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Enhanced ionic interaction in narrow pores and ion pair formation

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Abstract. The same physical phenomenon that gives rise to the increase in the electrostatic self-energy of an ion within a narrow water-filled pore is shown to result in interionic electrical interactions within the pore that are much stronger and of longer range than those between the same ions in the same solution in bulk. Because of the much enhanced attraction between ions of opposite charge within the pore the formation of ion pairs becomes likely, even for strong electrolytes that are fully dissociated in the same solution when not spatially confined. Some predicted consequences of ion pair formation in narrow pores that may be experimentally detected are discussed. It is shown that, in a simple passive pore, due to ion pair formation, an Ussing unidirectional flux ratio exponent of less than 1 is predicted. This is usually thought to characterize a carrier rather than a pore.

Key words: Ionic interactions – Ion pair formation – Ions in narrow pores – Ion channels and filters

Introduction

A major energy barrier to the passage of a small ion through a narrow water-filled pore is the rise in the electrostatic self-energy of the ion. This was first demonstrated as a simple model (Parsegian 1969) and the theory has since been elaborated to include more complicated geometry that may be numerically simulated on a computer and which allows for end effects in cylindrical channels (Levitt 1978) and for particular shapes of pore entrance (Dani 1986). The rise in self-energy is now routinely incorporated into models of ion transfer through narrow pores (Hille 1992). However the fact that exactly the same physical mechanism radically increases both the strength and the range of electrostatic interactions between different ions within a narrow water-filled pore is not widely recognized.

Because of its intrinsic difficulty, inclusion of the interaction between ions is seldom included in theoretical models of ion channels. The exception, which is much studied, is the case of "multi-ion channels" which are so narrow as to require that the ions proceed through the channel in single file. In the many simulations of such channels which use an Eyring "barrier-jumping" approach such as those of Hodgkin and Keynes (1955); Urban and Hladky (1979); Hille and Schwarz (1978), the repulsive interaction between ions of like charge is simply represented by not allowing two ions to occupy the same site and by changing the probability of entry or exit by an assigned amount depending upon whether the channel is occupied by another ion or not. In a recent heroic attempt to solve more exactly the problem of two like ions occupying a single channel using continuum diffusion theory (Levitt 1987), the solution was only possible by assuming that there was no component of electric field normal to the pore wall which removes the enhancement of interionic interaction discussed in this paper. The enhanced attraction between ions of unlike charge within narrow pores with the probability of ion pair formation has not to my knowledge been considered before.

The physical mechanism

Consider a cation within a pore filled with aqueous fluid with relative dielectric constant ε_f which penetrates through solid material of dielectric constant $\varepsilon_s < \varepsilon_f$. The electric field of the cation induces an electric dipole moment P per unit volume which points away from the ion. Within the aqueous fluid Div P is zero and the charge density due to the negative ends of the induced water dipoles cancels that due to the positive ends of other water dipoles at the same position so that no bound charge is induced within the fluid (Bleaney and Bleaney 1976). However at the interface between the fluid and the pore wall a bound surface charge is induced which is equal to the difference between the normal components of the induced electric dipoles moments in the two materials at the interface (Bleaney and Bleaney 1976). In this case, because the dipole moment induced in the aqueous fluid is larger than that in the confining solid ($\varepsilon_f > \varepsilon_s$), the induced charge has the same sign as the charge of the ion producing the polarization. The increase in the electrostatic self-energy of the cation in the pore compared with its value in the bulk fluid may be considered as due to the additional electrostatic interaction between the cation and the positive surface charge induced on the wall of the pore. Within the pore an anion induces a negative surface charge so that its self-energy is similarly raised.

If a second cation approaches the first cation within the pore it will be repelled by the like charge of the first cation as in bulk solution but it will also experience a repulsion from the positive charge induced at the pore wall by the first cation. Thus it is seen that the same physical mechanism that gives rise to the increase in the electrostatic self-energy of ions within narrow pores also increases the electrical interaction energies between ions in the pore. Clearly the attractive interaction between cations and anions within the pore will be similarly enhanced. The range of the inter-ionic interaction is also increased, as we shall see, due to the distributed nature of the charge induced at the pore wall.

