Structure and Stability of Self-Assembled Actin-Lysozyme Complexes in Salty Water

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Interactions between actin, an anionic polyelectrolyte, and lysozyme, a cationic globular protein, have been examined using a combination of synchrotron small-angle x-ray scattering and molecular dynamics simulations. Lysozyme initially bridges pairs of actin filaments, which relax into hexagonally coordinated columnar complexes comprised of actin held together by incommensurate one-dimensional close-packed arrays of lysozyme macroions. These complexes are found to be stable even in the presence of significant concentrations of monovalent salt, which is quantitatively explained from a redistribution of salt between the condensed and the aqueous phases.

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In the presence of multivalent cations, anionic biological polyelectrolytes can overcome their electrostatic repulsion and exhibit a mutual attraction. These “like-charge attractions” result from ion correlations that cannot be understood within mean-field theories such as the commonly employed Poisson-Boltzmann formalism [1–3]. The problem becomes more complex when the mediating multivalent cations are themselves macroions. Macroion-polyelectrolyte complexes occur in many physical systems, such as DNA-dendrimer complexes for nonviral gene therapy [4] and antimicrobial binding in cystic fibrosis [5]. Various factors affect their formation: the presence of salt can lead to an attraction driven by osmotic pressure [6]. Differential screening of positive and negative charges distributed on the surface of a macroion may significantly modify interactions at the macroion-polyelectrolyte interface [7]. Entropic gain due to mutual neutralization and consequent counterion release upon macroion-polyelectrolyte “adhesion” is expected to be important, but can be potentially modulated by the steric commensurability between the charge pattern on the polyelectrolyte and the macroion size [8]. The relative importance of all these interactions, and how they modify one another in their combined effect on the structural evolution of macroion-polyelectrolyte complexes, is generally unknown.

In this Letter, we examine the role of several of the above-mentioned interactions in the complexation of actin and lysozyme, a prototypical system of oppositely charged “rods” and “spheres,” over a range of monovalent salt concentrations. Using synchrotron small-angle x-ray scattering (SAXS), we show that self-assembled complexes are comprised of hexagonally coordinated columnar arrangements of actin held together by one-dimensional (1D) arrays of lysozyme macroions at the threefold interstitial “tunnels” of the columnar actin sublattice (Fig. 1). Molecular dynamics (MD) simulations using a realistic model of the actin helix provide a detailed confirmation of this picture, and reveal structural reconstructions and corresponding salt redistribution within an actin-lysozyme bundle as the inter-actin separation is varied. Both experiment and simulation show that the lysozyme is arranged in a close-packed manner, incommensurate with the actin periodicity. Moreover, the self-assembly of columnar actin-lysozyme complexes is enhanced for higher concentrations of monovalent ions. We believe that these results can be explained by significant partitioning of salt between

FIG. 1 (color). (a) Synchrotron 2D x-ray diffraction pattern of partially aligned actin-lysozyme bundles, formed in a solution containing 150 mM KCl. (b) 1D integrated slices along the \( q_z \) and \( q_r \) directions with arrows marking the actin-actin close-packed bundling peak (1), the actin helix form factor (2), and the lysozyme-lysozyme correlation peak (3). (c) Proposed structure of actin-lysozyme composite bundles (side and end views): lysozyme (orange) is close packed in threefold symmetric sites between actin filaments (blue).
the condensed and the aqueous phases, which strongly modifies screening effects.

F-actin is an anionic rodlike cytoskeletal polymer (diameter 7.5 nm, charge density $-e/0.25$ nm, persistence length 10 μm). Lysozyme is approximately an ellipsoid of size 2.6 nm × 2.6 nm × 4.5 nm with a net charge of +9e at neutral pH. Monomeric G-actin (MW 42 000) was prepared from a lyophilized powder of rabbit skeletal muscle. The nonpolymerizing G-actin solution contained a 5 mM TRIS (tris(hydroxymethyl)aminomethane) buffer at pH 8.0, with 0.2 mM CaCl$_2$, 0.5 mM ATP, and 0.2 mM DTT (dithiothreitol) and 0.01% KCl. Human plasma gelsolin was used to control the osmotic pressure to the osmotic pressure of the salt (100 mM KCl).

In order to elucidate the underlying mechanism for bundle formation and the structure of the resulting complex, we have performed MD simulations using a modified version of Moldy [10]. In these simulations, G-actin is modeled using the four-sphere model [11], which is based upon crystallographic measurements and provides a relatively accurate coarse-grained representation of the monomer charge distribution. F-actin is comprised of a sequence of these monomers, in which successive units have a separation of 27.5 Å and a relative rotation of 166.7° about the filament axis. This leads to a helical structure with a repeat unit of 13 monomers. The filaments are assembled into a parallel hexagonally coordinated bundle. An elementary simulation cell consists of a bundle fragment containing 2 × 2 filaments with a length of 6 repeat units (78 monomers) each. This cell is periodically replicated in all directions. Following the experiments, we set the lysozyme concentration in the bundle to neutralizing conditions, which corresponds to 352 lysozyme units per simulation cell. Each lysozyme is modeled as a rigid dumbbell structure of two spheres with diameter 25 Å and charge 4.5e, at a center-to-center distance of 20 Å, thus approximating the aforementioned ellipsoidal dimensions. Additional salt is modeled as monovalent spherical particles with a hydrated radius of 3.3 Å. Coulomb interactions are treated by means of Ewald summation, and excluded-volume interactions are represented by pair potentials of the form $k_BT(\sigma/r)^{12}$, where $\sigma$ is the sum of the effective radii of two interacting particles (ions, G-actin subunits, and lysozyme subunits). During each simulation, the actin separation is fixed, whereas lysozyme and all ions move freely. Thus, the simulations probe the stability of a swelling bundle while maintaining the filaments in a parallel configuration, ignoring their rotational degrees of freedom. This is justified by the observation that the calculations are confined to actin separations below 25 nm, i.e., less than 0.25% of the persistence length. Mutual sliding and axial rotation of the filaments are not taken into account.

