory in which the electrical potential is predicted from all of the charges present, particularly those in and near the binding region. A theory of this sort does not need an ad hoc description of ion-ion repulsion to predict the AMFE. Repulsion arises wholly from the self-consistent treatment of charge and repulsion by Poisson's equation. Constant-field, barrier (i.e., transition state), and most diffusion theories—including those of the AMFE (Eisenman et al., 1986; Armstrong and Neyton, 1992)—are not of this type, because they assume potential or barrier profiles independent of bath concentration or transmembrane potential (Eisenberg, 1996b; Appendix of Chen et al., 1997b). In such theories, applying either Poisson's equation or Coulomb's law (to all of the charges present) almost never predicts (or approximates) the potential or barrier profile actually employed by the theories in their computation of flux or current.

In the self-consistent PNP theory used here, a channel can have low or high occupancy and still show an AMFE, but in our calculations it must have localized ion-specific chemical interactions that change the local concentration of one ionic species. "Binding" in this model is electrostatic, not frictional. Ionic diffusion coefficients need not be reduced in the binding region. Any localized, chemically specific interaction seems to produce an AMFE: we can calculate an AMFE even if the localized ion-specific interactions with the channel protein are repulsive, i.e., if the channel has "negative binding." It seems that the electrostatic binding mechanism we compute in this paper gives an AMFE that occurs over a wide range of channel properties and experimental parameters.

METHODS

We describe conduction in the open channel by the generalized diffusion equation, the Nernst-Planck (NP) equation. The theory deals with temporal and spatial averages. It is not concerned with individual ions, their trajectories, or fluctuations.

$$J_k = -D_k \mathcal{A}(x)\left[\frac{dC_k}{dx} + \frac{C_k(x)\,d}{RT\,dx}(\mu_k^0 + zF\varphi(x))\right] \equiv \frac{-D_k \mathcal{A}(x)}{RT} C_k(x) \frac{d\mu_k(x)}{dx} \quad (1)$$

The NP equation describes the conditional probability of location of ions in a channel (i.e., their concentration) under very general conditions (Eisenberg et al., 1995). Occupancy is defined by

$$O \equiv \sum_k O_k \equiv \sum_k \int_0^d \mathcal{A}(x) C_k(x) dx \quad (2)$$

Note that the occupancy of all ions O (and the occupancy O_k of ions of type k) in this theory—like the concentration profile $C_k(x)$ from which they are derived—are outputs of our calculations. They are not inputs, assumed to have specific values, as in most traditional (e.g., state) theories. The occupancy is the temporal and spatial average of the concentration of ions in the channel, i.e., of the conditional contents of the channel. These average quantities are derived from the properties of trajectories of individual ions in Eisenberg et al. (1995, see equations 5.20, 5.24, and Section C). Trajectories of individual ions are computed, illustrated, and analyzed in Barcilon et al. (1993).

In these equations, J_x is the flux; x is the location along the axis of the channel; $\mathcal{A}(x)$ is the cross-sectional area at location x; $\varphi(x)$ is the electrical potential; D_k is the diffusion coefficient; C_k is the concentration of species k with charge z_k, e.g., K^+, Na^+, or Cl^-; and $\mu_k(x)$ is the electrochemical potential of species k, e.g.,

$$\mu_k(x) \equiv z_k F\varphi(x) + RT \ln C_k(x) + \mu_k^0(x) \quad (3)$$

Equation 3 consists of the terms used in earlier versions of PNP and an additional term, $\mu_k^0(x)$, the standard chemical potential of species k at location x, which describes the binding of the ion to the channel protein. In the calculation of this paper, $\mu_k^0(x)$ varies with location x and ionic species k, but with nothing else.

The PNP of Chen and Eisenberg (1993a) and Eisenberg (1996b) does not predict the AMFE unless it is modified (Chen, 1997). Here we have extended the original theory by supposing that each ion may have different chemical properties (i.e., an intrinsic chemical potential arising from different affinity or "binding") in a particular region of the channel, as is known to occur (by direct measurement) in gramicidin (Jing et al., 1995). We follow chemical convention (e.g., Krukowski et al., 1995) and describe the binding by assigning a different standard chemical potential $\mu_k^0(x)$ (or, equivalently, a different activity coefficient) to each ionic species. The different standard chemical potentials help the channel tell one ion from another and presumably arise from chemical interactions (see Ch. 3 of Brooks et al., 1988), many of which can in fact be described quantitatively and simply by the MSA (mean spherical approximation) theory of selectivity in bulk solutions (Durand-Vidal et al., 1996).

