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Phase Behavior and Coassembly of DNA and Lysozyme in Dilute Aqueous Mixtures: A Model Investigation of DNA−Protein Interactions

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Results from an experimental investigation of the phase behavior of an aqueous system of DNA from salmon testes and the protein lysozyme are presented. At very low concentrations of either or both of the macromolecular components, wormlike assemblies with a width of the order of 10 nm are formed. There are strong indications that direct interactions between the protein units are instrumental both in driving the phase separation and in controlling the morphology of the formed assemblies.

Introduction

Interactions between DNA and proteins are of the utmost importance for living organisms. The most immediate example is the intricate machinery centered around the nucleoprotein complex chromatin, which in eukaryotic cells mediates a highly efficient compaction of the DNA and plays important roles in organization of the genome and regulation of gene expression.[1]

From a physicochemical point of view, the underlying basis for the structure and function of nucleoproteins is largely a matter of the same types of intermolecular interactions that govern the behavior of any aqueous colloidal system. Thus, the mechanisms behind the organization and action of such complexes can, in a sense, be reduced to a dynamic balance between electrostatic, hydrophobic, and steric interactions.

A very useful approach for gaining fundamental understanding of a colloidal system is to investigate its phase behavior. Observables such as the number of phases, their range of existence, as well as their microscopic and macroscopic characteristics can give important clues regarding the forces at play on a molecular level.[2] Hence, phase studies on various colloidal systems, such as aqueous systems of amphiphiles, polymers, and/or proteins, have given substantial contributions to the general understanding of the relevant intermolecular forces. However, despite good potential, this approach has, to our knowledge, not been applied to investigate interactions between DNA and proteins.

Herein we present results from an experimental investigation of an aqueous system of DNA from salmon testes and the protein lysozyme. Lysozyme does, for multiple reasons, lend itself for use in a model investigation. It is an extensively studied, small (14.4 kDa) globular enzyme with a well-documented conformational stability; for instance, it retains a compact globular structure in the presence of high concentrations of surfactant.[3] In an unbuffered aqueous solution, it carries a net charge of +8 and gives a pH of 6.5.[4] Lysozyme has a multipolar charge distribution with distinct cationic and anionic patches, a common feature of proteins that strongly influence the interactions with other charged entities.[5] Salmon testes DNA has a size of ∼2000 ± 500 base pairs. To ensure the integrity of the double-stranded structure of DNA, all samples were prepared in the presence of a low concentration of simple salt.[6] In order to remove residual salts and other possible low molecular weight impurities, the DNA and lysozyme stock solutions were extensively dialyzed before the samples were mixed.

Results and Discussion

Figure 1 shows the dilute corner of a pseudoternary phase map for the investigated system. Concentrations are expressed in terms of negative charges from DNA, that is, one per nucleotide, and net positive charges from lysozyme, that is, eight per protein molecule. It can be noted that lysozyme has a significantly larger mass per charge than DNA, ∼1800 g/mol charges as compared to ∼330 g/mol. The investigated composition range is dominated by a two-phase region; samples are single-phase only at very low concentrations, below ∼0.01−0.05 mM with respect to net charge, of either or both DNA and lysozyme. Close to the phase border, phase separation is observable as a slight haziness of the samples, whereas at higher concentrations a flocculent precipitate is formed. At charge neutrality, a visible precipitate is formed up to very close to the water corner.

The phase border was determined turbidimetrically, by measuring the optical transmittance at 400 nm. To support the idea that the observed turbidity arises from phase separation and not the formation of soluble complexes,[7] samples were centrifuged; for all samples in the suggested two-phase region, a pellet could indeed be collected.

UV absorbance measurements on the supernatant from centrifuged samples show that both DNA and lysozyme are depleted from the solution as the precipitate is formed. This implies that the

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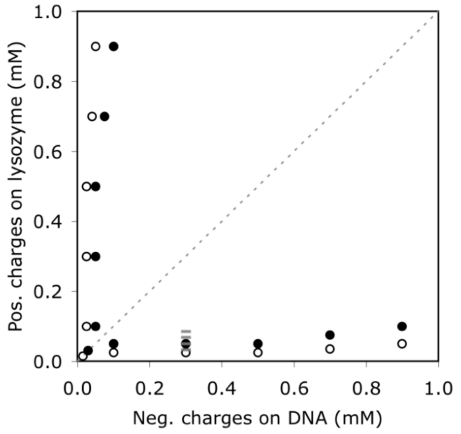

