Counterion Binding in Polyelectrolyte Theory

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A polyion is a polymer compounded of repeating ionized units. A polyelectrolyte solution contains the salt of a polyion, typically neutralized by small ions like sodium or magnesium. The chief distinction between an aqueous polyelectrolyte solution and an analogous small-electrolyte solution lies in the classification of electrolytes into strong and weak. Thus, dilute aqueous solutions of sodium acetate exhibit only the dissociated ions, but a substantial fraction of sodium ions are bound to the polyacrylate polyion at corresponding normalities. Polyelectrolyte salts can, therefore, be classified as weak electrolytes. An accurate description of the stoichiometry and mode of counterion binding to the polyion has been a central goal of the experimental and theoretical study of polyelectrolyte solutions.

In typical cases counterions are not bound to polyions by covalent bonds. Nevertheless, no ambiguity arises in the operational definition of bound counterions. If the self-diffusion coefficient of a small counterion is observed to be equal to that of a much larger polyion, for example, the counterion may be considered bound to the polyion.[1-4] In favorable cases, frequently encountered in practice, the close approach of a counterion and polyion occurs with mutual perturbation of hydration layers; the resulting volume changes can be either measured directly by volumetric instrumentation[5-8] or probed by ultrasonic absorption[9-11] or index of refraction measurements.[12] Counterions passing from a state of free hydration into an environment of perturbed hydration, as detected by these techniques, are said to pass from a free to a bound state. Magnetic and spin resonance signals are sensitive to the close approach of a polyion and a counterion; counterions giving rise to signals altered from a standard reference state (absence of polyion, for example) can be called bound to the polyion.[13-17]

In a typical competitive binding experiment, a polyelectrolyte salt immersed in excess aqueous 1:1 salt is brought into equilibrium with a solution containing a multivalent counterion, whereupon the latter competes with the univalent counterions for binding interactions with the polyion. In an equilibrium dialysis (membrane equilibrium) experiment, long-range ionically screened interactions of the multivalent counterion with the polyion are eliminated by the presence of excess 1:1 salt, and the difference in number of multivalent counterions on the "polyion side" of the membrane compared to that on the other side of the membrane is the number of multivalent counterions bound to the polyions.[7,18,19] Another possibility is the use of an indicator dye in a solution containing polyelectrolyte salt, 1:1 salt, and multivalent counterions.[20] Knowledge of the equilibrium constant for the complex between dye and multivalent ion, with appropriate consideration of activity coefficients, allows the determination of the concentration of free multivalent counterions, with the number bound to the polyion obtainable by difference.

The binding of proteins to DNA, which in some cases is entirely caused by electrostatic attraction between the DNA phosphates and cationic residues on the protein,[21-23] can be detected by a variety of techniques. A conceptually simple, but nonetheless effective and important, method is filter binding.[24,25] For example, nitrocellulose filters do not prevent the passage of uncomplexed DNA, but the H1 fraction of protein histone is retained by the filter.[26] Then, retention on the filter of a portion of the DNA molecules from a solution mixture of DNA and H1 is interpreted as complexation in solution of these molecules with H1 molecules.

In the older polyelectrolyte literature, the notion of counterion binding was also defined operationally, but in a physically unsatisfactory way. Thus, if the osmotic coefficient in a binary solution of a polyelectrolyte salt was measured to be 0.4, for example, then 60% of the counterions were reported as bound, with 40% free. Similarly, if the tracer diffusion coefficient of the counterions, relative to its value in polyelectrolyte-free

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systems, was 0.3, then 70% of the counterions were said to be bound. It is now understood that colligative and transport properties are strongly influenced not only by the extent of true binding, as defined by techniques such as those discussed above, but also by diffuse atmospheric screening of the Debye–Hückel type. Just as the oppositely charged ion cloud surrounding each ion in a small-electrolyte solution is not thought of as bound to the central ion, atmospheric effects in polyelectrolyte solutions should not be confused with physical binding. (Record and his colleagues, however, have introduced the notion of "thermodynamic binding", which includes atmospheric, or screening, effects; and, indeed, their definition has proved useful in thermodynamic calculations.[21])

The wide latitude offered in the operational definition of binding, even as restricted to exclude the diffuse atmosphere, augments the possibility that different techniques of measurement will respond to different states of binding. Two distinct binding states have been discovered,[10,15] and a commonly accepted nomenclature has become necessary to avoid confusion. Following Weill and his associates,[15] let us call "site bound" those counterions which are in direct contact with one or more charged groups on the polyion, with no intervening water molecules. Bound counterions which are not site bound will be called territorially bound. Territorial binding, thus defined, does not preclude the possibility of effects due to vicinal charge on the conformation of the polyion, or of effects specific to the species of counterion and polyion due to partial mutual disruption of the electrostricted local aqueous environment, or of some degree of nonuniformity in the distribution of counterions so bound along the length of the polyion or along the polar angle coordinate at constant radius (i.e., relatively slight localization effects). The term does suggest, however, that if the inner hydration layer of the counterion remains intact or if the counterion in its free state is only slightly hydrated, like K^+, then its interactions with specific locations on the polyion will be sufficiently weak to be dominated by the overlapping Coulomb fields of the charged groups. At this point, the counterion will be drawn into the polyion as a whole by the strong polyanionic field but, once there, will be more or less free to wander about the polyion surface, encountering only relatively small perturbations in its otherwise random trajectory.

