Electrostatic Exclusion of Neutral Solutes from Condensed DNA and Other Charged Phases

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ABSTRACT   Motivated by experiments on condensed DNA phases in binary mixtures of water and a low-dielectric solute, we develop a theory for the electrostatic contribution to solute exclusion from a highly charged phase, within the continuum approximation of the medium. Because the electric field is maximum at the surface of each ion, the electrostatic energy is dominated by the Born energy; interactions between charges are of secondary importance. Neglecting interactions and considering only the competition between the Born energy and the free energy of mixing, we predict that low dielectric solutes are excluded from condensed DNA phases in water-cosolvent mixtures. This suggests that the traditional continuum electrostatic approach of modeling binary mixtures with a uniform dielectric constant needs to be modified. The linking of solute exclusion to solute dielectric properties also suggests a mechanism for predicting the electrostatic contribution to preferential hydration of polar and charged surfaces.

INTRODUCTION

Because of their abundance, non- or weakly-interacting solutes can strongly influence biological reactions. Emphasizing excluded volume, these solutes could be said to "crowd" the solution, so as to push reactions toward more compact states (1). Typically, this stabilizes the folded structures of proteins and promotes intermolecular association. A solute may also have lower affinity for biomolecular surfaces, when compared to water. This "preferential hydration" creates an additional region of exclusion that enhances crowding effects (2). Often times it is useful to switch perspective from the solute to the solvent and think in terms of the solution osmotic pressure. Thus, crowding and preferential hydration can equivalently be described as "osmotic stress" (3). In any case, a key property that contributes to the effects of non- or weakly-interacting solutes on biochemical reactions is their nonuniform spatial distribution around reactants and products.

In charged systems, solutes are often treated in a completely different way. Here, added solute is assumed to distribute itself uniformly throughout the solution and modify electrostatic interactions via a uniform change in dielectric constant (4–9). This assumption of uniformity precludes the previously mentioned crowding or osmotic effects and consequently this electrostatic perspective has been viewed as mutually exclusive from the osmotic stress perspective.

Perplexingly, cosolute experiments on charged systems such as condensed DNA show hallmarks consistent with both an electrostatic (4–9) and an osmotic (10–12) interpretation. Motivated by this observation, we sought a link between the electrostatic influence of neutral solutes and their exclusion from highly-charged phases. We find that, precisely because a neutral solute modifies electrostatic interactions, it is excluded. This suggests that the electrostatic and osmotic stress perspectives, previously seen as mutually exclusive, are intrinsically linked.

In this article, we first demonstrate that the main electrostatic contribution to solute exclusion comes from the Born energy. We then use this to simplify the electrostatic free energy and obtain approximate analytical solutions that capture the main electrostatic effect on solute exclusion. Finally, using parameters from experiments on condensed DNA phases in binary mixtures of water and cosolute, we show that electrostatic exclusion of neutral solutes is likely significant under practical experimental conditions.

Dominance of the Born energy

In an ionic system, the total electrostatic energy consists of the Coulomb interactions plus the Born energies of all the ions. The Born energy or self-energy is the energy required to create charge on an ion within a particular environment. Analogous to the energy required to charge a capacitor, the Born energy for an ion depends on the polarizability of the medium in which it resides. In this section, we demonstrate that the Born energy dominates the electrostatics of solute exclusion and that Coulomb interactions are, to a first-approximation, negligible. Our argument is based on a system of only two charges; however, because of superposition, the result generalizes to an arbitrary charge distribution.

For simplicity, we assume that the two charges have the same magnitude, $|q_1| = |q_2|$ and the same radii a. In this case, the total electrostatic energy relative to infinite separation in vacuum E may be written as

$$E = \frac{q_1^2}{2a}\left(\frac{1}{\varepsilon} - 1\right) + \frac{q_2^2}{2a}\left(\frac{1}{\varepsilon} - 1\right) + \frac{q_1 q_2}{\varepsilon R}, \qquad (1)$$

where ε is the dielectric constant of the medium and R is the distance between the two charges. The first two terms are the

Born energies and the third term is the Coulomb interaction. To consider how the system energy changes with the dielectric constant of the medium, we consider $dE/d\varepsilon$,

$$\frac{dE}{d\varepsilon} = \frac{1}{\varepsilon^2}\left(-\frac{|q_1 q_2|}{a} - \frac{q_1 q_2}{R} \right). \quad (2)$$