The diffusion coefficients of Eq. 1, etc., can be made functions of position (e.g., Eisenberg et al., 1995, equation 3.1, p. 1768)—and, in fact, have been made such functions in our computer programs—but in Figs. 1, 2, and 3 *A*, the diffusion coefficients for all cations are set to be equal and independent of location in the channel.

PNP uses the Poisson equation to describe how the average potential profile $\varphi(x)$ is created by the average charge in and near the channel (Eisenberg, 1996b):

$$-\frac{\epsilon_0}{F}\left[\epsilon_p(x)\frac{d^2\varphi}{dx^2} + \left(\frac{d\epsilon_p(x)}{dx} + \epsilon_p(x)\frac{d}{dx}\ln \mathcal{A}(x)\right)\frac{d\varphi}{dx}\right] = \overbrace{P(x)}^{\text{Fixed Charge}} + \overbrace{\sum_k z_k C_k(x)}^{\text{Channel Contents}} \quad (4)$$

A numerically insignificant dielectric term has been left out of this equation (Barcilon et al., 1992). Here, $\epsilon_0\epsilon_p(x)$ is the permittivity of the channel's pore, and $P(x)$ is the spatial average of the fixed (i.e., the permanent structural) charge of the protein lining the wall of the channel's pore. Equations 4 and 1 have to be solved together, because both involve the same variables: the potential profile changes the concentration profile, and vice versa. Although this system of equations cannot be integrated by the standard numerical recipes distributed widely nowadays, they can easily be integrated by the Gummel iteration, described in our earlier papers (Chen and Eisenberg, 1993a; Eisenberg, 1996b). (The program that executes the original PNP theory is available on website http://144.74.27.66/pnp.html or by anonymous FTP from IP address 144.74.3.21.)

The use of the averaged potential on one side and the averaged concentration on the other side of the Poisson equation is biologically and physically justified. Biologically, it is clear that the averaged potentials, currents, and concentrations used in PNP describe current flow in some seven types of channels (Chen et al., 1995, 1997a,b; Tang et al., 1997) in solutions ranging from some 20 mM to 2 M. Physically, it is not unreasonable to state that the electrical potential (and potential energy) averaged over some microseconds is produced by the charge averaged the same way, on the same time scale. Indeed, were this not the case, one would need to include additional physics to account for the violation of the (mean-field) Poisson equation. We imagine that electrodiffusion of ions in open channels follows the same physical principles and mathematical laws as the electrodiffusion of charge carriers in many other physical