I have previously[27] referred to territorial binding as "delocalized", but the implication of a localized reference state, analogous to the isolated nucleus on which electrons are localized in solid-state theory, is inappropriate for polyelectrolyte solutions since the corresponding monomeric salt is usually completely dissociated in water. Weill and co-workers[14,15] have used the phrase "atmospherically trapped" as characteristic of territorially bound counterions, but this population of bound counterions would then be too easily confused with counterions in the Debye-Hückel screening atmosphere, which are not physically bound at all. A site-bound counterion, together with its site, can be called an "inner-sphere" complex,[10] in analogy to the corresponding state for a divalent metal ion and a sulfate anion; but since site-binding is probably usually accomplished by attachment to more than one group

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on the polyion,[7] and, moreover, since the "counterions" studied in polyelectrolyte solutions can be geometrically complicated objects like basic regions in proteins, the term "sphere" is not generally appropriate. Use of "outer-sphere" complex[10] to describe the mode of attachment of territorially bound counterions misses evocation of the nonlocalized nature of this type of binding. The important physical feature of the outer-sphere complex, namely, substantial disruption of all but the inner hydration, can quantitatively be incorporated into the definition of the reference-state chemical potential of territorially bound ions, as will be discussed subsequently.

The classification of bound counterions into two populations, site bound and territorially bound, applies as well to proteins bound to DNA. Regulatory proteins "recognize" specific base sequences, or sites, on the DNA molecule[28] and may be called site bound when attached there. The definition probably applies on the detailed molecular level as well, since it is unlikely that the complex between protein and its specific DNA site could be sufficiently stable if intervening water molecules prevented direct contact. On the other hand, many regulatory proteins and compacting proteins like histones also bind to DNA in the absence of specific "recognition sequences". Presently available data[21–23] are consistent with the interpretation of this "nonspecific" binding as territorial in nature.[23] If so, the binding free energy is entirely attributable to electrostatic forces.

This Account will be primarily devoted to recent developments in the molecular theory of territorial binding.[27,29,30] There is at present no corresponding theory for site binding, nor are quantitative molecular criteria known for the distribution of the total population of bound counterions between the two subpopulations. A tentative generalization at the present stage of development of the field would be that binding of univalent counterions to any polyelectrolyte is, with exceptional special cases, dominated by the territorial mode; divalent counterions are bound almost entirely in the territorial mode to polyions of low *surface* charge density (i.e., "fat" polyions like DNA and poly(styrenesulfonate), which, nevertheless, possess high *linear*, or axial, charge density), while a substantial fraction of bound divalent counterions are site bound if the polyion possesses both high linear and surface charge density ("thin" polymers like polyphosphate).[14,15] A program of compilation of detailed potential-energy profiles, which may mark the beginning of a theoretical attack on the problem, has recently been initiated.[31,32]

Counterion Condensation

Let the charge fraction f of a polyion be defined as the ratio of that part of its structural charge which is uncompensated by bound counterions to its total structural charge. As the result of extensive experimental binding studies, the following generalization may be formulated: in an environment containing counterions of only one valence type

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$$f = (N\xi)^{-1}, \xi > N^{-1}$$
$$= 1, \xi < N^{-1} \qquad (1)$$

where N is the counterion valence and ξ, a dimension-

$$\xi = q^2/\epsilon k_B T b \qquad (2)$$

less measure of linear polyion charge density, is defined by eq 2, with q the protonic charge, ϵ the bulk dielectric constant of solvent, k_B Boltzmann's constant, T the Kelvin temperature, and b the average linear charge spacing of the polyion (bearing univalent charged groups) taken along the contour length of the polymeric chain.

Two features of this empirical observation are immediately striking. The charge fraction, hence the number of bound counterions, does not depend on ionic strength or concentration of free counterions; thus, a conventional mass-action formulation does not hold for counterion binding in polyelectrolyte solutions. Instead, the term "counterion condensation" has been used to describe a situation in which, regardless of the ionic strength, the number of bound counterions is determined by the requirement that the net value of the polyion charge density, ξ_{net}, be always equal to $\min(\xi, N^{-1})$.[33] Moreover, the charge fraction ξ_{net}/ξ depends on no specific properties of the counterion and polyion other than the valence of the former and the linear charge density of the latter. It follows that the explanation for the observed charge fraction must involve only long-range electrostatic interactions between constituents of the solution. In particular, the finite thickness of the polyion chain is apparently irrelevant. Since site binding is determined by specific, short-range interactions, the polyelectrolyte charge fraction must be a manifestation of the properties of territorial binding; although some of the bound counterions may be site bound, at least some of the bound counterions must be territorially bound. (Let the structural charge density ξ be reduced by the factor α by site binding, and let P refer to total polyion charge. Then the charge-fraction rule implies $P_{net}/\alpha P = N^{-1}(\alpha\xi)^{-1}$, or $f = P_{net}/P = N^{-1}\xi^{-1}$, independent of α.)