The first term comes from the Born energies and is always negative, indicating that Born energy always decreases with increasing dielectric constant. In thermodynamic terms, the Born energy always favors the higher dielectric environment. The second term comes from the Coulomb interaction and its sign can be either positive or negative depending on the signs of the two charges. Thermodynamically, the interaction energy can favor either a high or a low dielectric environment depending on whether charges have the same or the opposite sign. However, because the ion separation R is always larger than twice the ion radius $2a$, the Born energy is always at least 50% larger than the interaction energy. This dominance of the Born energy is a consequence of the large electric field at the ion surface. Because of this, and regardless of the nature of ion interactions, the net electrostatic effect will always be to exclude low-dielectric solutes from highly-charged phases. Conversely, the electrostatic effect tends to include high-dielectric solutes in highly-charged phases.

Extent of exclusion

We consider exclusion of the low dielectric component of a binary mixture from a highly charged phase (Fig. 1). Following the argument above, we neglect the electrostatic interactions between charges and all nonelectrostatic interactions. In condensed DNA, these interactions are indeed small (~0.1 $k_b T$ per charge at equilibrium) (13,14), although they can become increasingly large when DNA is squeezed closer together (15). For simplicity, we also assume that the dielectric constant within each phase is uniform, that solvent and solute have equal molar volume, and that each phase is large enough to neglect boundary effects. Finally, the bulk phase is assumed to be much larger than the high-charge density phase so that changes in the composition of the high-density phase do not affect the composition of the bulk. Under these assumptions the only components of the total free energy are: 1), the Born energy, which tends to exclude low-dielectric solute; and 2), the mixing free energy, which tends toward perfect mixing. This model is adopted to capture the main electrostatic effect on solute exclusion in the simplest manner possible and precise quantitative predictions should not be taken seriously.

The solvent-cosolute mixing free energy density in the high-charge density phase is given by (16),

$$\Delta G_{\text{mix}} = N k_b T \left(\chi \ln \frac{\chi}{\chi_B} + (1-\chi) \ln \frac{(1-\chi)}{(1-\chi_B)} \right), \quad (3)$$
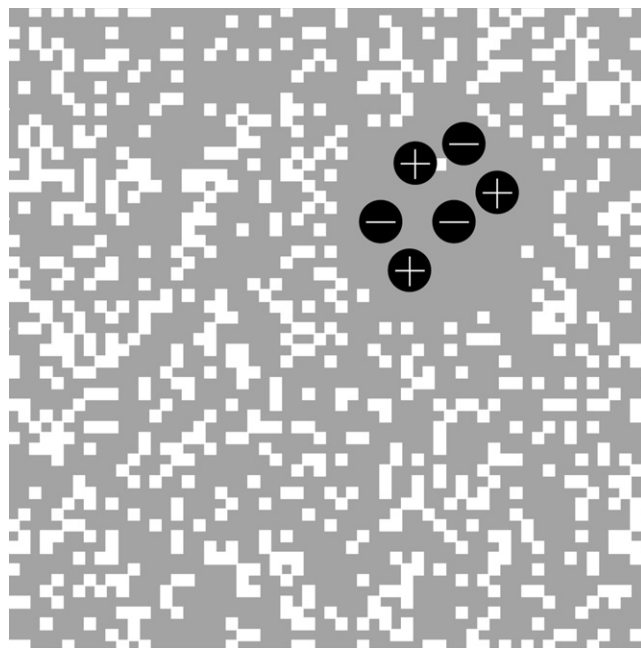
FIGURE 1 We consider a binary mixture containing a low-dielectric solute (*white squares*) and a phase with a high density of ions (*black circles*). The mole fraction of the low-dielectric component in the high-charge density phase will be reduced from the bulk value because of the higher density of Born energies in the high-charge density phase. We estimate the order of exclusion using an approximate analytical theory.

