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Electrostatics of Strongly Charged Biological Polymers: Ion-Mediated Interactions and Self-Organization in Nucleic Acids and Proteins

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Abstract

Charges on biological polymers in physiologically relevant solution conditions are strongly screened by water and salt solutions containing counterions. However, the entropy of these counterions can result in surprisingly strong interactions between charged objects in water despite short screening lengths, via coupling between osmotic and electrostatic interactions. Widespread work in theory, experiment, and computation has been carried out to gain a fundamental understanding of the rich, yet sometimes counterintuitive, behavior of these polyelectrolyte systems. Examples of polyelectrolyte association in biology include DNA packaging and RNA folding, as well as aggregation and self-organization phenomena in different disease states.

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INTRODUCTION

dsDNA: double-stranded DNA

DNA packaging: the process of compacting double-stranded DNA into an ordered structure for efficient storage

RNA folding: the process of compacting single-stranded RNA molecules into a functional, three-dimensional, biologically active structure

PB: Poisson-Boltzmann

All nucleic acids and most proteins carry uncompensated electrical charge. In biologically relevant physical situations, bare charges on these polymers are strongly screened by the large zero-frequency dielectric response of water, and by salt solutions containing coions and counterions. However, this simple picture can be deceptive. The entropy of these counterions can result in surprisingly strong interactions between charged objects in water despite short screening lengths, via coupling between osmotic and electrostatic interactions. Like charges repel because of the osmotic pressure of squeezed counterions; opposite charges repel because of the entropy gain of counterion release. For example, the free-energy gain upon binding between two macroions scales as kT multiplied by the number of counterions released, which can be a large number, even though the electrostatic interaction energy is much smaller at typical salt concentrations. The detailed behavior of the system also depends on other effects, such as the charge, size, hydration, concentration, and geometry of both macroions and counterions. Because of these and other considerations, the chemical physics of charged biological polymers is a rich field with often counterintuitive phenomena and has motivated widespread work in theory, experiment, and computation.

The salt-dependent behavior of short DNA duplexes is a simple illustrative example. Short double-stranded (ds)DNAs repel when the distance between macroions exceeds the screening length. As the ionic strength increases, screening occurs on length scales shorter than intermacroion distances, and the DNA molecules no longer interact (1). The addition of even small numbers of multivalent ions to solutions containing 1:1 salts results in a qualitative change and leads to the development of attractive forces between like-charged DNA and eventually to precipitation (2). This example illustrates some key physical principles that biology exploits: ionic-strength-dependent screening lengths and, importantly, attraction of like-charged objects.

In this review, we concentrate on the roles that counterions play in modulating the behavior of charged polymers (or polyelectrolytes) and discuss their connection to recent topics in biology and biomedicine, such as DNA packaging, RNA folding, and analogous forms of behavior in cytoskeletal organization, as well as give examples of pathological polyelectrolyte self-assembly, in which the rational control of the interplay between electrostatic and osmotic effects can have potential implications in human health.

COUNTERION DISTRIBUTION AROUND POLYELECTROLYTES

We begin with a simple picture of biological polyelectrolytes such as dsDNA, F-actin, and microtubules. Each polyelectrolyte has a distribution of counterions associated with it. The Manning counterion condensation theory (3) describes the localization of counterions to an infinitely long, infinitesimally thin line of charge using simple electrostatics and free-energy arguments. Significantly, this model predicts the fraction of the line charge compensated by ions of different valence. Many of these predictions have been borne out by more complex approaches (4). Mean field theories, including those based on the Poisson-Boltzmann (PB) equation, provide quantitative predictions of ion distribution about charged rods (5–7). This approach entails several approximations in its treatment of the ions. Most importantly, properties associated with discrete ions are not considered, including correlations between ions or finite ion size (e.g., 8). Recent work suggests that a fortuitous cancellation between these two effects results in approximate agreement with experiments (9, 10), as early work has shown (5, 11, 12). Within this mean field theory, however, like-charged objects such as polyelectrolytes repel in all salt conditions. Changing the valence of the ions in principle alters the screening contribution and thereby the strength of repulsion, but does not lead to attraction. Experimental work suggests that the nonlinear PB equation

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provides reasonably accurate descriptions of highly charged polyelectrolytes in 1:1 solutions (when corrected for ion size), but deviations have been detected as ion valence increases (1, 13).

