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Mathematical Modeling of the Concentration Dependence of Competitive Binding of Counterions in Polyelectrolytes

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ABSTRACT: In a previous study by ^{23}Na NMR, the preferential binding of Mg^{2+} over that of Na^+ in the system Mg^{2+}/Na^+/polyion, polyion = poly(galacturonate), was found to increase with the decrease in concentration of this mixed salt. The relative suppression of the binding of Na^+ by the presence of Mg^{2+} as its competitor was found to be linearly related to the ratio $P_{b,Mg}/P_{b,Na}$ where $P_{b,Mg}$ and $P_{b,Na}$ are the numbers of counterions per unit charge associated with or bound to the polyanion, in the pure (unmixed) neutral magnesium and sodium salts of the electrolyte, respectively, as calculated from the relationships based on the Poisson–Boltzmann (PB) model. The present work is a determination as to whether such a linear relationship is predicted by mathematical formulation of the PB model.

Introduction

Competitive binding in mixed counterion systems has been extensively studied by using ^{23}Na NMR in several polyelectrolyte systems.[1-3] These studies were performed in the presence of excess salts, a condition that, judging from considerable experimental evidence,[4] is well described by Manning's model.[5] Manning's theory is formulated in terms of the dimensionless charge-density parameter, ξ, defined by

$$\xi = e^2/4\pi kTl\epsilon_0\epsilon_r \qquad (1)$$

where e is the elementary charge, l the projection of the intercharge distance when the polyelectrolyte is considered as a cylinder, k Boltzmann's constant, T the kelvin temperature, ϵ_0 the permittivity in vacuum, and ϵ_r the dielectric constant of the solvent. The charge fraction of the polyion, that is, the charge per ionic group of the polyelectrolyte, equals the constant value of $(z\xi)^{-1}$, z being the valence (without sign) of a given counterion. Thus the number of counterions associated with or "atmospherically condensed" onto the polyanion per unit charge, P_b, is given by

$$P_b = 1 - (z\xi)^{-1} \qquad (2)$$

and is independent of concentration, excess salt, and radial dimension of the polyelectrolyte.

It appears now that territorial binding of counterions to polyanions when studied by NMR in the absence of salts is better described by the Poisson–Boltzmann (PB) model.[6] This was clearly demonstrated by the ^{23}Na NMR studies of sodium poly(galacturonate), poly(mannuronate), and poly(galuronate) in aqueous solutions by Grasdalen et al.[7] These authors extracted the correlation times, τ_c, from the spectral line shapes, which become distinctly non-Lorentzian for τ_c exceeding 0.5 ns. Using the concentration dependence of τ_c, they showed that the data were consistent with the (PB) model and a theory developed by Halle et al.[8] for the quadrupolar relaxation of counterions, as testified by the relative constancy of the ^{23}Na quadrupolar coupling constant at different polyelectrolyte concentrations, the latter being calculated from the mathematical formulation of this model, using certain simplifying assumptions and reasonable dimension parameters. The two additional parameters required for such calculations are a and Δ. The parameter a is the radius of the cylinder, which approximates the dimensions of the polyion, and Δ is the thickness of the region in which the counterions are considered to be "atmospherically or territorially bound" to the polyanion. The distance Δ is usually chosen to approximate the diameter of a given hydrated ion.[7] Also, P_b in the PB model is no longer concentration independent since another parameter R is used in its calculation. R is the radius of a cylindrical cell whose dimensions are chosen so as to give the correct overall concentration of the polyion, c_m (molar, on a monomer basis). It is thus related to concentration by the relation