where N is the number density of solvent and solute molecules. The mole fraction of the low-dielectric component in the high-charge-density phase χ will deviate from its value in the bulk χ_B because of the Born energy of the high-density phase (16),

$$\Delta G_{\text{Born}} = \frac{n}{a}\int_0^e \frac{q}{\varepsilon}\,dq, \quad (4)$$

where n is the number density of charges, a is the radius of the ions, e is the elementary charge, ε is the χ-dependent dielectric constant in the high-charge-density phase, and q is charge. The dielectric constant of a binary mixture depends linearly on the weight fraction of components (17) and, for small mole fraction, can be written $\varepsilon = \varepsilon_1(1 - \beta\chi)$. The value ε_1 is the dielectric constant of pure solvent and $\beta = (\varepsilon_2 - \varepsilon_1)/\varepsilon_1 \times MW_2/MW_1$. The value ε_2 is the dielectric constant of pure solute and MW_1 and MW_2 are the molecular weights of components of solvent and solute, respectively. Solute and solvent are also assumed to have equal mass densities so that molar volume scales as molecular weight.

In general, the integral in Eq. 4 needs to be carried out while χ is varied to minimize the total free energy. If we consider the limit $\chi_B \to 0$, however, the dielectric constant will change a vanishingly small amount and can be assumed constant. Integrating Eq. 4 then gives
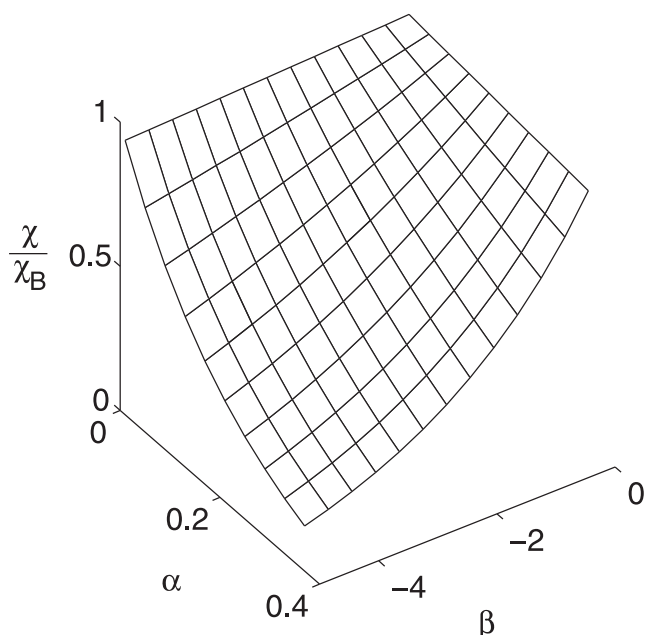
FIGURE 2 Fractional exclusion of the low-dielectric component χ/χ_B in the limit $\chi_B \to 0$. The mesh surface shows the dependence of χ/χ_B on the two dimensionless system parameters α and β, in general.

$$\Delta G_{Born}\Big|_{\substack{\lim \\ \chi_B \to 0}} = \frac{Ne^2}{2a\varepsilon_1(1 + \beta\chi)}. \tag{5}$$

Thus, the minimum of the total free energy, $\Delta G = \Delta G_{mix} + \Delta G_{Born}\big|_{\substack{\lim \\ \chi_B \to 0}}$, defined by $d\Delta G/d\chi\big|_{\substack{\lim \\ \chi_B \to 0}} = 0$, is given by the solution to

$$0 = \ln\frac{\chi(1 - \chi_B)}{\chi_B(1 - \chi)} - \frac{\alpha\beta}{1 + \beta\chi}\Big|_{\substack{\lim \\ \chi_B \to 0}}, \tag{6}$$

with

$$\alpha = \frac{e^2}{2\varepsilon_1 a}\frac{1}{k_b T}\frac{n}{N}, \tag{7}$$

$$\beta = \frac{\varepsilon_2 - \varepsilon_1}{\varepsilon_1}\frac{MW_2}{MW_1}. \tag{8}$$

Fig. 2 shows how the fractional exclusion χ/χ_B varies as a function of the two dimensionless system parameters, α and β, in the limit $\chi_B \to 0$.