In situations with multivalent ions or high–surface charge densities, more complex interactions between polyelectrolytes result from the organization and dynamics of condensed ions surrounding the polyelectrolyte. Attraction between like-charged DNA was demonstrated in a series of pioneering Monte Carlo simulations (14). Attractive interactions with different physical origins, such as van der Waals interactions, are qualitatively different because the attraction in these strongly charged systems is purely electrostatic in character. In the past few years, a large number of theoretical investigations have focused on the existence and form of like-charge attraction (10, 15–28) and on the collapse behavior of the polyelectrolyte (29–33). Different approaches have been employed, such as density functional theories, integral equations, and field-theoretical calculations (34–37). One important point of consensus is that correlations between counterions condensed on the polyelectrolyte can generate attractions (5, 9, 38, 39). Oosawa (40) showed that when the distance between two like-charged polyelectrolytes is large compared to the ion size, correlations between thermal fluctuations of the condensed counterion layers can result in attractions. These ideas have been refined by a number of groups (31, 41). At close distances, spatial correlations between the ions on the polyelectrolyte surfaces become important. Local fields due to the spatial arrangement of charges on a macroion can lead to patterns of counterion binding (42–44). If these ions form an ordered lattice, attractions can result as counterions arrange themselves along the surfaces of adjacent macroions in opposing patterns. An elegant picture of interacting Wigner crystals was developed by Rouzina & Bloomfield (17) and Shklovskii (22). More recent field-theoretic work has focused on predicting ion distributions in the so-called strong coupling limit, in which the counterion charge or surface charge density exceeds the range of applicability of PB theories (45). In this limit, in which correlations between counterions are very strong, the spatial dependency of attractive forces has been predicted (27, 45, 46).

As noted above, sophisticated approaches have been employed to investigate the electrostatic contributions to ion binding and ion correlation. In biological systems, other interactions (e.g., chemically specific interactions, macroion geometry, ion hydration) can have important influences (47). These are discussed in later sections of this review.

POLYELECTROLYTE CONDENSATION IN GENERAL

The condensation of polyelectrolytes by multivalent ions and/or macroions is important for a wide range of fundamental biological and biomedical processes. From a simple experimental viewpoint, polyelectrolytes chains or rods condense or collapse into a compact phase from solution as the concentration of oppositely charged multivalent ions or macroions increases. Examples include DNA packing in viruses (48, 49), bacteria (50, 51), or chromosomes (52–55). Synthetic gene-delivery systems based on cationic polymers (56, 57) and dendrimers (58, 59) are used to compact DNA, as are those based on cationic amphiphilic membranes (20, 60–62). In general, charged amphiphilic membranes can self-assemble with polyelectrolytes into a broad range of phases (63–65).

One of the goals of this field is to acquire a broadly enabling and fundamental understanding of how counterion behavior influences the interactions between polyelectrolytes, and to achieve rational control of these interactions. Electrostatic aggregation of biological polyelectrolytes is important to a number of disease states. For example, histones promote the aggregation and fibrillation of α-synuclein, which is thought to play an important role in the pathogenesis of Parkinson's disease (66). In cystic fibrosis, anionic inflammatory polymers such as DNA, F-actin, and extracellular bacterial filaments bind to and sequester endogenous antibacterial proteins, such as lysozyme, β-defensins, and lactoferrins, which are all cationic (67, 68), thereby inactivating

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them. This effect contributes to permanent and ultimately lethal airway infections. Recent experimental and computational work suggests that the osmotic pressure of counterions released during electrostatic binding of oppositely charged macroions leads to an anomalous stability of these complexes (**Figure 1**). This knowledge enables the design of charge engineered antimicrobials that resist binding and inactivation by airway-inflammatory polymers (69).

It has indeed been empirically observed that polyelectrolytes precipitate out of solution as the salt concentration exceeds a critical value as described above, but as the salt concentration is increased beyond a second critical value, the polyelectrolyte precipitate redissolves into