The phenomenon of counterion condensation appears at present to be remarkably general. Discovered first by Ikegami[12] by index of refraction measurements for the special case of polyacrylate and sodium ions, it has since been confirmed for univalent, divalent, and trivalent counterions[2] and nearly all of the commonly encountered polyionic species, both synthetic and naturally occurring.[15,29,34,35] No violations have been reported (except under certain extreme conditions to be discussed). A stringent test might involve a weak-binding system, such as the moderately weak binder poly(ethylenesulfonate) and the bulky tetrabutyl-ammonium cation (weak binding means minimal desolvation effects), on the grounds that counterion condensation, in some sense, is a strong effect. Tondre et al.[11] (see their Figure 4) have demonstrated conformance of this particular system to the general empirical rule by means of ultrasonic absorption measurements, which monitor in this case hydrophobic interactions among condensed tetrabutylammonium ions. (In contrast, the condensation rule does not hold for the in-

teraction of these cations with poly(styrenesulfonate) due to overriding hydrophobic interactions between polyion and counterion;[11] this system, then, is an example of the "extreme", or special, conditions necessary for breakdown of condensation.)

It could also be argued that a strong-binding system (maximal desolvation effects) would provide optimal conditions for breakdown of condensation, since the latter is a manifestation of nonspecific effects. Polyphosphate is perhaps the strongest binder of the commonly used polyions, yet Spegt and Weill[14] have confirmed condensation of Mn^{2+} in this system both from the EPR signal of the counterion and the magnetic relaxation rates of the water molecules influenced by the counterion. Strong binding of Mn^{2+} and Co^{2+} by polyphosphate is manifested not by deviations from the empirical charge-fraction rule but by complete dehydration, in the sense of complete release of electrostriction, of about half of the condensed counterions,[14,15] the other half being territorially bound.

Another rigorous test is to measure charge fractions for polyions with values of ξ close to critical for condensation. For example, a structure yielding ξ only slightly greater than unity might be expected not to conform well to the general rule that the charge fraction for univalent counterions is ξ^{-1}, since if the rule did not really correspond to a sharp phenomenon, perhaps no univalent counterions at all would bind under these conditions. But Magdelanat et al.,[2] in an elegant series of measurements of apparent polyion charges defined[2,3,36] through the Nernst–Einstein relation as proportional to the ratio of polyion electrophoretic mobility to polyion self-diffusion coefficient, with both quantities measured by laser light scattering, have found the charge fraction 0.83 for Na^+ and chondroitin ($\xi = 1.15$). Favorable comparison may be made to the value 0.88 from the general rule. (These authors also measured charge fractions for the same polyion in Ca^{2+} and La^{3+} environments, respectively. For Ca^{2+} they found $f = 0.42 \pm 0.04$, to be compared with 0.44 from the formula $f = (2\xi)^{-1}$; and for La^{3+} the measured value was 0.29 ± 0.02, with 0.29 obtained from $(3\xi)^{-1}$. Moreover, a small amount of Sr^{2+} added to the Na^+ salt of the polyion was observed to be abruptly released from the polyion when addition of La^{3+} ion had lowered ξ_{net} to $^1/_2$, the critical value for Sr^{2+} condensation; the tracer diffusion coefficient of Sr^{2+} rose steeply from a value equal to the self-diffusion coefficient of the polyion to the value characteristic of free Sr^{2+} in the absence of polyion.)

Nevertheless, there are aspects of the charge-fraction rule that have not yet been rigorously tested. Its dependence on the dielectric constant has been empirically inferred only from measurements in water; experiments in different solvent systems would therefore be of some interest. The formulation of eq 1 implies also that if the linear charge density of a polyion is varied, e.g., by titrating weak acid groups, then a discontinuous phenomenon occurs. As the charge density parameter increases across the value N^{-1}, the charge fraction abruptly changes slope as a function of ξ. That is, counterion condensation does not occur for values of ξ less than a critical value N^{-1} and abruptly sets in when ξ attains this value. Although a number of ex-

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periments have demonstrated that this behavior is close to being correct, none have been designed to test rigorously the hypothesis that the discontinuity is real and not simply a good approximation.