Comparison to experiments on condensed DNA

To determine whether electrostatic exclusion of solutes might be significant under practical conditions, we compare predictions of Eq. 6 to experimentally measured solute exclusion from condensed DNA phases (12). In these experiments, fractional exclusion was quantified as an equivalent number of waters per basepair that completely exclude the solute, N_w. Following Stanley and Rau (12), we estimate

the number densities, N and n, on a per-basepair basis. The experiments use ~1 M concentrations of solute in ~55 M water, so it is reasonable to approximate the number of solvent and solute molecules N from the number of water molecules in the void volume. We estimate a void volume of 1580 $\mathring{A}^3$ from the space between hexagonally packed 1-nm radii cylindrical DNA separated by a typical interhelical spacing of 30 $\mathring{A}$ (12). Using the 30 $\mathring{A}^3$ molar volume of water, this gives a total number of waters per basepair in the condensed phase of N ~53. The fractional exclusion of each solute is then given by

$$\frac{\chi}{\chi_B} = 1 - N_w/N, \tag{9}$$

where N_w is taken from Table I in Stanley and Rau (12). The value β for each solute is calculated from Eq. 8 using standard values for the dielectric constant of pure solute (18) to give the symbols in Fig. 3.

To make a theoretical prediction for fractional exclusion, we need an estimate for α. The most uncertain parameter required to calculate α is the ion radius a, which we nominally take as 2 $\mathring{A}$. Setting ε_1 to the dielectric constant of pure water, we have $e^2/(2\varepsilon_1 a) = 1.8\ k_b T$. This indicates that, in water, α will be roughly equal to the number of
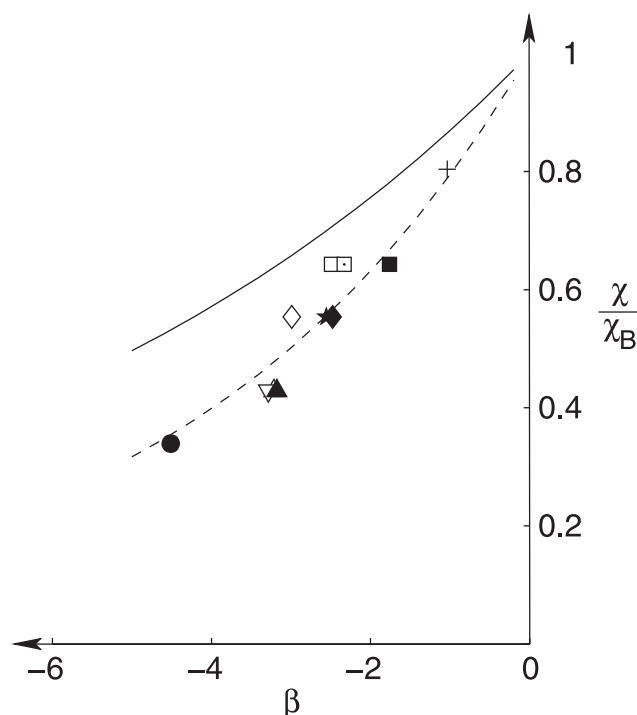

FIGURE 3 Predicted exclusion (*lines*) versus experimentally measured exclusion for condensed DNA (*symbols*). Experimental measurements from Table I in Stanley and Rau (12) are +, methanol; ■, ethanol; □, 1,2-propanediol; *, 1,3-propanediol; ◆, 1-propanol; ★, 2-propanol; ◇, 1,4-butanediol; △, 1-butanol; ▽, 2-butanol; ▲, t-butanol; and ●, MPD. The solid line is a nominal a priori prediction based on $\alpha = 0.13$. The dashed line is a best-fit with $\alpha = 0.23$ as the single free parameter.

charges per basepair n divided by the number of water molecules per basepair N. For DNA, there are two phosphate charges and two counterion charges per basepair, so $n = 4$ and $\alpha \sim 0.13$. The predicted exclusion, plotted as the solid line in Fig. 3, is ~40% smaller than the measured exclusion. If instead of estimating α, we allow it to vary to best-fit the data, we get $\alpha \sim 0.23$ (Fig. 3, *dotted line*). We have also compared the change in exclusion as the void volume is reduced and compared this to the distance dependence for exclusion measured in Stanley and Rau (12) (data not shown). Predicted exclusion decays approximately exponentially over the experimental range of interhelical distances, as in the experiments, but the theoretical decay constant at ~7.7 Å is twice the experimentally observed value. We conclude that the predicted electrostatic exclusion of neutral solute is of similar order to the measured exclusion of nonpolar alcohols from condensed DNA.