Figure 1. Partial phase map of the system DNA−lysozyme in 3 mM aqueous NaCl at pH 6.5 and 25 °C. Open circles represent the most concentrated one-phase samples, and filled circles the most dilute two-phase samples. The dotted line represents charge neutrality with respect to the macromolecular components. The gray dashes show the compositions of samples listed in Table 1.

new phase is concentrated in both these components; that is, the observed phase separation is of the associative type.[8] Associative phase separation is commonly observed in aqueous mixtures of oppositely charged polyions at low ionic strength and is predominantly a consequence of the entropic gain from release of the small counterions.[8] It is thus unlikely that the sodium or chloride ions contribute notably to the neutralization, and one can suppose that the precipitate essentially is composed by only the polyionic components.

The fact that the assemblies formed remain dispersed in solution for highly "unbalanced" DNA−lysozyme mixtures, as compared to the case for mixtures closer to charge neutrality (with respect to the polyionic components), where the new phase immediately separates, can reasonably be attributed to electrostatic stabilization by adsorption of excess DNA or lysozyme to the particle surface, which prevents clustering into a macroscopic precipitate.

Interactions with other charged entities can, by influencing the effective pK_a's of the acidic or basic amino acid residues, induce changes in the overall charge of a protein as well as in the charge distribution.[5] It is thus possible that the effective net charge of lysozyme could be different when it resides in assemblies with DNA as compared to the situation in solution. However, the finding that the border of the two-phase region is practically symmetric around the charge neutrality line suggests that the effective protein charge is indeed not altered on complexation with DNA. Furthermore, this suggestion is supported by the fact that pH remains unchanged as the precipitate is formed. It should be noted, though, that it is still possible, and likely, that the charge distribution is altered on formation of the mixed assemblies. The situation can be compared to that in aqueous mixtures of lysozyme and sodium dodecyl sulfate (SDS), where it has been found that lysozyme also retains a net charge of +8 but that the surfactant induces changes in the protein conformation (an increase in the fraction of β-structure was noted); however, as is also mentioned above, the protein remains compact and globular.[3]

Small-angle X-ray scattering (SAXS) experiments suggest that the precipitate has no well-defined molecular arrangement. Spectra of the collected solid show only weak, broad bumps centered at around $q = 0.17$ Å^{-1} (and in some cases a second bump at about twice this value) which corresponds to a characteristic distance of the order of 4 nm (calculated as $2\pi/q^*$, where q^* is the position of the first peak). The observed bumps reasonably arise from a distribution of protein−protein, DNA−protein, and DNA−DNA distances.

Table 1. Data on Selected Samples Used in Cryo-TEM and DLS Experiments

c_{DNA} (mM)	c_{lys} (mM)	r_{charge}[a]	r_{molar}[b]	$R_{H,app}$ (nm)[c]	PDI[d]
0.30	0.033	0.11	54	65	0.23
0.30	0.050	0.17	83	73	0.24
0.30	0.068	0.23	113	111	0.27
0.30	0.085	0.28	142	113	0.33

[a] r_{charge} = (net positive charges on lysozyme)/(negative charges on DNA). [b] r_{molar} = (moles of lysozyme molecules)/(moles of DNA molecules); calculated for a DNA size of 2000 bp. [c] $R_{H,app}$ = apparent hydrodynamic radius from DLS data evaluated using CONTIN analysis. [d] PDI = polydispersity index from a cumulant fit of DLS data.

To obtain information on the size and shape of the particles formed close to the phase border, cryo-transmission electron microscopy (cryo-TEM) and dynamic light scattering (DLS) experiments were performed on selected samples (see Table 1 and Figure 1).

Figure 2 shows examples of cryo-TEM images. The assemblies consistently show a wormlike appearance with a roughly constant width of the order of 10 nm and the length extending up to a few hundred nanometers; the average length seems to increase with increasing lysozyme concentration. These dimensions should be compared to the sizes of the lysozyme, which can be described as a slightly deformed sphere with a diameter of ~4 nm, and the DNA, with a diameter of ~2 nm and a contour length of 600−700 nm. Consistent with the SAXS results, the cryo-TEM images show no obvious order within the assemblies. However, the elongated shape of the aggregates clearly indicates a preferred anisotropic arrangement of the constituents. In addition to electrostatic attraction between DNA and lysozyme, it is very probable that both attractive and repulsive interactions between the protein units are important in governing this arrangement. Because of the significantly larger volume per net charge of lysozyme as compared to that of DNA (see above), the lysozyme globules will, by geometrical constraints, most likely be forced into a substantial degree of direct contact when residing in the assemblies, which implies that the effective charge distribution on the lysozyme surface should have direct consequences on the molecular organization. The considerable stiffness of DNA may also be important in dictating the structure of the aggregates, but the dimensions of the observed assemblies show that the polymer cannot be fully extended along their contour. It is known that the double-stranded structure of DNA is retained on complexation with lysozyme.[9]