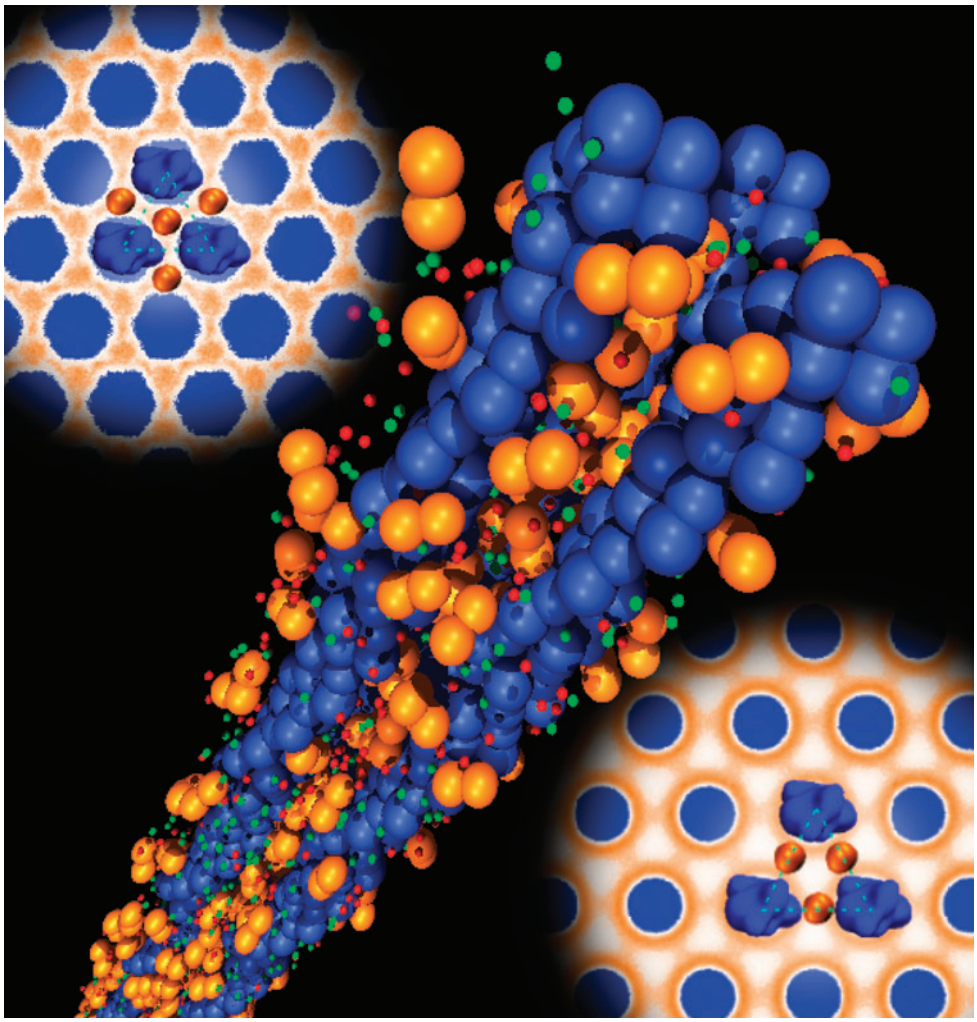

Figure 1

A bundle of actin filaments (*blue*) held together electrostatically by lysozyme (*orange*) in a salt solution, as obtained from molecular dynamics calculations in conjunction with synchrotron X-ray diffraction experiments. These complexes can contribute to persistent airway infections in cystic fibrosis by sequestering antimicrobials. Nonstick versions of lysozyme can be made: The reduction of lysozyme charge changes its geometric arrangement with actin, from a threefold to a twofold coordination, and destabilizes the bundle. Figure courtesy of Erik Luijten.

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solution (70). Recent experimental evidence illustrates the existence of both upper and lower limits on counterion concentration that result in condensed phases (e.g., 71). One explanation for this phenomenon is the inversion, or overcompensation, of polyelectrolyte charge (72, 73). Charge inversion has been directly observed for surfaces in the presence of multivalent ions (74). However, alternative proposals exist for polyelectrolytes. For example, a two-state model, collapsed or extended, that does not necessarily involve overcharging predicts instead a sensitive dependency on ion size (33). Other recent proposals focus on the importance of coions. One simulation links the occurrence of overcharging with ion size via coion interactions (75). An alternative description of these phenomena, relying on incomplete dissociation of counterions from coions at high salt concentrations, has also been proposed (64).

CONDENSATION OF POLYELECTROLYTE CHAINS: THREE PROTOTYPICAL EXAMPLES

F-actin

Rod-like biological polyelectrolytes have been recently used as experimental systems for the study of like-charge polyelectrolyte attraction. These include the filamentous bacteriophages, microtubules, and F-actin (60, 76–78). Owing to their simple geometry, these idealized rod-like polyelectrolytes (with persistence lengths of a micrometer or more) are particularly well-suited for comparison with theory.

In simple, abstract line-charge models, attractions between like-charged polyelectrolytes have been found. The finite diameter of polyelectrolytes has been considered in a number of models (21, 28, 79). A recent Monte Carlo simulation study of polyelectrolyte cylinders found that the finite polyelectrolyte diameter and the resultant angular degrees of freedom for condensed counterions significantly change the nature of the induced attractions relative to simple line-charge models (80). Interestingly, the net charge of the polyelectrolyte together with its associated ions does not always have to be neutral for like-charge attraction to occur, and the threshold fraction of charge that needs to be neutralized on the polyelectrolyte surface before attractions are observed depends on the valence of the ions (79).

It is well-known that a system of neutral rods will exhibit a first-order isotropic-nematic transition (81, 82) as a function of rod concentration. When the rods are charged, the spatial distribution of the counterions and coions becomes important. Onsager originally modeled electrostatic interactions within the Debye-Huckel approximation, in which he worked in the limit of high ionic strength $kd \gg 1$, where k is the Debye screening length and d is the rod diameter. In this formulation, the role of electrostatics is to increase the effective rod diameter and hence favor the nematic phase (83, 84), without qualitative changes to the isotropic-nematic transition. However, as two charged rods at low ionic strengths approach one another, they prefer to be perpendicular to one another to minimize electrostatic repulsion (83–86). Considerable experimental (87–89) and theoretical work (83, 84, 86, 90–93) has been done on this topic.