$$R = (\pi lc_mN \times 1000)^{-1/2} \qquad (3)$$

where N is Avogadro's number and l has been previously defined. Thus, since P_b for each ion is also dependent on its charge, the competitive binding between the counterions of different valency would be expected to be concentration dependent in the absence of salts. This was indeed experimentally observed by Qian et al.,[9] who estimated the relative suppression of the binding of Na^+ by the presence of Mg^{2+} in the mixed-salt system Mg^{2+}/Na^+/polyanion, polyanion = poly(galacturonate) (PG), in the absence of excess salt, to be strongly concentration dependent. The relative suppression of the binding of Na^+ ion by the presence of an equivalent amount of ion M in the mixed salt, also in the absence of excess salt, was mathematically defined in this work as Γ_M:

$$\Gamma_M = X_B(M/Na)/X_B(Na) \qquad (4)$$

where $X_B(M/Na)$ is the actual or experimental fraction of sodium ion territorially bound in the mixed salt M/Na^+/polyanion and $X_B(Na)$ the actual or experimental fraction of sodium ion bound in pure sodium salt Na^+/polyanion at the same concentration in aqueous solution. Γ_{Mg} in the poly(galacturonate) system was estimated from the ^{23}Na NMR line widths at half-height using the following relation, which is based on a two-site model:[1-3,6]

$$\Delta\nu_{1/2} = (1 - X_B)\Delta\nu_{1/2,F} + X_B\Delta\nu_{1/2,B} \qquad (5)$$

where $\Delta\nu_{1/2,F}$ and $\Delta\nu_{1/2,B}$ are the line widths that characterize the free and the bound states, respectively, and X_B is the mole fraction of bound sodium ions, while $\Delta\nu_{1/2}$ is the experimentally observed line width. Assuming that the presence of a competing ion does not affect the value

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of $\Delta\nu_{1/2,B}$ for any given concentration, by measuring $\Delta\nu_{1/2}$ for a mixed salt of the polyion, which is denoted by $\Delta\nu'$, and the corresponding $\Delta\nu_{1/2}$ for the pure salt of it at the same concentration, $\Delta\nu$, one can determine Γ_M by combining eq 4 with eq 5

$$\Gamma_M = \frac{\Delta\nu' - \Delta\nu_{1/2F}}{\Delta\nu - \Delta\nu_{1/2,F}} \tag{6}$$

and estimating $\Delta\nu_{1/2,F}$ from a salt of NaCl in aqueous solutions, at the same temperature.

It was found that the experimentally determined Γ_{Mg} decreased monotonically and dramatically with the decrease in concentration but that there was no simple functional relationship between Γ_{Mg} and c_m. However, what appeared to be a linear trend was observed between Γ_{Mg} and a calculated parameter κ, which was defined as the ratio

$$\kappa = P_{b,Mg}/P_{b,Na} \tag{7}$$

where $P_{b,Mg}$ and $P_{b,Na}$ are the calculated P_b's for pure magnesium and sodium poly(galacturonate), respectively. It should be noted that the symbol $P_{b,Na}$ as used in this paper differs in meaning from X_b in eq 4, the former being the theoretically calculated and the latter the experimental or actual fraction of territorially bound sodium in the absence of excess salt. The rationale for making the plot of Γ_{Mg} versus κ was that $P_{b,Mg}$ and $P_{b,Na}$ should reflect separately the bonding affinities of Mg^{2+} and Na^+, that κ could be viewed as the extent to which Mg^{2+} competes for bonding with Na^+, and that therefore one would expect the lowering of Γ_{Mg} with the increasing κ. The authors could not determine whether the linear relationship between Γ_{Mg} and κ was fortuitous or whether it was the result of the mathematical formulation of the PB model, since the exact solution for Poisson–Boltzmann equation is presently known only for one counterion of a given valency. However, a numerical method of solving the problem of two ions of different valency in any given ratio of equivalents is available, and the present work deals with the application of this numerical method to the problem of the relationship between Γ_{Mg} and κ.

