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 \[1, 2, 3\]. 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 non-viral 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 three-fold 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 repartitioning of salt.
between the condensed and the aqueous phases, which strongly modifies screening effects.

F-actin is an anionic rod-like cytoskeletal polymer (diameter 7.5nm, charge density $-e/0.25\text{nm}$, persistence length $10\mu\text{m}$). Lysozyme is approximately an ellipsoid of size $2.6\text{nm} \times 2.6\text{nm} \times 4.5\text{nm}$ 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 non-polymerizing G-actin solution contained a 5mM TRIS buffer at pH 8.0, with 0.2mM CaCl$_2$, 0.5mM ATP, and 0.2mM DT and 0.01% NaN$_3$. G-actin (2mg/ml) was polymerized into F-actin upon the addition of salt (100mM KCl). Human plasma gelatin was used to control the average F-actin length to $\sim 1\text{mm}$. The F-actin filaments were treated with phalloidin (MW 789.2) to prevent depolymerization. Hen egg white lysozyme (MW 14,300) was mixed with F-actin in 1.5mm diameter quartz capillaries to form isoelectric actin–lysozyme complexes. SAXS experiments were performed both at Beamline 4-2 of the Stanford Synchrotron Radiation Laboratory as well as at an in-house x-ray source. The incident synchrotron x-rays from the 8-pole Wiggler were monochromatized to 8.98KeV ($\lambda=1.3806\text{Å}$) using a double-bounce Si(111) crystal, focused using a cylindrical mirror. The scattered radiation was collected using a Bruker 2D wire detector (pixel size 79μm). The 2D SAXS data from both systems are mutually consistent.

A 2D diffraction pattern for partially aligned isolectric F-actin–lysozyme bundles and its associated 1D integrated slices along the $q_z$ and $q_r$ directions are shown in Figs. 1A and 1B. Examination of the slice along the equatorial ($q_r$) direction shows a correlation peak at $q=0.07\text{Å}^{-1}$ that corresponds to close-packed composite actin–lysozyme bundles. The inter-actin spacing of 90Å is consistent with a columnar actin lattice expanded by lysozyme in three-fold interstitial “tunnels,” aligned with its long axis parallel to the actin. This inter-actin spacing is significantly larger than the 75Å spacing for close-packed actin condensed with multivalent ions. No other arrangement of lysozyme and actin will reproduce this diffraction pattern, given their respective sizes. Along the meridional ($q_z$) direction, a weak, mosaic-smeared actin form factor feature at 0.113Å$^{-1}$ is observed, as well as a new, strong correlation peak that differs from expected actin form factor features (layer lines) in position, orientation, and relative intensity. The appearance of this peak at $q_z=0.130\text{Å}^{-1}$ corresponds to an inter-lysozyme distance of 48.3Å, comparable to the length of lysozyme along its major axis, which suggests that lysozyme is close-packed along this direction within the bundles. Interestingly, this lysozyme periodicity is incommensurate with the projected actin periodicity ($\sim 56\text{Å}$), in contrast with the behavior of divalent ions on actin. This incommensurate arrangement permits charge matching between actin and lysozyme within the bundle, and indicates the important role of entropy gain from counterion release in this system. Figure 1C shows schematic representations of a condensed bundle.

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. In these simulations, G-actin is modeled using the four-sphere model, 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 27.5Å and a relative rotation of 166.7° around 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 25Å and charge 4.5e, at a center-to-center distance of 20Å, thus approximating the aforementioned ellipsoidal dimensions. Additional salt is modeled as monovalent spherical particles with a hydrated radius of 3.3Å. Coulomb interactions are treated by means of Ewald summation, and excluded-volume interactions are

![FIG. 2: Osmotic pressure of a hexagonally-coordinated bundle of actin filaments in excess solution, as determined from MD simulations. Inset: lysozyme pair correlation function along the filament axis. For discussion see the text.](image-url)
represented by pair potentials of the form \( k_B T (\sigma/r)^2 \), 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 25nm, 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 to the osmotic pressure of the salt \( \Pi_{\text{salt}} \). A negative osmotic pressure difference \( \Delta \Pi \equiv \Pi - \Pi_{\text{salt}} \) 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 rod-like polyelectrolytes by counterions. Figure 2 shows that, under salt-free conditions, an inter-actin spacing of 0.25% of the persistence length.

**FIG. 3: Contour plots showing the lysozyme distribution in actin–lysozyme complexes without added salt.** Darker shading corresponds to higher concentrations. In the equilibrium configuration (A), lysozyme is predominantly located in the three-fold interstitial regions. The free-energy inflection point occurs at a slightly expanded lattice (C), in which lysozyme is depleted from these regions and occupies the bridging regions instead. Panels (B) and (D) are the counterparts of panels (A) and (C), respectively, in the absence of electrostatic forces.
increased to 150mM, the turbidity increases and the intensity of the lysozyme–actin diffraction peak increases without significant changes in its peak width (Fig. 4A), indicating the formation of more bundles (rather than bundles that are more ordered and have larger coherent domains). At higher salt concentrations, the trend reverses and a weakening of the bundling peak is observed. The same results are found using KCl (Fig. 4B), showing that this is not a cation-specific binding effect.

Since regular screening is likely to play a role in the ultimate disappearance of the bundle, we concentrate on the stability at low and intermediate salt levels. Simulations of bundles with additional salt show that the osmotic pressure within the complex rises more rapidly as a function of salt concentration than the bulk pressure of salt at the same concentration, leading to destabilization once the salt concentration exceeds ∼10mM. This apparent discrepancy with the experimental findings already suggests a redistribution of salt ions. To quantify this further, grand-canonical simulations of the bundle can be employed 

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 repartitioning 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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