For example, in a titration of polyphosphate with Co^{2+}, eq 1 predicts that the condensation of Co^{2+} would abruptly cease when 82% of the phosphate charge has been compensated; water proton relaxation data[14] are indeed sufficiently dense to establish the region 0.8–0.9 as at least one of transition from bound to free Co^{2+} but do not sharply define an actual discontinuity. For a carboxymethylcellulose sample of degree of substitution 2.1, Na^+ condensation was determined by ultrasonic absorption[9] to set in for a value of ξ not less than 0.9 and not greater than 1.2, but no intervening data points were gathered in an attempt to pin down more closely the predicted value $\xi = 1.0$ for the onset of condensation. Similarly, it is clear from quadrupole relaxation data on the ^{23}Na nucleus that Na^+ condensation on polyacrylic acid does not occur at $\xi = 0.8$ and does occur at $\xi = 1.1$,[37,38] but the present absence of a denser grid of data precludes the statement that Na^+ condensation begins at $\xi = 1.0$.

To the degree that a macroscopic system may be considered of infinite extent, discontinuous phenomena occur in nature (first-order phase transitions, for example). To the degree that a polyion may be considered infinitely long, therefore, it is reasonable to suggest that the discontinuous aspect of eq 1 is real and not simply a good approximation to a curve with continuous derivative. Given the current interest of chemical physicists in sharply defined transitions, an attempt to confirm this conjecture, which, as will be reviewed in the next section, has some theoretical support, would presumably be well received.

As mentioned, the polyelectrolyte charge-fraction rule (eq 1) is known not to be completely general. Evidently, since its validity requires that at least some of the bound counterions be territorially bound, if a strong complex with substantial pK exists between counterion and discrete sites on the polyion, then practically all bound counterions will be site bound, and the charge fraction will reflect effects specific to the complex. The most obvious example is a weak polyacid for which protons are regarded as counterions. The covalent binding of these counterions to the polyacid groups then implies, as observed, a charge fraction for the unneutralized acid close to zero. More subtle considerations are required to explain the observed charge fraction of 0.32 in silver poly(vinylsulfonate),[8] which is slightly smaller than the value of 0.36 predicted from eq 1; perhaps a small component of covalency in the site binding of the highly polarizable Ag^+ ion is present, but the dependence of charge fraction on ionic strength needed to test this hypothesis[8] has not yet been determined for this system. The typical polycation poly(4-vinylpyridinium) has a charge fraction of 0 in 0.8 M KBr and actually becomes anionic at still higher ionic strengths, migrating toward the anode in an electrophoresis measurement.[39] A similar charge reversal of DNA occurs in an environment of about 1 N aqueous

$MgCl_2$ (unpublished data of Papon and Strauss, cited in ref 27), even though eq 1 correctly describes the binding of Mg^{2+} to DNA at more moderate ionic strengths.[27] The effect of strong hydrophobic interaction in the tetrabutylammonium poly(styrenesulfonate) system[11] has already been mentioned.

For present purposes, however, the primary point is that all known cases of deviation from eq 1 involve extreme conditions, whether of very high ionic strength or of substantial covalent or other "special" contributions to the binding free energy. In particular, the rule persists even at surprisingly high ionic strengths, up to at least 0.6 M NaCl in the NaDNA system[17] and at least 0.3 M NaCl in sodium carboxymethylcellulose[9] (the charge fraction obeyed eq 1 over the entire range of ionic strengths used in the cited experiments, so that the point at which it fails for these systems is still unknown).

Theory of Territorial Binding[27,29,30]

Since the most obviously distinctive physical aspect of a polyion is its spatially linear correlation of (univalent) charged groups with uniform average axial charge spacing, an idealized, but reasonable, model for theoretical purposes is a linear array of unit point charges q with constant spacing b between charges. In the range of practical ionic strengths, ionic forces are characterized by interaction distances shorter than the lengths of typical polymers, so the linear array of charges may be taken of infinite length, thereby eliminating end effects from the model. Uncomplicated though it may be, this model will clearly produce results consistent with eq 1, at least insofar as the latter contains as a structural variable only the linear charge density of the polyion and, in particular, is independent of the polymer length, or molecular weight.

If the electrostatic free energy G_{el} of the system is approximated by ionically screened (Debye–Hückel) potentials of type $r^{-1} \exp(-\kappa r)$, summed over all pairs of charges on the same polyion (neglecting, thus, interactions between polyions), one finds

$$G_{el} = -n_P RT\xi \ln (1 - e^{-\kappa b}) \qquad (3)$$

where n_P is the total number of moles of univalent charged groups on the polyions, and ξ is defined by eq 2. In order to abstract from this formula its purely polyelectrolyte component, let the exponential be expanded

$$G_{el} = -n_P RT\xi[\ln (\kappa b) + 0(\kappa)] \qquad (4)$$

In this form it becomes clear that, apart from the distinctively logarithmic "polyelectrolyte" term, other contributions to the electrostatic free energy are small-electrolyte effects, in the sense of a limiting behavior at low ionic strengths (the Debye screening parameter κ is proportional to the square root of the ionic strength) identical with that of small-electrolyte free energies. Since the task at hand is to find the molecular origin of the empirical polyelectrolyte charge-fraction rule, embodied to within at least a good approximation by eq 1, and, moreover, since this rule is qualitatively at variance with small-electrolyte behavior, it is reasonable to emphasize the ln (κb) term in eq 4 while neglecting the effect of the terms of order κ.