Mathematical Model

The mathematical model used in this work was essentially the one devised by Dolar and Peterlin.[10] These authors started with the same assumptions of the (PB) theory that are still in use today[6-8] and applied them to a strong polyelectrolyte containing mono- and divalent ions only in the absence of excess salts. For this case, the Poisson–Boltzmann equation reads

$$\frac{1}{r}\frac{d}{dr}\left(r\frac{d\Psi}{dr}\right) = -\frac{4\pi e}{\epsilon}\left[n_1{}^0 \exp\left(\frac{-e\Psi}{kT}\right) + 2n_2{}^0 \exp\left(\frac{-2e\Psi}{kT}\right)\right] \tag{8}$$

where Ψ is the electrostatic potential; r is the distance from cylinder axis; $n_1{}^0$ and $n_2{}^0$ are the numbers of mono- and divalent counterions per unit volume at $\Psi = 0$, respectively; and other constants have been previously defined in the introduction. Using the usual boundary conditions, these authors transformed eq 8 into an integral equation whose approximate solution was found by using a quadrature method based on Lagrange interpolation through three consecutive points. The interval from a to R was divided into 40 subintervals by using 41 points. The values of Ψ (expressed as was done in ref 10 by dimensionless $\phi = e\Psi/kT$) at these 41 points were estimated by solving a set of 41 nonlinear equations by the Newton–Raphson method. Iteration was continued until the increments in the ϕ were less than 0.0001.

Once a solution for the electrostatic potential for each of the two ions (a set of Ψ values coresponding to 41 points mentioned above) was obtained for any given set of input parameters and for any arbitrary pair $(N_1{}^0, N_2{}^0)$, the latter being defined by

$$N_1{}^0 = n_1{}^0/(n_1{}^0 + 2n_2{}^0), \qquad N_2{}^0 = 1 - N_1{}^0 \tag{9}$$

the fraction of either ion bound to the polyelectrolyte could be obtained from

$$P_b = \frac{\int_a^{a+\Delta} \exp(-e\Psi/kT) r \, dr}{\int_a^R \exp(-e\Psi/kT) r \, dr} \tag{10}$$

by numerical integration. In eq 9, Δ is the thickness of the region where the ions are considered "bound" to the polymers.

The way the program is constructed for any given pair $(N_1{}^0, N_2{}^0)$, one obtains the corresponding pair (N_1, N_2) defined by

$$N_1 = n_1/(n_1 + 2n_2), \qquad N_2 = 1 - N_1 \tag{11}$$

where n_1 and n_2 are the average numbers of counterions with single and double charge, respectively, per unit volume. Varying $(N_1{}^0, N_2{}^0)$ pairs over a range of values and obtaining the corresponding (N_1, N_2), a table of (N_1, N_2) pairs with the corresponding P_b values for each ion can be constructed and P_b corresponding to any N_1 determined by cubic spline interpolation. N_1 represents the equivalent mole fraction of the singly charged ion in a mixed salt before it is placed in solution. Thus the program allows one to calculate the theoretical P_b for both ions for any given composition in the mixed salt, for any concentration, and for any set of input parameters.

We have now made this program operational after having performed the following tests:

1. With the same parameters for the structure of poly(sulfonic acid) that were used by Dolar and Peterlin, their calculations were reproduced within the precision of the graphical representation of their results.

2. A set of initial guesses for the values of the Ψ function at each of the 41 points was used as in Dolar and Peterlin. We have used for this initial guess the exact solution for Ψ for either one of the two ions when present alone ($N_1 = 1$ or $N_2 = 1$), which is given by[7]

$$\Psi(r) = \frac{2kT}{ze} \ln\left(\frac{r}{R}\left(\cos\left(s \ln \frac{R}{r}\right) + \frac{1}{s} \sin\left(s \ln \frac{R}{r}\right)\right)\right) \tag{12}$$

where the dimensionless parameter s is the smallest positive solution to the transcendental equation

$$z\xi = \frac{1 + s^2}{1 + s \cot\,[s \ln (R/a)]} \tag{13}$$

In the case $N_1 = 0.5$, the values of the Ψ function at each subdivision converged to the same numbers, irrespective of whether the initial guess was made using the monovalent or divalent ion alone. Furthermore, it was found that any arbitrary set of reasonable Ψ used as the original guess would result in the same convergence, albeit with longer computer time.