In the presence of multivalent ions, the phase behavior of polyelectrolytes becomes even more complex owing to the possibility of different types of ion-induced attractions. The mechanisms described above for multivalent ion-induced polyelectrolyte attraction are all relatively short ranged, whereas the imperfectly compensated polyelectrolyte rods are mutually repulsive at large distances. [For example, counterion correlations on polyelectrolyte surfaces disappear beyond ~1 nm in simulations (79).] The competition between long-range electrostatic repulsion and short-range attractions can drive the formation of qualitatively different structures. The behavior of linear polyelectrolytes in dilute and semidilute salt solutions is a useful starting point

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of comparison (21, 94, 95). Macroscopic phase separation is predicted for both regimes, but a gelation transition is observed in systems with strong short-range attractions. Owing to the competition between gelation and phase segregation in the presence of multivalent ion linkers (94), gelation tends to be suppressed because of electrostatic repulsions between cross-linked charged chains: Dilute polyelectrolyte solutions macroscopically phase segregate, whereas gels can form in semidilute solutions only if the ion size is smaller than both the Bjerrum length and the distance between charged monomers. This is consistent with the empirical observation that ions with larger hydrated sizes are less multivalent and require larger threshold concentrations before inducing polyelectrolyte condensation.

Ion size effects can be investigated using simple model systems, such as dumbbell-shaped divalent ions. Using a family of homologous diamine ions (molecules with two cationic amine groups connected by a spacer of variable length), a recent experiment demonstrated that the small diamines condense polyelectrolyte rods, whereas the larger diamines cannot (96). The addition of a single CH_2 group to the spacer will suppress multivalent-ion-driven polyelectrolyte condensation. Ion size and shape effects for different ions with the same valence can strongly influence polyelectrolyte condensation. These results have been confirmed in a recent computer simulation, which laid the groundwork for a general, microscopic understanding of prototypical dumbbell ions (97).

With rod-like polyelectrolytes in multivalent salt solutions, liquid crystalline effects can have strong consequences. As the divalent ion concentration is increased, F-actin rods organize into a lamellar liquid crystalline network phase of cross-linked rafts before fully condensing into bundles comprising close-packed F-actin rods (60). This phase behavior of F-actin in physiological salt solutions may have potential implications for the biology of the cytoskeleton, as these are the baseline nonspecific interactions on top of which architecture-altering actin-binding proteins exert their influences.

Multi-axial liquid crystalline gels and networks have been investigated in a variety of contexts (98). Interestingly, it was predicted in the 1970s that a cubatic phase of polyelectrolytes (a positionally ordered phase with cubic symmetry) may exist with no added salt because of the electrostatic repulsion between rods that enforce mutually perpendicular rod orientations (99). The phase was never observed. However, in the presence of strong linkers such as multivalent ions that can form cross-links between polyelectrolyte rods, and thereby maintain a high local rod density, the repulsive interactions responsible for the orientational behavior are expected to be stronger. Bruinsma (100) generalized the Onsager theory of nematic liquid crystals to multi-axial phases of rod-like polyelectrolytes. He found that multi-axial liquid crystalline phases (such as cubatics, tetratics, and trigatics) can exist near regions of phase coexistence between the isotropic phase and dense bundle phase. The sequence of experimentally observed phases with increasing multivalent salt concentration, from isolated rods to aggregated networks of rods and aggregated bundles of rods, was recently confirmed in an explicit ion, continuum dielectric molecular dynamics simulation (101).

The dynamics of these exotic orientationally ordered polyelectrolyte systems, as seen in the above liquid crystalline network phases, is largely unexplored. Slow modes have been observed using light scattering in high-density polyelectrolyte solutions with no added salt, which has been attributed to the possible formation of star-like complexes (102, 103). The orientational ordering behavior of polyelectrolyte rods at high densities has been recently studied using Monte Carlo simulations. These are inherently frustrated systems. As a system of like-charged rods condenses, the rods will reorient to minimize the total energy. Because electrostatic interactions are long ranged, there are a large number of configurations with similar total energies that are far apart in configuration space. Owing to this electrostatic frustration, Fazli et al. (104) found that a simple system of charged rods exhibits exotic chiral phases with different twist angles as well as slow dynamics.

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As the concentration of multivalent ions increases, the open multi-axial phases will collapse into a close-packed bundle. F-actin is usually approximated as a cylinder with a diameter of 7.5 nm and a linear charge density of –1e/0.25 nm. Although an idealized charged cylinder is a useful guide to our intuition, F-actin, like most biological polymers, is significantly more complex. Each monomer (∼5.5 nm in size) has heterogeneous charge distribution of anionic as well as cationic amino acids, which is repeated along the symmetry of a 13/6 helix, or 13 monomers in 6 full helical turns. In bundles of F-actin, the F-actin helix will overtwist to match its local charge density with the counterion distribution (60), as described below.

DNA

dsDNA has a linear charge density of −2e/0.34 nm, corresponding to two electrons per base pair. In contrast to rod-like polymers, the DNA molecule is described as a semiflexible polymer with three independent degrees of freedom: bending, twisting, and contraction/extension (105). A detailed description of factors that contribute to the elasticity of DNA is presented in a recent review (47). It is well-known that the bending and twisting of DNA are linked to its function (106), and the energetic cost of bending is an important consideration in DNA condensation. Because dsDNA has a long elastic persistence length, of order 500 Å or 150 bp, long dsDNA chains are effectively modeled as worm-like chains (107). dsDNA that are short relative to the persistence length are commonly used in biophysical studies because they are effectively treated as rigid rods (108). Recent work has focused on identifying deviations from this conventional elastic model (109). Here, the application of novel, single-molecule techniques to this active experimental field (e.g., 110–112) may be illuminating.