Two illustrative examples

To illustrate the effect, calculations will be performed for two simple geometries, a parallel sided aqueous slab and a cylindrical aqueous pore for which analytical results are possible. Because such calculation assumes continuous fluids with constant dielectric constants the results of the calculations are unlikely to be numerically accurate but are useful in a discussion of the effect. Because the enhanced interaction stems from the same mechanism that gives rise to the raised self-energy of the ion, it will be significant in all cases in which a raised self-energy is found.

We first consider the case of a single ion within a parallel sided aqueous slab of dielectric constant ε_f confined between two solid blocks with dielectric constant ε_s. The induced bound surface charge may be readily calculated using the method of images (Neumcke and Lauger 1969) in this geometry. Before calculating the interaction between two ions we will calculate the excess energy DU that the single ion experiences due to interaction with the induced surface charge as it moves across the aqueous slab. For a univalent ion the ratio DU/kT, where kT is a typical thermal energy at 25 °C, is plotted as a full line in Fig. 1 a as a function of its position along a line perpendicular to the faces of the slab. The fluid slab width was taken as 10 Å and ε_f was taken as 80 representing the aqueous fluid and ε_s was taken as 2 representing the plastic of a filter or membrane protein or lipid in the biological case. For a divalent ion the energy DU is 4 times as large. Thus there is a repulsion from the interfaces for ions of either sign such that the probability of finding an ion in the centre of the aqueous slab is 3.4 times and 133.6 times more likely than finding it 1 Å from the slab wall for univalent and divalent ions respectively.

The presence of such an ionic depletion layer at a solution-air boundary is thought to provide an explanation of the change in surface tension of electrolyte solutions with concentration and calculations of the induced surface charge like that above have been shown (Onsager and Samaras 1934) to provide a good first approximation to the size of the effect. In bulk solution the surface charge is thought to be screened by counterions so that it decays exponentially to zero in a distance from the surface of order the Debye length. Assuming counterion screening with a Debye length of 10 Å for the parallel slab, leads to calculated values of DU/kT for univalent ions shown by the broken line in Fig. 1 a. The probability of being on the median plane of the slab is a factor 3.7 higher than that of being 1 Å from an interface much like the case without screening. Thus the first effect predicted by the calculation is that within narrow aqueous sheets ions are repelled from the confining walls.