DISCUSSION

The effects of weakly interacting solutes on biological reactions are frequently ascribed to either a change in dielectric constant (4–9) or solute exclusion (10–12). We have pointed out that these two phenomena are intertwined and that, precisely because a solute lowers the local dielectric constant of a solution, it will be preferentially excluded from regions of high-charge density. This indicates that cosolutes should be expected to affect biological reactions through a common mechanism involving the dielectric constant and solute exclusion.

The model that we developed captures only the main electrostatic contribution to solute exclusion, within the continuum approximation of the medium. This main effect is due to the Born energy. Within the continuum electrostatic picture, we demonstrated that the interactions between charges can contribute a maximum of 1/3 of the total electrostatic energy, when ions are close-packed. In reality, aqueous polyion phases are often diffuse, the charges several ionic radii apart, and the electrostatic interactions will contribute $\ll 1/3$ of the total energy of exclusion. Ionic screening further reduces the magnitude of electrostatic interactions and would further decrease the magnitude of interactions between charges. In condensed DNA phases, for instance, experimentally measured interaction free energies are ~0.1 k_bT per phosphate charge, at equilibrium (13,14). For comparison, the Born energy for transferring one ion from water to pure ethanol is ~4 k_bT. The dominance of the Born energy leads to the fortuitous simplification that the electrostatic interactions can be neglected when considering the electrostatic contribution to solute exclusion. This simplification is our primary result and is important because predictions for electrostatic interactions in soft-condensed matter systems, even well-studied systems like condensed DNA, remain controversial and uncertain (13,19).

A number of refinements in our model for calculating the electrostatic contribution to solute exclusion could be imagined and some work on this was done in the context of a different application (20–23). We have not done so for pedagogical reasons and because fundamental uncertainties in electrostatic interactions in aqueous solution would limit the physical significance of these refinements. For example, it would be straightforward to generalize our approach of taking the mean extent of exclusion within the charged phase by calculating a position-dependent exclusion around each charge. However, the real distance-dependent exclusion present in experiments is likely convoluted with a distance-dependent polarizability for water itself (24). This distant-dependent polarizability is poorly understood and cannot be accurately predicted at this time. Because of these uncertainties, our conclusion that electrostatics exclusion of low dielectric solutes can significantly change the composition of charged phases, is probably the most precise statement possible at this time.

The simplification that electrostatic interactions are negligible facilitates calculations of the electrostatic contribution to solute exclusion. However, solute exclusion cannot be predicted from electrostatics alone. Other contributions—in particular, solute packing and hydrogen bonding—are also known to be important in the solvation of charged species (25). For example, the ~4 k_bT Born energy predicted for the transfer of a Cl^- ion from water into pure ethanol accounts for only half of the experimentally measured transfer energy of 7.7 k_bT (25). Similarly, we finding that the Born energy predicts roughly half of the total experimentally measured exclusion of ethanol from condensed DNA phases (Fig. 3). The other half is likely contributed by hydrogen bonding or solvent packing that is outside the purview of our continuum electrostatic model. The ability of our model to capture differences between different nonpolar alcohols (Fig. 3) may be a consequence of capturing, reasonably accurately, the variation in electrostatic exclusion against a background of constant nonelectrostatic exclusion. However, for many solutes such as glycerol, sorbitol, and threitol, there is no correlation between extent of exclusion from ionic phases and molar dielectric increment (11,26). Here, the electrostatic contribution to solute exclusion is likely very small and our model—or any exclusively continuum electrostatic model (20–23)—will fail to accurately predict solute exclusion.

Finally, we note that many biological molecules have a region near their surfaces that is preferentially hydrated (2). Because most solutes have lower molar polarizability than water, our model suggests that preferential hydration could be, in part, a consequence of the electrostatic preference of charged or polar biomolecular surfaces for a high dielectric environment. Conversely, molecules with positive dielectric increments, such as glycine, are predicted to be enriched around polar and charged surfaces. More accurate elaborations of our theory may therefore be generally useful for predicting the partitioning of solution components in heterogeneous biological solutions.

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