Genomic DNAs are long but efficiently packaged. For example, DNA in the T4 phage genome contains 160 kbp and has a contour length approaching 54 μm, yet it can be packaged into a viral capsid of 100-nm diameter (113). It is interesting to consider the forces involved in DNA packaging. The high linear charge density leads to strong Coulomb repulsion between adjacent strands. Other important considerations include the loss of DNA configurational entropy and the energetic expense associated with deforming the stiff DNA helix. In addition, counterions contribute both enthalpic and entropic terms to the overall free energy. In vivo DNA packaging in bacteriophages requires assistance from ATP-powered motors that pump DNA into the capsid against high pressures (e.g., 114). In eukaryotic genomes, multiple proteins are involved in DNA packaging, notably the histones that DNA encircles.

Biophysical studies have benefited from the induction of condensed DNA phases in vitro. Various phases have been identified, depending on the concentration and length of the DNA, as well as on the presence of condensing agents, including multivalent ions, basic proteins, crowding polymers, cationic liposomes, and alcohols (113, 115). DNA strands containing more than 400 bp pack into dense toroids (**Figure 2**) or rod-like structures in the presence of multivalent ions alone. Within these tightly organized structures, some of the DNA is found in a highly organized hexagonal lattice, which can model densely packaged genomes (116).

DNA condenses when multivalent ions (including polyamines) are added (e.g., 117–119). At the simplest schematic level, multivalent ions are thought of as electrostatic bridges between negative charges on DNA, and such ions can induce the formation of compact phases. However, electrostatic arguments alone do not explain many observed ion-specific effects in DNA condensation. For example, some liquid crystalline phases observed in the presence of the trivalent polyamine spermidine do not occur in the presence of trivalent cobalt hexammine (70). Short DNA strands also form liquid crystalline aggregates, either hexagonal or cholesteric, in the presence of spermidine or spermine (120). In general, the interhelical spacing of DNA within such complexes has

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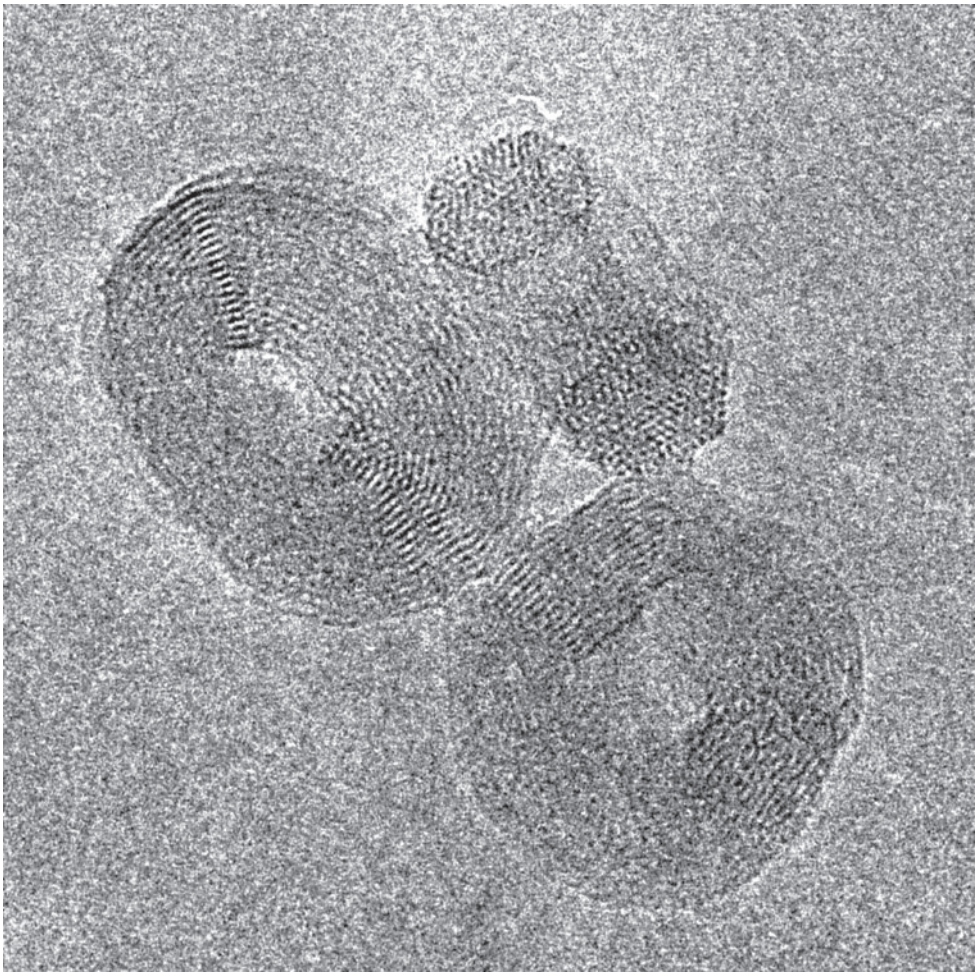