3. In the limits of $N_1 = 1$ and $N_2 = 1$, the values obtained for P_b of the mixed salt calculated by using this

Table I
Theoretical and Experimental Γ_{Mg} in Mixed (Mg/Na) PG at Different Concentrations

	Γ_{Mg}			Γ_{Mg}	
c_m, mM	theor	exptl	c_m, mM	theor	exptl
50	0.719	0.86	8.3	0.520	0.61
25	0.640	0.76	4.2	0.451	0.55
16.7	0.593	0.69			

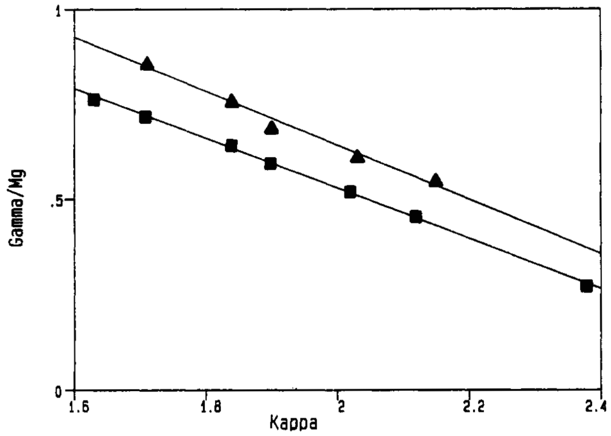

Figure 1. Dependence of the theoretical and experimental ratio of the amount of Na^+ territorially bound in a mixed neutral salt of poly(galacturonate) containing Na^+ and Mg^{2+} in equivalent amounts to the Na^+ bound in pure sodium poly(galacturonate); Γ_{Mg} (as defined by eq 4) versus the ratio $\kappa = P_{b,Mg}/P_{b,Na}$, where $P_{b,Mg}$ and $P_{b,Na}$ are the number of counterions per unit charge calculated by eq 14 bound in pure magnesium and sodium poly(galacturonate), respectively, at the same concentrations. Symbols: (■) theoretical; (▲) experimental.

program were in complete agreement to four significant figures with the exact solution for both ions given by[6]

$$P_b = 1 - \frac{1}{z\xi}\left[1 - \frac{1 - s\tan\left[s\ln\dfrac{R}{(a+\Delta)}\right]}{1 + (1/s)\tan\left[s\ln\dfrac{R}{(a+\Delta)}\right]}\right] \tag{14}$$

4. In order to test whether the 41 points were sufficient to obtain results of acceptable precision, Γ was calculated at $N_1 = 0.5$ and $c_m = 0.50$ mM by using 21, 31, 41, 51, and 61 points. The calculated Γ's were in agreement to four significant figures over this range of numbers of points. Thus, the use of 41 points is more than adequate, because the precision of the experimental Γ's is ±0.03.

This program, which is written in Fortran WATFIV, is available upon request through BITNET; CMRIEDL, Clemson. Double precision was used and the machine was NAS AS/XL-V60 with a Fortran WATFIV compiler.

Results of Numerical Calculations

The set of parameters that was used is the same as in ref 9 for the calculation of κ in the poly(galacturonate) system. Table I lists the theoretical values of Γ_{Mg} numerically calculated for different concentrations, c_m, of the mixed salt, using the same parameters, as well as the corresponding values of Γ_{Mg} obtained experimentally from the ^{23}Na NMR line width measurements in ref 9.