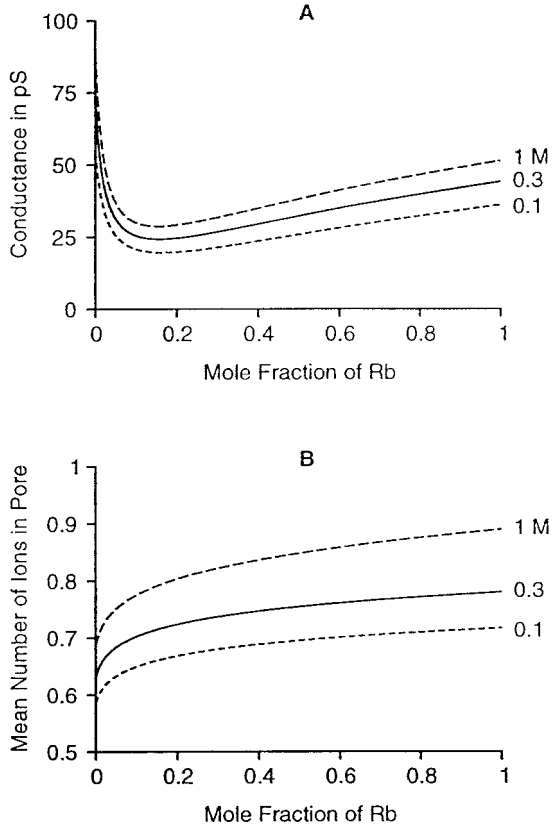

FIGURE 1 Anomalous mole fraction effect (AMFE) at low ionic occupancy. (*A*) AMFE on single-channel conductance computed from PNP theory for a hypothetical pore surrounded by identical bath solutions ("symmetrical solutions") containing varying mole fractions of RbCl and NH_4Cl. The pore was assumed to have Rb binding just in its left half-side. NH_4 does not bind anywhere. The structural charge along the whole pore was assumed to have a uniform density of -5 M (like that found in a number of channels), corresponding to 0.79 charges altogether, or 0.038 charges/Å, spread uniformly along the 21-Å length of the 4-Å-diameter pore of volume of 2.64×10^{-25} liters. Three total salt concentrations (0.1, 0.3, 1 M) are used. Diffusion coefficients were spatially uniform and equal for both cationic species at 10^{-6} cm^2/s. There was no affinity or binding assumed for NH_4 anywhere in the channel. Rb was assumed to have a standard chemical potential of -120 m-eV (i.e., -4.7 kT) in the internal (left) half of the pore, but none in the external (right) side of the pore (illustrated in Fig. 2 *A*). Anionic flux was minimized by assigning Cl ion a repulsive standard chemical potential of 120 m-eV and a small diffusion coefficient (10^{-9} cm^2/s). Conductances were computed as the slope conductance between transmembrane potentials of ± 10 mV. (*B*) Total number of ions present in the pore (see Eq. 2) as a function of the Rb mole fraction (same conditions as in *A*). Note that this average occupancy was smaller than one ion per pore.

systems (see citations in Eisenberg, 1996b), most notably semiconductors (Seeger, 1991; Lundstrom, 1992).

RESULTS AND DISCUSSION

Fig. 1 *A* plots the conductance of a hypothetical pore (25 Å long by 4-Å diameter) with a low occupancy bathed in identical solutions (on both sides) containing 0.1, 0.3, or 1 M total salt with a variable percentage (i.e., mole fraction) of Rb and NH_4. Parameters used in the PNP calculation are given in the figure captions.

The names of ions were chosen in deference to Eisenman et al. (1986); we have used this paper as a historical as well as an experimental definition of the AMFE. Thus, in the graph labeled 0.3 M, solutions range from pure NH_4 {300 mM NH_4Cl; 0 mM RbCl} (shown in the figures as mole fraction 0), through {200 mM NH_4Cl; 100 mM RbCl} (shown as mole fraction 0.33), to pure RbCl solutions {0 mM NH_4Cl; 300 mM RbCl} (shown as mole fraction 1.0). Note that the conductance in pure RbCl solutions is less than in pure NH_4Cl solutions, but the conductance in 15% RbCl (0.15 on the abscissa) is less than either.

This electrostatic/binding AMFE was calculated for uniform structural charge along the whole pore, giving a density of -5 M (like that found in a number of channels, *op. cit.*), corresponding to 0.79 charges altogether, or 0.038 charges/Å, spread uniformly along the 21-Å length of the cylindrical pore proper (4-Å diameter), which itself (without atria, etc.) has a volume of 2.64×10^{-25} liters. Rb binding is placed in just the left half of the pore, and NH_4 does not bind anywhere.

A wide range of pore parameters gives the electrostatic AMFE, provided the channel is longer than ~5 Å. The AMFE seems to be present whenever the two ions have different affinities at some location. We can calculate similar AMFEs in symmetrical (but not uniform) channels, in channels with tiny (<0.1 ions/pore) or with multiple occupancy, with a structural charge of anywhere between 0 and 50 M, with structural charge located only in the binding region of the channel, with standard chemical potentials (of binding) of -20 to -200 m-eV, in bath concentrations of 30 mM to 3 M. To our surprise, the AMFE is predicted even when the left-hand side of the channel (the "binding" region) actually repels Rb: both binding and repulsion (i.e., a standard chemical potential with sign opposite that of binding) can reduce conductance (Fig. 3 *A*).