Figure 2

DNA in toroids. Cryo-electron microscope picture courtesy of Nicholas Hud.

been observed to vary from 2.8 nm to 3.3 nm and depends on the precise ionic conditions (121), attributed to the different ionic components in the DNA aggregate itself. Osmotic pressure can be used to drive DNA into ordered arrays (122) and can influence electrostatic effects. In a recent set of experiments (123, 124) combining osmotic stress measurements with single-molecule magnetic tweezer measurements, the authors extracted the ratio of attractive to repulsive contributions to the condensing force.

In addition to electrostatics and osmotic pressure, chemical interactions between ions and specific binding sites on DNA can also be important. Different ions of the same valence have different interaction modes (Mn^{2+} versus Ca^{2+} versus Mg^{2+}) or prefer different binding sites on the DNA (47). Clear effects are also attributed to differences in ion size and geometry (70, 125). Ion hydration and site-specific binding also contribute (e.g., 126). Additional considerations involve the structure of the underlying surfaces, such as the geometry of the grooves (127) and the helical pitch of the backbone (47). Ion bridging (44) may also be relevant [even when transient (128)]. Finally, hydration forces, although not explicitly connected with ion distributions, must also be considered (123).

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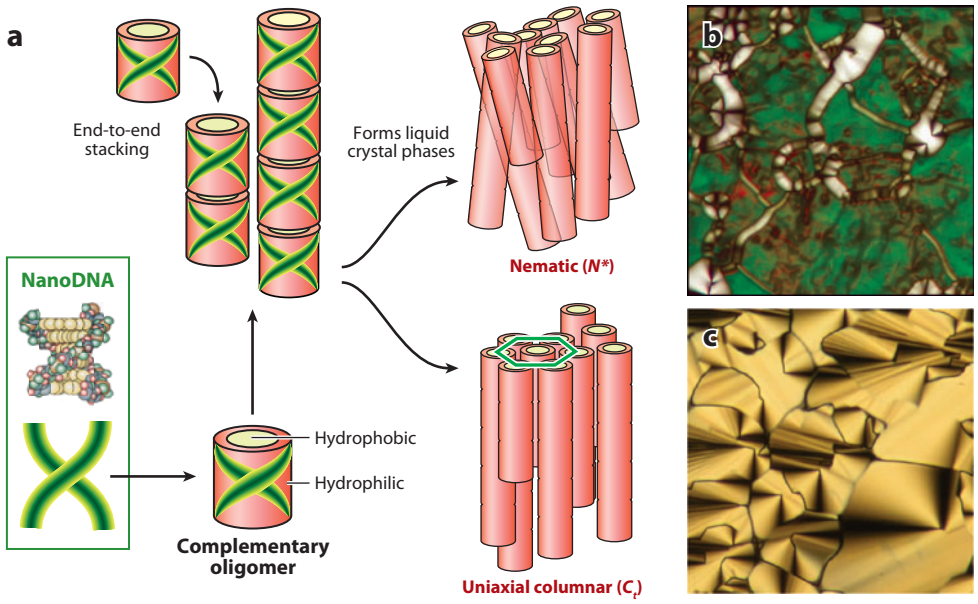

Figure 3

(*a*) Short DNA duplexes at high concentration self-organize into liquid crystal phases. B-form DNA duplexes can stack end to end to create anisotropic units. Depending on the DNA concentration, either a nematic phase (*top*, *right*) or a columnar phase (*bottom*, *right*) has been observed. (*b*,*c*) These longer structures can self-assemble into macroscopically observable phases. This work is described in Reference 129. Figure courtesy of Noel Clark, adapted for publication by Annual Reviews.

Recently, systematic studies have employed numerous copies of manufactured dsDNA with identical sequences (e.g., 129, 130). These duplex DNA are much shorter than a persistence length (150 bp); the shortest molecules studied contain only 6 bp. Studies of short duplexes are favorable for direct comparison with simulation [although end effects may be present (131)]. As in previous studies, condensation of these short DNA can be induced either by increasing the concentration of the DNA (129, 132), and observing the rich, liquid crystalline phase behavior, or by adding multivalent ions to semidilute solutions (130). Short DNA strands stack end to end when surrounded by either monovalent or divalent ions (129, 133) because of a favorable interaction between exposed terminal base pairs. **Figure 3** illustrates the self-organization into macroscopically observable phases of short DNA duplexes at high concentration. By providing optimized conditions for packaging (and by extension unpackaging), these experiments on short nucleic acid strands have applications to small-molecule therapies (134) involving aptamers or short dsRNA strands for RNA interference. In this latter process, the delivery of 20–26-bp dsRNA can be exploited for therapeutic applications (135). It is intriguing that the differing topologies of RNA and DNA lead to different interactions with ions (127).