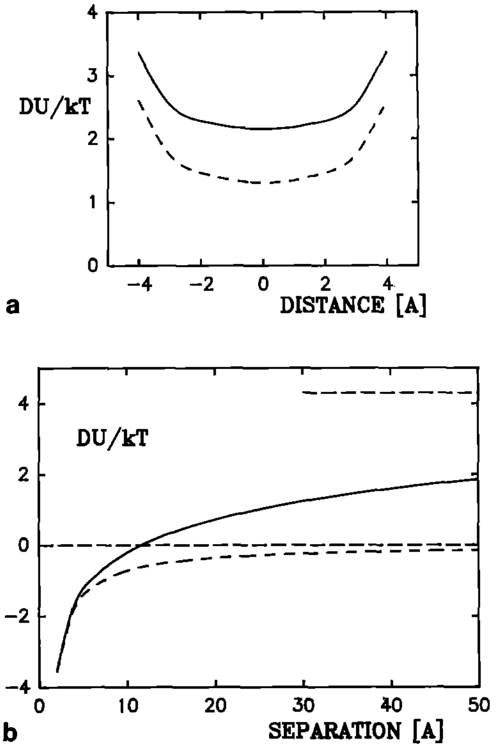

Fig. 1. a The full line gives the calculated increase in self-energy in units of kT of a univalent cation or anion within a narrow aqueous slab over that found in bulk solution. The energy is plotted as a function of its position along a line normal to the faces of a 10 Å thick plane slab of fluid of relative dielectric constant 80 confined between the parallel faces of two infinite solid blocks of solid material with relative dielectric constant 2. The broken curve gives the result of the calculation when counterion effects are included which result in a Debye length within the fluid of 10 Å. **b** The full curve shows the calculated electrical energy in units of kT of a univalent cation-anion pair situated in the mid-plane of the fluid block described in Fig. 1 a as a function of their separation measured in Å units. The broken line shows the calculated direct interaction energy as in bulk solution and the difference between the two lines is the excess energy of the two ions when in the narrow aqueous slab. The zero of energy, shown by the lower broken straight line, represents the state with the two ions well separated and fully hydrated in bulk solution. The upper straight broken line gives the limit of the electrical energy of the ion pair at large separation

The second and more significant prediction is of an increased interaction energy between pairs of ions within confined water-filled spaces. In Fig. 1 b the full line shows the electrical energy DU in units of kT of a univalent cation/anion pair situated in the median plane of the slab as a function of the distance between the ions. The dimensions and dielectric constants assumed are as in Fig. 1 a. The broken curved line shows the effect of the direct electrostatic attraction between the ions (as in bulk solution) and the difference between the full and broken lines is due to the induced surface charge. The zero of energy is that of the two ions well separated and fully hydrated in bulk solution. At their distance of closest approach of 2 Å the energy DU of the ions within the pore is close to the interaction energy in bulk solution. This is due to the almost complete cancellation of the two induced surface charge distributions of opposite sign when the ions are close together. Thus the excess energy required to separate such an ion pair within the narrow aqueous slab may be equated to the rise in the electrostatic self-energies of the two ions when they are separated, which is absent when they are close together due to the surface charge cancellation.

At large separation the electrostatic energy reaches 4.3 kT shown on the graph by the upper broken straight line so that the calculated change in energy of the ion pair moving from close proximity to wide separation is more than twice as big in the slab as it is in bulk solution. Also the electrical attraction between the ions is much longer in range than the direct electrostatic interaction due to the distribution of the induced surface chare over an appreciable area. Although the predicted effect is large in this geometry where the solution is confined in one dimension it is much larger in a pore where the aqueous solution is confined in two dimensions and we now turn to this cases.

A cylindrical pore is probably the most relevant geometry when discussing biological channels or filters. The voltage within and without a long cylindrical pore due to a charged particle on its axis and charge induced on the pore wall may be calculated by numerical integration of an expression involving modified Bessel functions (Smythe 1968; Parsegian 1975). We will consider a cylindrical pore in an infinite solid block with dielectric constant of 2 filled by a fluid with relative dielectric constant 80. For a pore of diameter 10 Å the electrostatic energy in units of kT at 25 °C of a univalent cation/anion pair, both on the axis of the pore, as a function of their separation in Å units is plotted as a full line in Fig. 2 a. The energy of the interaction predicted for bulk solution is shown as a broken curve. Again the difference between the two curves gives the contribution of the excess interaction energy of the two ions within the pore over that in bulk solution. Once again the zero of energy is that of the two ions well separated and fully hydrated in bulk solution. The excess electrostatic self-energy of each isolated univalent ion is +9.7 kT in the 10 Å diameter pore so that the energy required to separate the two ions rises to about +19.2 kT at large separation as indicated by the broken line at the top of the figure. Again, as with the slab, the cancellation of the surface charges induced by the oppositely charged ions leads to a virtual elimination of the excess electrostatic self-energy when the ions are close together. Thus it is seen that to separate the ions within the pore requires 6.6 times the energy required to overcome the direct interaction. The effect of the counterion clouds in bulk solution is to lower the energy of an ion below that of an isolated fully hydrated ion by reducing the electric potential at its surface (Lakshminarayanaiah 1984), so that the difference between the energy required to separate the ions in the pore and that in bulk solution is made even larger when counterion effects in the bulk solution are taken into account. As in the case of the slab the interaction within the pore is long range due to the distribution of the induced surface charge over an appreciable area of the pore wall. Even at a separation of 40 Å the effective electric field of one ion acting on the other is 4.24×10^7 V/m. To produce such a field at this distance due to a univalent ion in a bulk fluid would require that the effective dielectric constant of the fluid was less then 3. We have considered the attraction between univalent cations of opposite sign but the same arguments predict a similarly enhanced long-range repulsion between uni-