Fig. 2 shows the profiles of standard chemical potential and electrical potential, and the concentrations of Rb and NH_4 that produce the conductances plotted in Fig. 1; we look inside the channel to see how different affinities produce the AMFE. In the simple case shown and analyzed in Figs. 1 and 2, the applied voltage is zero and diffusion coefficients are assumed to be equal and spatially uniform. Varying the diffusion coefficients gives a variety of shapes for the AMFE curves (see Fig. 3 *B*), because the D_j determine the vertical location of the left- or right-hand ends of the curves.

The conductance for each ion is determined (mostly) by the region where it is present in the lowest concentration, its depletion zone. The overall resistance of the channel arises, in effect, from resistors in series, one from the binding region, one from the depletion zone, and one from the unbinding region. The highest value resistor (which occurs in the depletion zone) limits the overall series conductance. For example, the conductance for Rb is limited by its concentration just to the right of center (outside its binding

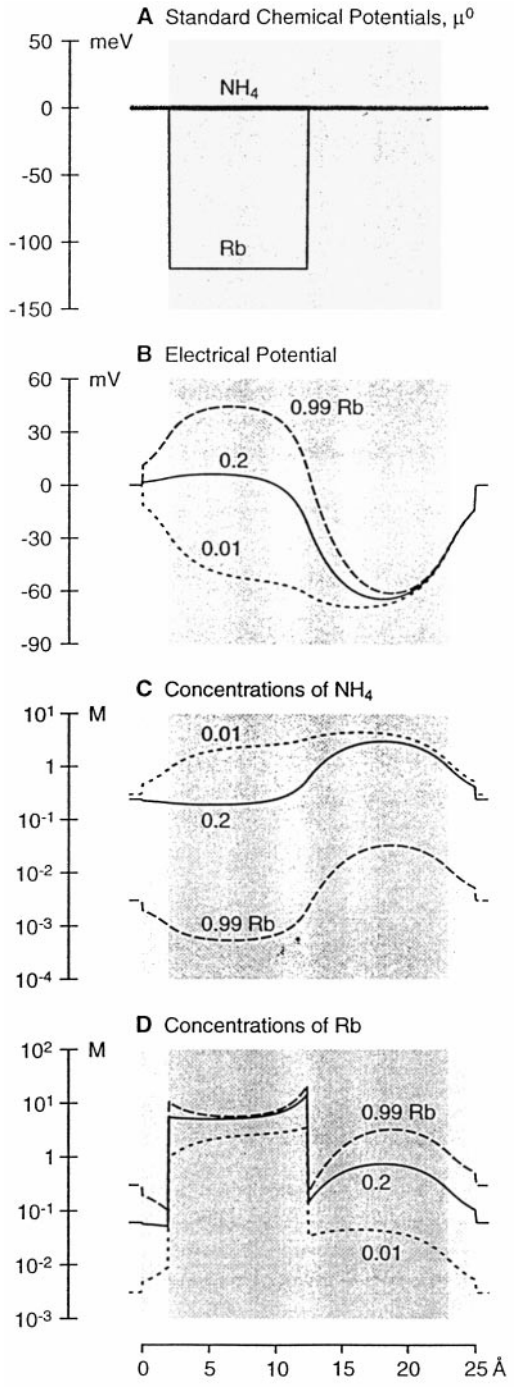

FIGURE 2 Redistributions of pore electrical potential and ionic concentrations underlying the AMFE shown in Fig. 1. (*A*) Profiles of cationic chemical potentials used. These and all other pore parameters were identical to those used in Fig. 1. The abscissa plots distance along the axis of the pore; the pore region is marked by gray shades (*dark*, the cylindrical interior; *light*, a short atria that opens with a rounded taper to the membrane surface. The atria and bulk solutions had no structural charge or affinity for ions and so diffusion coefficients for ions in aqueous solutions were used. (Diffusion coefficients of ions in the proper cylinder are given in the caption to Fig. 1.) The origin of the abscissa is at the "intracellular" end of the pore. The PNP equations were integrated in the pore itself and in hemispheres extending 100 Å into both bulk solutions. These are represented with a compressed abscissa scale. Computed profiles are shown for three different mole fractions of Rb, each in a symmetrical bulk solution with 0.3 M total salt concentration.