RNA

Like DNA, RNA is uniformly negatively charged and attracts oppositely charged counterions. Unlike DNA, the majority of physiological RNAs are single-stranded molecules. Functional RNA structures contain rigid, short helical regions joined by more flexible single-stranded regions, loops, hinges, or junctions (136). Because of the electrostatic effects discussed above, RNA structure

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is intricately linked with ions. Most RNAs fold in the presence of small, typically millimolar quantities of Mg^{2+}. The energetics of ion-RNA interactions has been recently reviewed (6) and suggests at least two different roles for ions in interactions with RNA structures, distinguished by ion hydration. Fully hydrated ions stabilize folded RNA structures by screening the backbone charge, enabling the close approach of RNA helices while other stabilizing interactions take hold. Dehydrated ions make specific contacts to the backbone and forge strong connections between different regions of the chain (137).

Because functional RNAs form compact structures, there has been speculation about a Mg^{2+}-induced attractive force between double-stranded regions in a larger molecule. It has been suggested that this force might arise from ion correlations (138), tight ion binding (42), or from advantageous changes to the free energy resulting from counterion delocalization (139). Experiments devised to measure attractive forces between tethered DNA helices found no evidence of a Mg^{2+}-induced attraction (140). More recent experiments probed interactions between isolated RNA helices and found only end-to-end stacking and no evidence for side-by-side packing (127).

Compact or folded RNA structures are stabilized by long-range or tertiary contacts that form within an electrostatically relaxed ensemble (141). The fully hydrated counterions effectively screen the charge on the backbone, facilitating the formation of contacts between remote parts of the molecule, but they do not generate attractive forces on their own. Site-bound ions, conversely, do forge strong links between remote regions on the chain; however, very few ions of this type are found in RNA structures (142).

COUNTERION ORGANIZATION AND DYNAMICS NEAR BIOLOGICAL POLYELECTROLYTES

Because counterions play a central role in the generation of interpolyelectrolyte forces, it is important to quantify their spatial distribution, correlations, and dynamics. We end this review with a summary of recent progress made toward addressing this challenging problem.

Several detection methods provide information to distinguish associated from free counterions. These techniques include changes to nuclear magnetic resonance relaxation rates due to the localization (5), measurements of energy transfer between luminescent ions that depends on collision frequencies (12), and the use of ion-sensitive dyes that provide a precise measure of the free-ion concentration (143). Progress has been made by ion-counting experiments (13, 144) that report the different numbers of ions that are attracted (counterions) or repelled (coions) because of the presence of a charged macroion.

To obtain information about the spatial distribution of the ions, intimately linked with the mechanism for counterion-induced attraction, techniques with spatial sensitivity on the angstrom length scale are required. X-ray scattering methods have provided an important perspective on this problem.

Small-angle X-ray scattering provides information about the spatial distribution of all components in the system. Counterions contribute in a unique way to the scattering of the DNA-counterion system. Early measurements of ionic distributions around rod-like DNA structures relied on heavy atom replacement to emphasize the scattering from the ions relative to the nucleic acid (145). More recently, anomalous small-angle X-ray scattering has been used to highlight scattering originating from the counterions. The distributions around DNA of monovalent, and divalent ions, alone (146) and in competition (147), have been measured. The ion-size corrected nonlinear PB equation was found to adequately account for the spatial distribution of monovalent ions around DNA (147). **Figure 4** illustrates the association of monovalent ions to DNA. The importance of finite ion size was validated by subsequent ion-counting measurements (13). The

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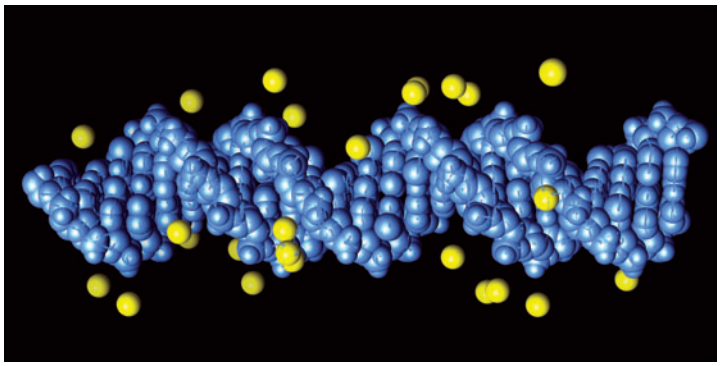

Figure 4

Counterions around DNA, showing the spatial distribution of monovalent ions (*yellow*) around DNA (*blue*). Ion locations are determined by computing the electrostatic potential around the DNA, using anomalous small-angle X-ray scattering (127). As described in Reference 1, monovalent counterions are then randomly distributed, consistent with Boltzmann factors for this potential. One possible ion distribution is shown here.

