42c Metastable Mesoscopic Clusters in Low-Ionic Strength Protein Solutions

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Mesoscopic disordered clusters have been theoretically predicted and experimentally evidenced in several recent works dealing with colloid and protein solutions 1,2. The interest in them is underscored by their participation in the formation of crystals and other ordered solid phases 1, by their effects on the rheological and other properties of the solutions. The existence of these clusters in protein solution is of particular interest because phase transitions with proteins underlie several debilitating diseases: Alzheimer's, eye cataract, sickle cell anemia, and others. For tests of the mechanism of cluster formation and in search of other sources of the repulsive maximum and to better understand the stability of the mesoscopic clusters and their participation in phase transitions we studied solution of the protein lysozyme with low ionic strength. We found that in lysozyme solutions at ionic strength of 0.1 M and less two types of clusters exist: of sizes ~ 10 nm and of size ~ 100 nm. The larger clusters occupied ~ 10-4 of the total solution volume, while the smaller ones - ~ 10-7. Increasing the concentration of lysozyme leads to an increase of both volume fractions and not of the sizes of the clusters. The consistence of cluster size indicates that the clusters represent a well defined phase in metastable equilibrium (a stable phase would grow to macroscopically detectable dimensions) with the solution. Increasing of the ionic strength by the addition of 50, 100 and 150 mM NaCl results in complete disappearance of the clusters, i.e., this observation indicates that the clusters are due to a Coulomb repulsive maximum, “hump”, at intermediate separations in the intermolecular interaction potential, and an attractive minimum at small separation Comparing the clusters sizes, lifetimes and population in phosphate and citrate buffers, we found differences. This suggest that the attraction that is also a part of the cluster formation mechanism is not only van der Waals, but also involves specific chemical forces, mediated by the buffer species. Increasing the ionic strength by increasing the phosphate concentration does not lead to cluster disappearance. The clusters observed at phosphate concentrations > 150 nM cannot be due to Coulomb interactions and we conclude that the source of the hum in this case is structuring of the water molecules around the lysozyme molecules assisted by the phosphate, also called hydration force. The lifetimes of the clusters exiting at this higher ionic strengths are shorter: < 20 s, and their size vary in significantly broader boundaries. Investigating the participation of the clusters in crystal formation, few found that at low ionic strength, no crystals form, i.e., the clusters with long lifetimes observed at these conditions are not a part of the crystallization mechanism. However, crystals form in the solution with higher concentrations of phosphate, where clusters with short lifetime were observed. We conclude that clusters which are due to the structuring of the water around protein molecules and which have short lifetimes can be part of a mechanism of formation of ordered solid phases.

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