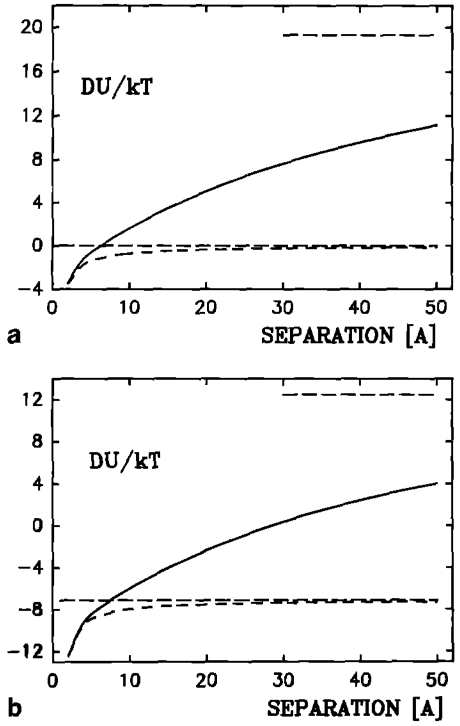

Fig. 2. a The full curve gives the electrical energy of a univalent cation-anion pair in units of kT as a function of their separation in Å units along the axis of a 10 Å diameter pore containing fluid with dielectric constant 80 surrounded by solid with a dielectric constant 2. The broken curve gives the interaction energy in bulk solution in the same units. The zero of energy is that of the two ions well separated and fully hydrated in bulk solution and is indicated by the lower broken straight line. The difference between the two curves gives the calculated excess energy of the pair of ions when within the pore. The upper broken straight line gives the total electrical energy of the pair at large separation within the pore. **b** The full curve gives the electrical energy in units of kT of a linear univalent anion-divalent cation-univalent anion triplet as one anion is removed along the pore axis. The broken curve gives the interaction energy due to the anion separation which would be experienced in bulk solution. The limit of the total electrical energy of the three ions with the anion well separated along the pore is given by the upper broken straight line

valent ions of the same sign the narrow pore. The calculated excess self-energy of a single ion in a narrow pore is inversely proportional to the pore diameter and proportional to the square of the ionic valence.

A neutral univalent ion pair once formed is only weakly attracted to other univalent ions through its dipole moment. A divalent cation is most strongly attracted to a divalent anion with 4 times the energies shown in Fig. 2a, but it can also form a stable neutral triplet with two univalent anions. As an example of this behaviour in Fig. 2b is plotted (full line) the electrical energy in units of kT of a linear array of univalent anion-divalent cation-univalent anion on the axis of the pore as one anion in moved along the axis of the pore away from the other two ions considered fixed. The distance of closest approach of neighbouring ions is taken as 2 Å. The direct interaction energy of the ions is shown as the broken curved line and the difference between the two lines is the extra energy of the ions when within the narrow pore. The broken straight line at -7.1 kT gives the direct interaction energy of the intact pair with the other anion remote and the upper broken straight line at 11.8 kT gives the total energy of the three ions when the third ion is far removed along the pore. It can be seen that the total excess energy of the three ions in close proximity is near zero so that the total energy approximates closely to the direct interaction energy. The forces tending to complete the neutral triplet are comparable with those tending to form a neutral univalent ion pair within the pore.

The calculations reveal the following properties of ions in narrow aqueous pores in material of lower electric polarizability.

(1) The ions are repelled by the pore walls.

(2) The total electrostatic energy of a pair of oppositely charged ions of the same valence in a narrow pore tends to the value of their total electrostatic energy in bulk solution when the charges approach one another. At larger separations the total electrostatic energy of the pair rises due to the increased electrostatic self-energy of the two ions when well separated.

(3) As a consequence of (2) the energy required to separate a neutral pair or neutral cluster of ions is many times larger in a narrow pore than in bulk solution.