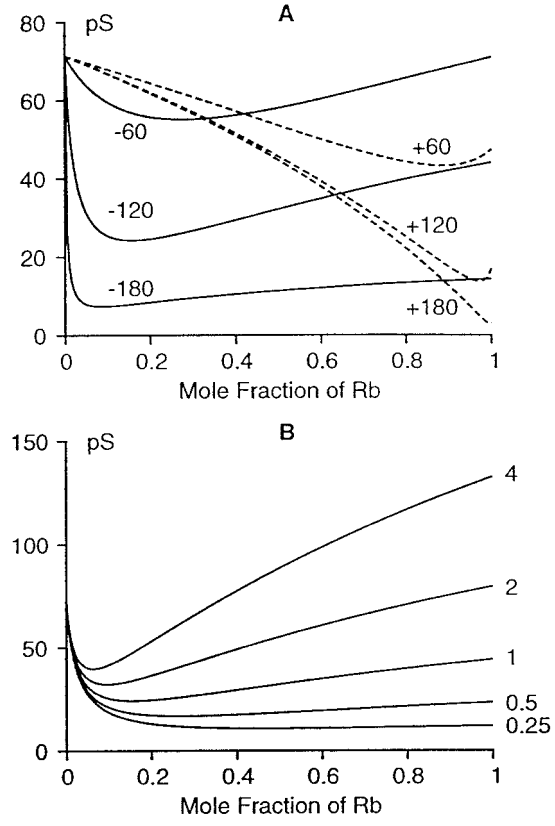

FIGURE 3 Variations in the AMFE resulting from a varied Rb affinity (*A*) or Rb diffusion coefficient (*B*). (*A*) The Rb affinity is varied in the left half of the pore proper from negative values of μ^0_{Rb} (indicated in units of m-eV), indicating attraction (——), to positive values indicating repulsion (– – –). (*B*) The Rb diffusion coefficient in the entire pore proper is varied by successive factors from the value in Fig. 1, namely 10^{-6} cm^2/s. The factors were 0.25 in the bottom curve, then 0.5, 1, 2, and, finally, 4 in the top curve. The Rb affinity in the left half of the pore proper was −60 m-eV. All other parameters were as in Fig. 1. The salt concentration was 0.3 M.

region, in the region $12.5 < x < 17$ Å in Fig. 2 *D*), which is much less than the Rb concentration in the region of binding.

Not surprisingly, Rb (Fig. 2 *D*) is concentrated in its binding region in the left-hand side of the pore, and its concentration is buffered. This Rb "neutralizes" the negative structural charge in this region, or even creates a net positive space charge: note the sometimes positive potential in this region (Fig. 2, *B* and *D*). In the binding region, the bath Rb concentration has a fairly small effect: the concentrations in the binding region are more or less buffered.

The buffered, bound Rb controls the Rb in the unbound region by repelling nearby cations, creating a depletion zone (when bath Rb concentration is larger than ~0.02 M). Rb conductance is limited by this region of low concentration, which is adjacent to (but not in) the binding region.

NH_4 is not bound in the left region. Indeed, it is not bound anywhere. NH_4 interacts with the bound Rb ions because it experiences the electrical potential created by their space charge; there are no specific interactions of pairs of ions in our model. In our model, NH_4 and Rb interact

only through the mean electrical field, the gradient of the electrical potential.

The mean field interaction can be strong. When Rb is mixed into the bathing solutions (i.e., its mole fraction is increased from zero), the NH_4 concentration in the left-hand region is reduced drastically (see lowest curve in Fig. 2 *C*). The binding region contains a large concentration of the bound ion Rb, which creates a positive net charge; that, in turn, makes the potential more positive (Fig. 2 *B*) and thereby excludes NH_4. More crudely, the bound Rb repels nearby cations. Bound Rb repels NH_4 in the binding region. It repels Rb nearby, just outside the binding region. Of course, if Rb is absent from the solutions, this effect cannot occur.

Repulsion effects of this sort arise from the Poisson equation of PNP. They do not have to be ascribed to specific electrostatic interactions of pairs of ions. They arise from the average interaction of averaged densities of charge that occur in any self-consistent mean field theory. A theory that did not calculate the electric potential from all of the charges present, including those in the channel's pore, would have difficulty describing such depletion layers and repulsion effects.