Although the DNA−lysozyme assemblies sometimes (see Figure 2a) show a ringlike appearance, they probably have a molecular organization fundamentally different from that in the toroidal constructs often formed with DNA and multivalent cations.[10] It is unlikely that the large lysozyme to DNA volume ratio at charge neutrality is compatible with the hexagonal packing of DNA generally found in toroids.

In the concentration range used in the cryo-TEM experiments, DLS measurements give apparent hydrodynamic radii, $R_{H,app}$, of the aggregates ranging from 65 to 113 nm; the polydispersity

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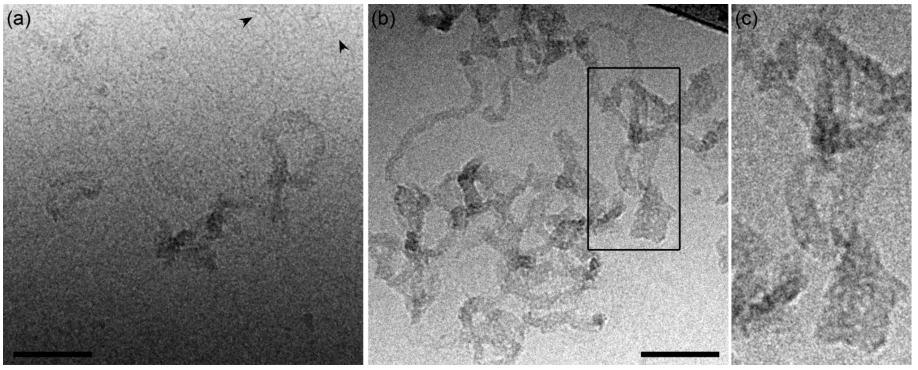

Figure 2. Cryo-TEM images of samples containing 0.30 mM DNA and 0.033 (a) or 0.085 (b) mM lysozyme. Scale bars are 100 nm in (a) and (b), and arrow heads in (a) indicate excess free DNA. Panel (c) is a magnification of the frame in panel (b).

increases slightly with size. The obtained $R_{H,app}$ values suggest that, at these conditions, the aggregates are dispersed individually rather than in clusters as suggested by the cryo-TEM micrographs (the latter can probably be attributed to flow, and the associated shear forces, during sample preparation) and confirm a slight increase in apparent aggregate size with increasing lysozyme concentration at low +/− charge ratios (r_{charge}). For samples with a lysozyme concentration above 0.12 mM, that is, above an r_{charge} value of 0.40, DLS data reveal multimodal size distributions and a significant increase in the average size. This observation can tentatively be ascribed to clustering and/or entangling of the primary assemblies.

It is valuable to compare the findings from the present study with results on the related system of salmon testes DNA and poly(amido amine) (PAMAM) dendrimers of generation 4 (G4).[11] The size of these molecules is comparable to that of lysozyme (Ø ∼ 5 nm), but they carry 64 cationic surface groups. With a constant DNA concentration of 0.45 mM and a varying dendrimer concentration, soluble aggregates with a $R_{H,app}$ value of ∼50 nm are formed from an r_{charge} value of 0.4 up to charge neutrality, where phase separation occurs. Thus, although one expects a stronger electrostatic attraction of DNA to the dendrimers than to lysozyme, phase separation occurs at a significantly lower r_{charge} value in the latter case at otherwise comparable conditions. This finding is a direct manifestation of the importance of attractive interactions (electrostatic and/or hydrophobic) between the protein units in governing the behavior of the DNA−lysozyme system.

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Concluding Remarks

With a study on a model system, we have demonstrated a novel approach for investigating DNA−protein interactions. Our results suggest that direct interactions between the protein units strongly influence the behavior of the studied system. It is intriguing to find that, although one does not expect the presence of specific sites for interactions between DNA and lysozyme, its components arrange into wormlike assemblies with a quite consistent, narrow width. Incidentally, the width of the assemblies is similar to that of the so-called 10 nm fiber, the first level of organization of chromatin.

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Supporting Information Available: Detailed descriptions of sample preparation and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.