This model indeed predicts electrostatically driven complex formation. Since the water is modeled as a dielectric continuum, the osmotic pressure $\Pi$ can be obtained directly from the virial involving all interparticle forces [12]. Bundle formation takes place in excess solution, and hence the bundle stability follows from a comparison of the osmotic pressure of the salt $\Pi_{salt}$.
A negative osmotic-pressure difference $\Delta \Pi = \Pi - \Pi_{\text{salt}}$ implies bundle contraction and the free-energy minimum ($\Delta \Pi = 0$ and $\partial (\Delta \Pi)/\partial V < 0$) yields the stable actin separation. A comparable approach has been employed before [see Refs. [13,14] and references therein] to study the condensation of rodlike polyelectrolytes by counterions. Figure 2 shows that, under salt-free conditions, an inter-actin spacing of $\sim 100 \, \text{Å}$ is predicted, in quite close agreement with the experimental observations.

Having established that our model captures essential aspects of actin-lysozyme complexation, we exploit it to elucidate the structural properties and evolution of the resulting complexes. Figure 3 shows contour plots of the lysozyme center of mass, projected on a plane perpendicular to the bundle axis. In order to minimize artifacts resulting from the finite actin length, the calculations employed filaments consisting of 12 repeat units (156 monomers). In the equilibrium configuration [Fig. 3(a)], the maximum lysozyme concentration occurs in the threefold interstitial regions between the actin filaments, supporting the interpretation of the SAXS diffraction data in Fig. 1. Furthermore, the lysozyme pair correlation function (inset of Fig. 2) along the actin axis shows a clear peak at $z = 50 \, \text{Å}$, corroborating the experimentally measured close-packed value of 48.3 Å [15]. Minor enhancements of the lysozyme concentration can also be seen in the bridging regions between pairs of neighboring filaments. The lysozyme distribution is governed both by entropic effects and by a competition between electrostatic protein repulsions and actin-lysozyme attractions. Also, the helical actin structure imposes an excluded-volume repulsion with a circular regions) also has a more pronounced hexagonal structure in panel A, which is caused by the precessing highly charged regions on the monomers. Consideration of larger lattice spacings provides information on intermediate states that may arise during the complexation process. As illustrated in Fig. 3(c), significant rearrangements occur in the final stages of the bundle formation: at the osmotic-pressure minimum (free-energy inflection point), i.e., at an actin separation that is increased by merely 16 Å, lysozyme is depleted from the interstitial regions and instead predominantly occupies the bridging sites. Figure 3(d) confirms that this lysozyme distribution is again dominated by electrostatic effects. Thus, the complex evolves from lysozyme at bridging positions between pairs of actin rods to the final threefold positions observed by experiment. Lysozyme and actin "contact" interactions are maintained during this structural relaxation, indicating that their mutual electrostatic attraction plays an important role.

It is interesting to consider how this self-assembly is affected by the addition of monovalent salt. The counterion release mechanism implicit in actin-lysozyme binding will strongly modify qualitative arguments based on screening. The partitioning of salt between the condensed and aqueous phases implies a different degree of screening inside and outside the complex. In addition, it can lead to a stabilizing external osmotic pressure. Such a redistribution of ions is often not taken into account theoretically. To study these effects, a series of SAXS measurements were performed on actin-lysozyme complexes at different NaCl concentrations. As [NaCl] is increased to 150 mM, the turbidity increases and the intensity of the lysozyme-actin diffraction peak increases without significant changes in its peak width [Fig. 4(a)], indicating the formation of more bundles (rather than bundles that are more ordered and have larger coherent domains). At higher salt concentra-

![FIG. 2 (color online). Osmotic pressure of a hexagonally coordinated bundle of actin filaments without added salt, as determined from MD simulations. Inset: lysozyme pair correlation function along the filament axis. For discussion see the text.](image-url)
In summary, by studying self-assembled actin-lysozyme complexes via a combination of small-angle x-ray scattering and molecular dynamics simulations, we have shown that salt partitioning impinges strongly on the structure and stability of the complex, and qualifies commonly invoked mechanisms such as counterion release and differential screening.

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FIG. 4. Series of diffraction data showing the evolution of bundle structure as a function of (a) NaCl and (b) KCl concentration with maximum bundling occurring around 120 mM. (c) Simulated (○) bundle vs bulk ion concentrations for a stabilized actin-lysozyme complex. Salt partitions into different concentrations inside and outside the bundle. The dashed line is a guide to the eye.

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[15] To avoid cross correlations between different threefold interstitial regions, the pair correlation function is computed for each region separately.