(4) The attractive electric field between oppositely charged ions is much longer range in the pore than in bulk solution.

The simple calculations performed here are for pores with lengths much greater than their diameter and do not include end effects. In plastic track-etch filters such as Millepore filters, the diameter of the cylindrical pores may be as small as 10 Å and the length is typically 5 μm so that the length to diameter ratio may be 5000 and end effects are entirely negligible. For shorter biological pores, numerical calculations that include end effects (Levitt 1978) show that the excess self-energy is reduced in short pores, particularly towards the end of the pore. However for a pore of length 50 Å and diameter 10 Å with the same dielectric constants as we assumed above, the excess self-energy of each univalent ion in the centre of the pore is still 4.6 kT so that the energy required to separate a neutral univalent ion pair is still about 3.7 times as large as in bulk solution without considering counterion effects. As all ions that pass through the pore must pass through this central region the probable effects of the enhanced interaction which we discuss below, including ion pair formation, are present even when allowing for end effects.

Experimental evidence of the enhanced strengths of electrical interaction in narrow water-filled pores was obtained in a recent study of acid dissociation in the phoE porine channel (Gutman et al. 1992). The pore is elliptical in cross section (12 × 17 Å) at one end and cylindrical with a 10 Å diameter at the other. The chief conclusions were (i) that electric interactions within the pore are unusually strong and long range as if the dielectric constant of the water had been reduced and (ii) that proton mobility is reduced within the pore. Some of this may well attributable to the ordering of water in the pore (Edmonds 1984) but surface charge effects as discussed here must also play a role.

The probability of ion pair formation within narrow pores

Perhaps the most interesting consequence of the enhanced interaction between oppositely charged ions within narrow pores is the increased probability of neutral ion pair formation in narrow pores even with strong electrolytes which are normally fully dissociated in bulk water. Ion pair formation in bulk solution has been much studied both theoretically and experimentally (Robinson and Stokes 1970). An important parameter is the Bjerrum distance $\boldsymbol{q} = z_1 z_e \boldsymbol{e}^2/[2(4\pi \varepsilon_0 \varepsilon_f)\,\mathrm{kT}]$ at which the electrostatic interaction energy between the two ions is equal to 2 kT. Here z_1 and z_2 are the ion valences, $\boldsymbol{e}$ is the proton charge and ε_f is the dielectric constant of the bathing solution. The equilibrium dissociation constant $\boldsymbol{K}$ of the ion pair is strongly dependent upon the value of $\boldsymbol{q}$. In dilute solutions and to a first approximation, the dissociation constant is proportional to $\boldsymbol{q}^{-3}$. This is equivalent to a dependence of $\boldsymbol{K}$ on the third power of the dielectric constant of the fluid ε_f. This dependence of the dissociation constant upon the dielectric constant of the fluid has been studied for very dilute solutions of tetraisoamylammonium nitrate in dioxan-water mixtures (Fuoss and Kraus 1933). In water the salt is fully dissociated but by varying the liquid mixture the dielectric constant may be varied over a wide range. For fluid mixtures of dielectric constant 2.65, 8.5 and 38 the values of the dissociation constant $\boldsymbol{K}$ were found to be 10^{-14}, 10^{-4} and 0.25 respectively with many intermediate values. Throughout the range, the value of $\boldsymbol{K}$ predicted theoretically from the measured value of the dielectric constant is in excellent agreement with the value determined experimentally by measurement of the electrical conductivity. Even a strong electrolyte such as potassium bromide has a dissociation constant of only 1.1×10^{-7} in acetic acid which has a dielectric constant of 6.2 (Jones and Griswold 1954) and a dissociation constant of only 1.89×10^{-3} in ammonia which has a dielectric constant of 22 (Hnizda and Kraus 1949).

The data leading to Fig. 2a show that the attractive interaction between two univalent ions within the pore is considerably greater than 2 kT even when they are separated by a distance along the pore of 100 Å which is a very large Bjerrum parameter *q*. As shown above the enhanced interaction at large distances is as strong as in a bulk solution when the dielectric constant of the fluid is less than 3 so that the probability of ion pair formation under these circumstances must be high as judged by the results obtained in bulk solution.