A free energy of the Debye–Hückel type, such as that in eq 4, cannot, by itself, account for binding phenom-

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ena, which, by definition, are manifestations of interactions more intimate than simple ionic screening. Since counterions are empirically observed to possess a bound state, there becomes available the standard statistical mechanical procedure of assigning parameters to characterize this state and then attempting to evaluate the parameters as those which minimize the total free energy of the system.

Let θ_N be the number of bound counterions per charged group fixed to a polyion, with the understanding that all counterions in the system have the same valence, N. The mode of binding is assumed to be territorial, so that each charged group has its structural charge q effectively lowered to the same extent by the bound counterions (as opposed, for example, to a situation for which a pair of charged groups is completely neutralized by a site-bound divalent counterion, while others retain their full structural charge). If the structural charge is lowered to the value q_{net}, then

$$q_{net}/q = f = 1 - N\theta_N \qquad (5)$$

where it is also noted that q_{net}/q is, by definition, the polyion charge fraction f. The effect of territorially bound counterions on the electrostatic free energy is obtained by replacement of q by q_{net} in eq 4.

$$G_{el} = -n_P RT(1 - N\theta_N)^2 \xi \ln(\kappa b) \qquad (6)$$

Other contributions to the total free energy are obtained from interaction of each territorially bound counterion with its immediate environment (exclusive of other territorially bound counterions and of the long-range ionic interactions built into eq 5) and from the ability of these counterions to mix translationally with each other. Analogous contributions are provided by the free counterions. With μ_f and μ_b written for the chemical potentials of free and bound counterions, respectively, one finds from thermodynamic identities relating chemical potentials to the total free energy that

$$\mu_f = \mu_f{}^\circ + RT \ln c_f \qquad (7)$$

and

$$\mu_b = \mu_b{}^\circ + RT + RT \ln \gamma_b c_b \qquad (8)$$

where

$$RT \ln \gamma_b = 2RTN\xi(1 - N\theta_N) \ln(\kappa b) \qquad (9)$$

and

$$c_b = 10^3 \theta_N / V_P \qquad (10)$$

In eq 7 $\mu_f{}^\circ$ represents the interaction of a free N-valent counterion with pure solvent, as well as translational, rotational, and internal contributions to the free energy of the isolated ion, while c_f is the molarity of free counterions. In eq 8 the quantity $\mu_b{}^\circ$ has a meaning for the bound state analogous to that of $\mu_f{}^\circ$; hence, the increment

$$\delta\mu^\circ = \mu_b{}^\circ - \mu_f{}^\circ \qquad (11)$$

primarily measures the change in solvation free energy as a counterion passes from the free to the bound state, since the translational, rotational, and internal contributions are the same in both states for territorial binding. The extra term RT in eq 8 originates in the standard osmotic contribution to the chemical potential of bulk solvent. The activity coefficient γ_b of bound counterions, detailed in eq 9, obviously derives from the

electrostatic free energy, given in eq 4, required to form the linear arrays of charge together with the counterions bound to them. Note the "small-electrolyte" activity coefficient corrections are neglected in both eq 7 and eq 8. Finally, c_b in eq 8 and 10 is the *local* molarity of territorially bound counterions, since their translational mixing occurs in a region of volume $n_P V_P$ close to the polyion (V_P is the volume of the "bound region" in units of cm^3/mol of polyion equivalent).

Additionally specified in the derivation of eq 7–10 is the condition of excess salt, that is, the number of moles of N-valent counterion added as a simple salt is much greater than that originating from the polyelectrolyte salt, or, more simply, $c_f \gg c_P$. In this case c_f is approximately equal to νc_s, where c_s is the molarity of added small electrolyte and ν is the number of N-valent ions per formula salt. Moreover, the Debye screening parameter κ is then approximately independent of the number of moles of bound counterions and may be computed as equal to κ_s, the corresponding parameter for a small-electrolyte solution of salt molarity c_s.