The NH_4 concentrations along the channel are shown in Fig. 2 *C*. The top curve shows that NH_4 concentration is quite uniform when Rb is absent and NH_4 is the only cation. When Rb is mixed into the bathing solution, and thus the NH_4 is reduced, its concentration drops in the left binding region of the channel, and the maximum of the concentration curve becomes more prominent in the right nonbinding region (see *middle curve*). The behavior on the right is unimportant for the overall conductance, because the resistance (to NH_4 movement) there is low. This resistance is determined by the region of lowest NH_4 concentration, the left-hand region in which the concentration is depleted by the repulsion effects previously described. The low concentration/high resistance region for NH_4 is the left, where Rb (but not NH_4) is bound. NH_4 and Rb are chemically equivalent on the right-hand side of the channel—neither is bound there. Nonetheless, the ions behave very differently there because the binding region (on the left) determines the properties of the nearby nonbinding (right-hand) region, by creating a depletion zone. The bound ion determines the conductance of the unbound ion.

Turning back to Fig. 1, we notice that the conductance in pure NH_4 solution (*left-hand side* of Fig. 1 *A*) is larger than the conductance in pure Rb solution (*right-hand side* of Fig. 1 *A*), even though their diffusion coefficients are the same and Rb is bound, because Rb (even in pure Rb solutions) has a zone of low concentration, a depletion zone that limits current, irrespective of the (low) resistance of the binding region. If Rb is absent from the solutions, depletion zones and other repulsion effects (resulting from excess concentrations of bound ions) cannot occur. Thus NH_4 does not have a depletion zone in pure NH_4 solutions, because it is not bound and Rb is not present.

In Fig. 1, the AMFE is seen as the mole fraction of Rb in the bath gets larger. Bath Rb has little effect on Rb concentration in the binding region of the channel, but the NH_4 concentration in the binding region is significantly reduced, as the mole fraction of Rb in the bath gets larger, thus reducing the contribution of NH_4 to the overall channel conductance. For these reasons, the overall conductance decreases as the mole fraction of Rb is increased in the bath from zero to ~15%. Beyond 15%, the NH_4 concentration in the left side decreases slightly below its bath concentration, and the Rb concentration (on the right) is of similar magnitude. NH_4 and Rb contribute (roughly) equally to the overall reduced conductance. A further increase in the mole fraction of Rb slightly increases the overall conductance (toward the conductance in pure Rb) because of changes in the size and shape of the Rb depletion zone. The resulting minimum in the curve defines the AMFE as anomalous.

Simply stated, the sticky ion Rb displaces the nonsticky NH_4 from the binding region and a depletion region nearby. The sticky Rb punishes itself by creating a depletion zone for itself outside the sticky region. The nonsticky NH_4 is reduced in its concentration (and so is its contribution to occupancy) in the sticky zone. Both ions are reduced in the depletion region. Both ions give little flux, because each one is depleted somewhere.

The discontinuity in binding—in the central region of the channel—has large quantitative effects on the overall properties of the channel, and so more detailed study of the AMFE may reveal different properties of this crucial region in the many different types of channels (Conley, 1996). We would not be surprised if the functionally important properties of channels—and other types of proteins that transport ions across membranes—are controlled by the structure of the binding region, and the resulting depletion layers, just as the function of transistors (whether acting as resistors, diodes, voltage amplifiers, or current amplifiers) is controlled by their doping profile and the resulting depletion layers.

In the calculations reported in Figs. 1 and 2, binding is equal and uniform because of our ignorance of the atomic details of binding in real channels. Diffusion coefficients and the profile of structural charge are also assumed to be equal and uniform, to keep the number and complexity of figures manageable.