Both theoretical and experimental Γ_{Mg} are plotted versus κ in Figure 1. As can be seen, the plot of theoretical Γ_{Mg} versus κ is very nearly linear. There is but a very slight curvature, the best linear fit resulting in the slope of −0.660 with a standard deviation of 0.010. The best linear fit of the plot of the experimental Γ_{Mg} versus κ is very close to being parallel to the theoretical one, the slope being −0.710 with a standard deviation of 0.042. Thus, there is a remarkable agreement between the trends in the theoretical and experimental competition parameters in this system containing the monovalent and divalent ions in a 1:1 equivalent ratio, as related to concentration via the calculated parameters, κ.

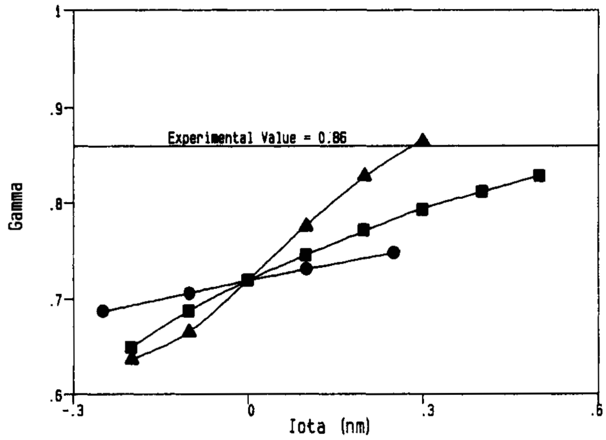

Figure 2. Plots of theoretical Γ_{Mg} for c_m = 50 mM versus incremental changes (I) one at a time in parameters l, a, and Δ, the intersection point in the plots representing the values of these parameters listed in Table I. Symbols: (▲) l; (■) a; (●) Δ.

As to the absolute magnitudes, the theoretical Γ_{Mg} are about 17–20% lower than the experimental. Since the selection of parameters a and Δ may involve some arbitrariness, in fitting the noncylindrical molecule to idealized cylindrical dimensions, we have carried out a study of how the change in various parameters would affect the theoretical Γ_{Mg}. Starting with the set of parameters used in ref 9, we have systematically varied them, one parameter at a time, by small increments in nanometers, which we denote by I (positive I meaning positive increment and vice versa). The results of these studies are exemplified in Figure 2 by plots of calculated Γ_{Mg} versus I, for l, a, and Δ, at one given concentration, c_m = 50 mM. These plots clearly demonstrate that it is not the inaccuracy of selection of either one of the above-mentioned parameters that results in the discrepancy between the absolute magnitudes of theoretical and experimental Γ_{Mg}, since in order for the theoretical values of Γ_{Mg} to converge to the experimentally observed value of 0.86 the parameters l and a would have to be increased by unrealistic amounts, incompatible with the dimensions of the poly(galacturonate) model.[11]

As to parameters Δ_1 and Δ_2, the value of Γ_{Mg} is clearly insensitive to their incrementation; and increasing both of them by as much as 0.25 nm leads to P_b of 0.42, which is a value greater than 0.39 predicted by Manning's theory, whereas in the absence of excess salts P_b is estimated to be lower than this limiting value. Thus, apart from the fact that there are several simplifying assumptions in the PB model[6] and that complete correspondence between the theory based on it and actual experimental results cannot be expected, other factors must be considered that may be responsible for the difference in magnitude between theoretical and experimental Γ_{Mg}. Superimposed on territorial binding there may be other effects such as specific interactions of the two chemically different ions with the polyanion and with each other that are not accounted for by the PB theory.

Despite the discrepancy between the theoretical and experimental Γ_{Mg}, the near linear dependence of Γ_{Mg} on κ at an appropriate N_1 may eventually serve as a useful test as to whether the binding of selected pairs of counterions to a polyion is basically territorial. However, such tests should be limited to certain equivalent ratios of the