The territorial binding of counterions M^{N+} to a polyion may now be formulated as a binding reaction $M^{N+}(\text{free}) \rightarrow M^{N+}(\text{bound})$, with reaction affinity $\Delta\mu$ (defined as $\mu_b - \mu_f$) given by

$$\Delta\mu = \delta\mu^\circ + RT + RT \ln(\gamma_b c_b/c_f) \qquad (12)$$

or, with eq 9 and 10 and the excess salt condition,

$$\Delta\mu = RT \ln[(10^3 e/V_P{}')\nu^{-1} c_s{}^{-1} \theta_N(\kappa_s b)^{2N\xi(1-N\theta_N)}] \qquad (13)$$

where e is the base of natural logarithms and

$$V_P{}' = V_P \exp(-\delta\mu^\circ/RT) \qquad (14)$$

The condition for equilibrium of the binding reaction, $\Delta\mu = 0$, is equivalent to minimization of the free energy with respect to the parameter θ_N, with $V_P{}'$ and c_s held fixed. At first glance, it appears from eq 13 that the equilibrium condition might not yield much relevant information, because a solution θ_N exists as a function of c_s and $V_P{}'$, in contradistinction to the charge-fraction rule eq 1. The full implications of eq 13, however, are not seen without consideration of the "limit of infinite dilution", or "limit of zero ionic strength".

Suppose, then, that pure solvent be added to the system sufficiently slowly for the continuous maintenance of equilibrium. This procedure (suggested by C. F. Anderson) provides the operational meaning of the mathematical statement

$$\lim_{c_s \to 0} \Delta\mu = 0 \qquad (15)$$

Note that the condition of excess salt, if initially satisfied, remains satisfied throughout the limiting process. Since $\kappa_s{}^2$ is of order c_s, it follows from application of eq 13 to eq 15 that a unique value

$$\theta_N = N^{-1}(1 - N^{-1}\xi^{-1}) \qquad (16)$$

is required if $\Delta\mu$ is not to have limiting values of $\pm\infty$. For the particular limiting value zero, characteristic of equilibration of the binding reaction, substitution of eq 16 into eq 13 yields a unique value of $V_P{}'$, namely, that for which the bracketed expression is unity.

$$V_P{}' = 10^3 e\nu^{-1} N^{-1}(1 - N^{-1}\xi^{-1})(\kappa_s b)^2 c_s{}^{-1} \qquad (17)$$

Note that $V_P{}'$ is independent of c_s since the ratio $\kappa_s{}^2/c_s$ is independent of c_s. (Numerical calculation of $V_P{}'$ is most easily performed from formulas recorded in eq 14

of ref 29 or eq 16 of ref 30 for salts with constituent ions of arbitrary valence and eq 15 of ref 27 for 1:1 salts.) With V_P', the structural parameter characteristic of territorially bound counterions, thus unambiguously determined from passage to the limit of zero ionic strength, one merely notes that eq 16 for θ_N uniquely satisfies the equilibrium condition $\Delta\mu = 0$ for all values of ionic strength, or c_s, within the domain of validity of eq 13, since use of this value of θ_N eliminates the dependence of the bracketed expression on c_s. Thus, a single equilibrium condition $\Delta\mu = 0$ imposed on the three parameters c_s, θ_N, and V_P', when supplemented by the requirement that binding equilibrium be maintained upon indefinite dilution of the polyelectrolyte solution and by the interpretation of V_P' as a structural parameter (hence, independent of c_s), yields unique values of both θ_N and V_P' which are independent of c_s. Since, according to eq 5, the charge fraction f equals 1 $- N\theta_N$, the value of θ_N given by eq 16 yields $f = N^{-1}\xi^{-1}$, in agreement with the charge-fraction rule eq 1. (Equation 16 is clearly restricted to the case $\xi > N^{-1}$ for θ_N to satisfy the physical requirement of being a positive number or zero; for $\xi < N^{-1}$, it follows from eq 13 that with negative values excluded for θ_N, the condition $\Delta\mu = 0$ cannot be satisfied in the limit $c_s \rightarrow 0$ unless θ_N also tends to zero, again in agreement with the charge-fraction rule eq 1.)

Since $\delta\mu°$, by definition, does not depend on c_s, the same property for V_P follows from eq 14; hence, the local molarity of territorially bound counterions, given by eq 10, is also independent of bulk ionic strength. More insight into this theoretical phenomenon is obtained from inspection of eq 12. An expression of this type for $\Delta\mu$, when constrained to the value zero by the equilibrium condition, would normally be expected to yield a routine mass-action expression, in the limit of infinite dilution, of the form $c_b/c_f = K$, where, in this case, the equilibrium constant K would be given by the expression $\exp[-(\delta\mu°/RT) - 1]$. Indeed, the mass-action law does follow from the rigorously correct assertion that all activity coefficients must yield unity as their limiting values. For the case at hand, however, it has just been established that c_b = constant. The reason for this apparent violation of mass action is the unusual, and apparently "incorrect", behavior of the activity coefficient γ_b, which, as follows from eq 9 and 16, is of order $c_s \approx c_f$ (the constancy of c_b at equilibrium then being obvious from eq 12) and, hence, possesses limiting value zero instead of unity. The problem is resolved with the observation that, as noted previously, polymer end effects have been neglected in the derivation of the expression for γ_b upon appeal to interaction distances short compared to the polymer length over the range of practical ionic strengths. Therefore, the "limit of infinite dilution" must be interpreted as a range of ionic strengths that remains in the regime of practical experiments with polymers. Since, for example, electrostatic interactions are not effectively screened at distances greater than 100 Å only when ionic strengths are less than 10^{-3} M, no operational restriction at all is thereby imposed for typical polyelectrolytes.