Fig. 3 shows the effects of varying Rb affinity in just the binding region (Fig. 3 *A*) or the Rb diffusion coefficient everywhere (Fig. 3 *B*). In real channels, profiles of structural charge and diffusion coefficients are unlikely to be equal and uniform, of course (Chen and Eisenberg, 1993a; Chen et al., 1995, 1997a,b; Eisenberg, 1996b; Tang et al., 1997). And binding is likely to be more complex as well. Thus real channels are likely to have a wider variety and more complex forms of the AMFE than those illustrated in this paper, in which we have computed a most uniform case. Nonetheless, we have not been able to produce an AMFE by introducing a spatial variation of the diffusion coefficients, without binding, and we think we know why. As defined here, the AMFE can and does occur when ion concentra-

tions and transmembrane potentials are (nearly) equal on both sides of the channel. That is to say, it occurs close to equilibrium when ions hardly flow. In that situation, ion distributions are minimally altered by fluxes, and so are minimally sensitive to the ionic diffusion coefficients. Ion binding, however, affects ion concentrations, even in the absence of flux, and thus can produce an AMFE, even under conditions close to equilibrium. In this way, diffusion coefficients can change the shape of the AMFE (Fig. 3 *B*), but they cannot create it.

Our model grows from that of Eisenman et al. (1986), in the sense that both models include electrostatics and binding. Their model was heuristic, however: it did not predict *IV* curves. Their theory also did not compute ionic flux and/or the electric field self-consistently. It only considered the field produced by some of the charges present (cf. Armstrong and Neyton, 1992), and it used transition state theory to link the height of supposedly large potential barriers to the conductance of the channel. We have discussed the difficulties of such theories at some length (e.g., Eisenberg, 1996b; Chen et al., 1997b).

Our model is also heuristic, but in a different sense. The binding of ions is described here by the ions' standard chemical potential, its affinity. This is a description—not a theory or model—and needs to be replaced by a more specific physical model that derives the dependence of the affinity on the interactions of ions with water, the channel protein, and each other. In bulk solutions, these interactions produce a significant dependence of the standard chemical potentials on concentration, electric field, or even flux. In the highly charged pipes formed by ionic channels, the dependence of standard chemical potential on concentration, etc., is not known. However, the very fact that channels are such highly charged pipes means that their internal environment is buffered against changes in the external environment. In particular, changes in the concentration of counterions in the bath (ions of sign opposite that of the fixed charge) are unlikely to have large effects on the concentration of counterions in the channel, in most cases. The MSA (mean spherical approximation; Durand-Vidal et al., 1996), which generalizes Debye-Hückel/Gouy-Chapman/Poisson-Boltzmann theory to allow for the finite volume of ions, allows quite accurate predictions of the properties of ionic solutions in the full physiological concentration range (including ion interactions in mixed bulk solutions (Blum et al., 1996) and in narrow planar pores (Bratko et al., 1991)) and should be incorporated into PNP to see if the resulting theory predicts the AMFE, perhaps even in spatially uniform channels (see Anderson and Wood, 1973; Chen, 1997).

Interestingly, Blum and his colleagues have shown (using general derivations widely discussed in the physical chemistry literature that do not depend on the MSA) that theories like Poisson-Boltzmann and PNP "become exact for large electric fields, independent of the density of hard spheres" (Henderson et al., 1979, p. 315), and "independent of interactions of molecules in the fluid phase" (Blum, 1994, p. 972). Large electric fields are very likely to exist in proteins and channels, because their surfaces/walls contain substantial structural charge (Warshel and Russell, 1984; Davis and McCammon, 1990; Honig and Nichols, 1995), and pores are very small (Chen et al., 1997b). The existence of such fields and charge—and the slow time scale of biological systems—may allow mean field theories like Poisson-Boltzmann and PNP to be more successful descriptions of channels and proteins than of bulk solutions (in which the structural charge density is zero and the relevant time scales are fast).

CONCLUSION

We have shown that the AMFE can occur in channels with neither single filing nor multiple ionic binding sites. We have shown instead that the AMFE can arise as a consequence of spatial variation in specific ionic affinity and nonspecific mean field electrostatic interactions captured by the PNP equations. Local attraction of an ion species leads to nonuniform distributions of charge carriers in the pore, which can result in low overall conductance. The AMFE can occur while the mean ionic occupancy of the pore is very low; this AMFE does not reflect variations in the total ionic occupancy of the pore. The total ionic occupancy is determined by the need for (approximate) electroneutrality.

Contrary to the common view, the presence of an AMFE in a channel does not imply that permeation is through a single-file, multiply occupied pore with identifiable interactions between individual bound ions.

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