Predicted consequences of ion pair formation in narrow pores

Theoretical predictions of the effects of ion pairing in bulk solution on experimentally determined properties such as electrical conductivity and surface tension are much simplified (Robinson and Stokes 1970) by the assumption of equilibrium, so that the electro-chemical potential of each type of carrier is the same at any location. During the rapid passage of ions and ion pairs through a narrow pore, equilibrium is unlikely and it is doubtful if a meaningful electro-chemical potential can be defined within the pore. For this reason it is more difficult to make a detailed theory of the effects of ion pair formation within narrow pores but some experimentally verifiable effects are predictable and they are listed below.

1. Although neutral overall, a pair of small ions will still have a large hydration energy (Parsegian 1969) and so, like a single ion, will partition strongly into the polarizable aqueous phase within a pore.
2. High selectivity for positive ions over negative ions or vice-versa will reduce the possibility of ion pair formation within a pore due to the small probability of finding both positive and negative ions at the same location within the pore.
3. The major differences between a neutral ion pair and a single ion are that the pair transports no current and that it is not driven by a voltage gradient, leading to reduced ion flow through a pore when voltage driven and much reduced current flow whether it is driven by a voltage or a concentration gradient. Because of the inverse dependence of the excess self-energy on the diameter of the pore and the absence of adequate counterion screening in narrow pores, the tendency toward pair formation becomes stronger the narrower the pore.
4. Because of 3. above the ion flux through a narrow pore as measured by radioactive ion tracer will be greater than that expected from the current flow.
5. Changes in voltage and ionic concentration that resulted in equal changes in the electrochemical potential of a given ion between the ends of the pore will not result in equal changes in the measured flux of that ion.
6. Although they may impede flow, ion pairs or neutral triplets are unlikely to cause physical blockage of a pore as there is no self-energy barrier to leaving the pore provided they do so intact and then dissociate in the bulk solution. A major barrier to transfer of the pairs is likely to be their initial entry as single ions into the pore.
7. The ratio of the unidirectional fluxes J_{12} and J_{21} of one particular type of ion with valence z passing through a pore when the voltage across the pore is V_{12} may be written

$$J_{12}/J_{21} = [(C_1/C_2)\exp(z\,e\,V_{12}/\mathrm{kT})]^n ,$$

where C_1 and C_2 are the ionic activities at the ends of the pore, e is the proton charge and n is known as the flux ratio exponent. Ussing (1949) showed that if the ions do not interact within the pore $n = 1$, and Hodgkin and Keynes (1955) showed that if there is interaction between the particular ions within the pore leading to single file transfer, n may be greater than 1. For a pore containing neutral ion pairs, because the voltage gradient does not act as a driving force upon the neutral pairs, a flux ratio exponent n less than 1 is possible. Such a flux ratio exponent has in the past been considered to be the characteristic of a carrier and not of a pore. Previously we have shown (Berry and Edmonds 1992) that a value of n less than 1 is possible in a pore in which the transient ion interacts with the electrical structure of the pore. Here we have shown that due to ion pair formation, values of n less than 1 are possible even in a passive narrow pore so that the use of the measured flux ratio exponent to distinguish between a carrier and a pore is without foundation.

Conclusions

An ion passing from aqueous solution into a narrow water-filled pore in material of low electric polarizability experiences a large rise in its electrostatic self-energy. The effect may be considered to be due to the interaction between the ion and the bound polarization charge induced at the wall of the pore by the electric field of the ion. This same induced charge results in a strong enhancement of the strength of electrostatic interactions between ions in narrow pores and an increase in their range. Like ions will repel each other more strongly than in bulk solution and ions of opposite charge will strongly attract each other leading to the probability of ion pair formation even between ions that are fully dissociated in bulk solution. Some of the consequences of neutral ion pair formation in narrow pores which are experimentally verifiable are listed.

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