If $\delta\mu°$ is taken as zero, that is, if territorial binding is assumed to proceed with negligible desolvation, numerical values of c_b may be obtained from eq 10, since V_P is then equal to V_P'. Let us consider DNA in an

environment of aqueous 1:1 salt as an example.[27] The value of ξ based on the average axial phosphate spacing is 4.2, with the corresponding values $\theta_1 = 0.76$ (or charge fraction $f = 0.24$) and $V_P = 646 \text{ cm}^3/\text{mol}$ of P. (If DNA is modeled by an impenetrable cylinder of 10-Å radius, then this value for V_P corresponds to location of the territorially bound counterions within a cylindrical shell of thickness 7 Å coaxial with the DNA molecule.) The value of c_b is then 1.2 M.

The value $\theta_1 = 0.76$ for DNA, which does not depend upon the assumption that $\delta\mu° = 0$ (or even that all bound counterions are territorially bound—see discussion following eq 1) has been confirmed to within about 10% by NMR measurements.[17] There is suggestive empirical evidence to support the assumption that $\delta\mu°$ is at least quite small for binding of the alkali metal cations to DNA.[16,27] The value of V_P, and hence of c_b, predicted on the basis of negligible $\delta\mu°$, has been confirmed indirectly by an analysis of the excluded-volume effect on Donnan salt-exclusion measurements.[34] The predicted value $\theta_2 = 0.44$ (charge fraction $f = 0.12$) for the aqueous MgDNA–MgCl$_2$ system has been confirmed to within about 5%,[27] but the corresponding value $V_P = 1120 \text{ cm}^3/\text{mol}$ of P (thickness of coaxial cylindrical shell equal to 11 Å) is too large for consistency with Donnan salt-exclusion data for this system.[7] Since Mg^{2+} and Ca^{2+} binding data allow at most a small fraction of these ions to be site bound to DNA,[27] the most likely explanation for this discrepancy is failure of the assumption of negligible $\delta\mu°$. Indeed, the extent of dehydration observed when the Mg^{2+} ion binds to DNA, although small, is significant.[7] The dehydration is observed as a positive volume change for the forward binding reaction, presumably due to release of some of the electrostriction, and, hence, probably correlated[19] with a predominantly entropic negative value of $\delta\mu°$; accordingly, eq 14, in which V_P' is a constant, would predict a value of V_P lower than the value corresponding to $\delta\mu° = 0$. Values of $\delta\mu°$ of about -2, -1.4, and -0.5 kcal for territorial Na^+ binding to polyphosphate, polyacrylate, and poly(styrenesulfonate), respectively, have been crudely estimated from NMR data.[29] (The value $d = 9.2$ Å in Table 5 of ref 29 should have been 10.4 Å.) The order given correlates well with extents of dehydration measured by dilatometry for these systems,[5] and, in particular, the value near zero for poly(styrenesulfonate) is consistent with the well-known inability of this "fat" polyion to produce significant dehydration of conterions bound to it.[15]

The validity of the results discussed here are limited to the domain of validity of eq 13 for $\Delta\mu$, which may be defined by $\kappa_s b \ll 1$ (see eq 3 and 4 and the discussion immediately following them). In practice this restriction may not be severe, since even when $c_s = 1$ M, $\kappa_s b = 0.56$ for DNA in 1:1 salt. If $\delta\mu°$ is neglected, it is easy to verify that $c_f/c_b = e(\kappa b)^2$, so that the theory can also be said to be restricted to the range $c_s \ll c_b$ in which the invariant local concentration of territorially bound counterions is much greater than the bulk ionic strength.[27] Since c_b for DNA in 1:1 salt is 1.2 M, again the range of allowable ionic strengths is seen to approach 1 M. The treatment is also restricted to the case of excess salt, $c_f \approx c_s$; Iwasa has initiated a more general analysis.[40,41]

(40) K. Iwasa, *J. Phys. Chem.*, **81**, 1829 (1977).