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distribution of divalent ions also appears to be well described by the nonlinear PB equation, although other measurements suggest that divalent ions compensate more charge than expected based on the PB equation alone (1, 13). The distribution of trivalent ions, including cobalt hexammine and spermidine, can also be extracted when these multivalent ions are in competition with monovalent ions (2). Anomalous small-angle X-ray scattering experiments indicate that more highly charged ions are more closely localized to the nucleic acid surface than their lower-charged counterparts. Finally, differences in the ion distribution around DNA and RNA molecules of identical sequence have been reported, highlighting the importance of helix geometry to ion condensation (127).

Although experiments show unambiguously that an attractive interaction exists, there has been little done on measuring actual counterion correlations, which are necessary to generate attractions. The organization of divalent Ba ions on actin filaments was studied using synchrotron X-ray diffraction (60). Interestingly, the counterions do not form a lattice that simply follows actin's helical symmetry; rather, they organize into one-dimensional counterion density waves parallel to the actin filaments (**Figure 5**). Moreover, this counterion charge density wave is coupled to torsional distortions of the oppositely charged polyelectrolytes, so that attractions are optimized via charge alignment between the counterion domains and polyelectrolytes. It will be interesting to see how this counterion organization is modified under different conditions. For example, it has been shown that the structure of a counterion lattice within a condensed polyelectrolyte rod phase undergoes a series of shearing transitions as the spacing between the rods decreases (148).

The spatiotemporal correlations and collective dynamics of counterions that mediate binding between F-actin in aqueous solution have recently been directly measured using high-resolution inelastic X-ray scattering (149). The counterions exhibit a new acoustic-like phonon mode, which is strong evidence for the existence of counterion correlations. The dynamics of ions interacting with their cages of nearest neighbors can be inferred from deGennes narrowing effects seen in the widths of Brillouin peaks at large wave vectors, suggesting that the counterions are in a liquid-like phase. These results suggest that counterions are hierarchically organized: At large length scales compared to the actin monomer, they modulate their density into domains or density waves that follow charge variations of the F-actin surface. At small length scales within the domains, counterions exhibit liquid-like correlations and dynamics. The measured speed of sound and collective relaxation rates in the liquid-like domains agree well with model calculations. Interestingly, similar

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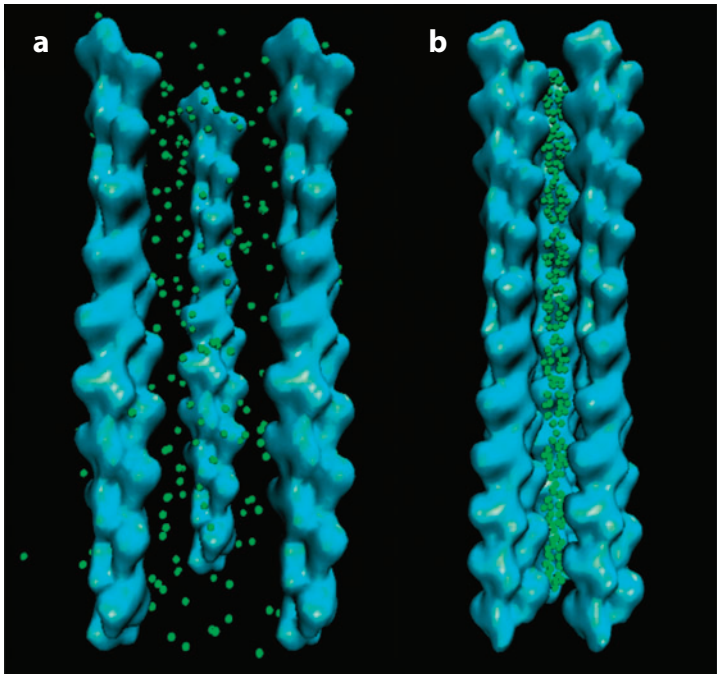

Figure 5

Schematic representation of (*a*) uncondensed F-actin and (*b*) condensed F-actin bundles. At high multivalent ion concentrations, the ions on F-actin organize into counterion density waves that couple to twist distortions of the F-actin helix. Recent inelastic X-ray measurements show that ions in such counterion density waves are in a liquid-like state and exhibit caging dynamics.

distributions of ions are observed between RNA kissing loops, a common structural motif found in many RNA-mediated processes, such as antisense recognition, riboswitches, and viral replication (150).

CONCLUSION

In this article, we review ion-mediated polyelectrolyte interactions. Validations and modifications of unifying concepts are beginning to emerge in measurements and simulations of prototypical systems, such as DNA, RNA, and cytoskeletal polymers. Owing to recent progress in theory and computation, and the application of novel experimental tools, it is now possible to make contact between the fundamental physical chemistry of highly charged systems and a broad range of biological and biomedical processes.

SUMMARY POINTS

1. Polyelectrolyte association occurs in a broad range of biological phenomena. Such association is often mediated by oppositely charged counterions (such as divalent Mg ions) or macroions (such as charged proteins).
2. Theoretical approaches beyond commonly used mean-field models are required to explain the attraction between like-charged biological polymers.

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3. Novel experimental and computational approaches are being applied to quantify the role of counterions in polyelectrolyte association.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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