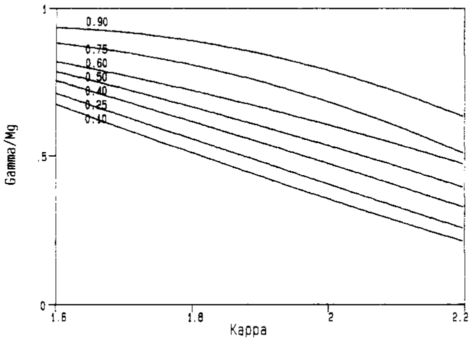

Figure 3. Plots of theoretical Γ_{Mg} versus κ at different N_1 values, using the values of parameters listed in Table III. The values of N_1 are indicated on the curves.

two ions. The dependence of the theoretical Γ_{Mg} on κ was calculated for N_1 values in the range 0.10–0.90. The resulting curves are shown in Figure 3. The curvature is large and negative at $N_1 > 0.6$ but is very small for $N_1 < 0.6$, eventually becoming positive at $N_1 < 0.3$. These results suggest that Γ_{Mg} versus κ will be linear enough for a test of this type for territorial binding when N_1 lies between 0.25 and 0.50. This condition was satisfied in the PG experiment using a 1:1 equivalent ratio of Mg^{2+} to Na^{2+} that did produce a linear relationship between Γ and κ.

Registry No. PG, 9046-38-2; Na, 7440-23-5; Mg, 7439-95-4.

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A Lattice Statistical Mechanics Model of the Conformational and Sequence Spaces of Proteins

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ABSTRACT: We develop theory to explore the relationship between the amino acid sequence of a protein and its native structure. A protein is modeled as a specific sequence of H (nonpolar) and P (polar) residues, subject to excluded volume and an HH attraction free energy ϵ. Exhaustive exploration of the full conformational space is computationally possible because molecules are modeled as short chains on a 2D square lattice. We use this model to test approximations in a recent mean-field theory of protein stability. Also, exhaustive exploration permits us to identify the "native" state(s) in the model, the conformation(s) of global free energy minimum. We then explore the relationship between sequences and native structures by (i) further exhaustive exploration of the full space of all sequences, for short chains, and (ii) random selection of sequences, for longer chains, in some cases exploring exhaustively only the fully compact conformations. The model has the following properties. For small ϵ, the chains are unfolded. With increasing HH attraction, molecules with certain sequences fold to a state with relatively few conformations that have (i) low free energy, (ii) high compactness, (iii) a core of H residues, and (iv) substantial secondary structure. The potential of a molecule to fold to this state is predicted largely by the composition, but for intermediate compositions it depends also on the specific sequence of residues. Some folding sequences have multiple native states; those native structures are broadly distributed throughout the conformational space. However, a most interesting prediction is that, even with only the H and P discrimination among residues in this model, a folding sequence is most likely to have only a single native conformation, a predominance that increases with chain length.

Introduction

A problem of long-standing interest in biology has been that of predicting the three-dimensional structure of a globular protein from knowledge of its amino acid sequence. If the "thermodynamic hypothesis" of protein folding is correct, i.e., that the native structure of the globular molecule is that conformation which has the lowest free energy, then the native structure could be identified in principle simply by systematic evaluation of the free energy of every possible conformation. The problem is that this calculation is not yet practical using current force-field algorithms because the computer time required is far too great. The computer time scales with the number of conformations, which has an exponential dependence, a^n, on chain length n, where $a > 1$ is a constant that depends on chain flexibility and excluded volume.[1] As a consequence, force-field studies at near-atomic resolution are presently limited to explorations of very small regions of conformational space and thus to problems involving dynamics,[2] binding, or catalytic mechanisms[3-4] wherein structural perturbations of the protein are small. Computer limitations currently preclude application of high-resolution methods to problems of (i) large conformational changes, (ii) predictions of thermodynamic properties of folding, which also require knowledge of the ensemble of unfolded reference states, or (iii) the prediction of the native structure from the primary amino acid sequence.

In the absence of high-resolution methods for large-scale exploration of conformational space, two alternative approaches have emerged to predict conformations of proteins from primary structures: (i) semiempirical methods and (ii) methods based on simplifications of conformational

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