Relation to Poisson–Boltzmann Distribution

The counterion distribution about a charged cylinder predicted by numerical solutions of the Poisson–Boltzmann (PB) equation is a continuous function of distance from the cylinder surface. Any attempt to divide the distribution into bound and free counterions must necessarily, therefore, suffer from lack of uniqueness, in contrast to the theory of the preceding section. It is possible, however, that the distribution may have a structure that suggests a fairly narrow range of distances as plausible cut-off points for bound counterions. An early attempt to use the PB distribution as the basis for the definition of a bound fraction of counterions was made by Kotin and Nagasawa.[42] They noted that the radial distribution function for counterions relative to the cylindrical axis of the polyion possesses a minimum, and defined as bound those counterions located at distances less than that marking the minimum. Their result, in present notation, is $f = (2\xi)^{-1}$ for 1:1 salt; thus, these authors were able to capture the qualitative essence of the charge-fraction rule eq 1, namely, its invariance to ionic strength and size, but missed its actual value by a factor of two, far outside the limits of experimental error.

Another approach has recently been tried by Fixman,[43] who has defined an excess charge density in the region outside a polyion as that over and above the density predicted by the linearized PB equation (i.e., the Debye–Hückel approximation). Substantial excess charge (territorially bound counterions) close to a cylinder with a high value of ξ is predicted by the full, nonlinearized, PB equation. Fixman's definition, therefore, attempts to make contact with the theory outlined in the previous section, which attributes departure from the Debye–Hückel free energy of formation of a linear charge array to territorial counterion binding. The continuous PB excess charge is then replaced, by a purely numerical procedure, with a step function equal to the excess charge at the surface for small distances and to zero for larger distances; the width of the step function roughly corresponds to the distance at which the PB excess charge has fallen to the fraction e^{-1} of its surface value. In this way Fixman has derived a two-state theory for the counterions that is numerically consistent with the PB equation. Following his instructions and using the numerical work in his Table 1, I have calculated polyion charge fractions for this two-state PB theory. With $\xi = 4$ and the cylinder radius $a = 12$ Å, values chosen by Fixman to simulate DNA, the charge fractions thus obtained are 0.53 and 0.34 at ionic strengths 0.064 and 6.4×10^{-4} M, respectively, in 1:1 salt. The latter value is approximately maintained at least down to 6.4×10^{-8} M. By contrast, the empirical charge-fraction rule eq 1 predicts the value 0.25 regardless of ionic strength, as does the two-state theory of territorial binding reviewed in the previous section. NMR experiments[17] on the NaDNA–NaCl system measure a charge fraction invariant to ionic strength in the range 0.006–0.6 M and possessing a value between 0.15 and 0.35. Thus, Fixman's procedure, like Kotin and Nagasawa's, fails to reconcile the PB equation with observed charge fractions.

The primary value of Fixman's paper, however, is in its careful statistical mechanical derivation of the PB equation, which marks the first occasion in the field of polyelectrolyte theory that the PB equation has been quantitatively revealed as an approximation to a more exact formulation. For the surface counterion concentration, the quantity of greatest relevance to the present review of counterion binding to polyions, the PB prediction, according to Fixman's approximate computations, represents an underestimate of about 6% at 1:1 salt concentration 0.00064 M and 26% at 0.064 M for $\xi = 4$ and $a = 12$ Å, with worse errors expected at higher ionic strengths. The discrepancy between the charge fraction 0.53, obtained above as the PB prediction for the latter ionic strength, and the observed value 0.25 ± 0.10 can therefore be attributed, at least partially, to errors intrinsic in the PB equation.

Concluding Remarks

I have sought to make a definitive statement about the current status of a single, well-defined aspect of polyelectrolyte behavior in solution—counterion condensation. An idea of the breadth of possible generalizations and applications may be obtained from other sources.[21,27] I hope to have clarified several points. Since the bound state for counterions can be operationally defined, it cannot be doubted that a portion of the counterions in a polyelectrolyte solution are bound to polyions of sufficiently high charge density, usually encountered in practice. A continuum of bound states may exist, ranging from covalent complexation to complete territoriality. If covalent binding and other exceptional cases are excluded, the polyion charge fraction is found empirically to conform closely to eq 1, although existing measurements are not sufficiently extensive to allow the statement that this formula is sensibly exact.

Although a two-state theory of the territorial mode of counterion binding has eq 1 as a consequence of its assumptions, a fundamental theoretical problem remains. A territorially bound counterion is not intrinsically different from those counterions which are not bound. It resides in the vicinity of a polyion only because of the high charge density of the latter and may exchange rapidly with a free counterion. It should be possible to formulate a single-population theory which gives rise to eq 1. Fixman's analysis[43] appears to be a major step in this direction, but stops short of a solution, possibly because the question, as posed here, was not asked. Presumably, simplification in the unipopulation theory may be achieved by adoption of the one-dimensional lattice model for the polyion; refinements in the model can well be deferred until final theoretical resolution of the status of eq 1 for the simplest model available.

(41) K. Iwasa, *Biophys. Chem.*, **9**, 397 (1979).
(42) L. Kotin and M. Nagasawa, *J. Chem. Phys.*, **36**, 873 (1962).
(43) M. Fixman, *J. Chem. Phys.*, **70**, 4995 (1979).