New and Notable

Staying Together: Protein Molecules in Mesoscopic Clusters

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Collective behavior of protein molecules in solutions is critically important for understanding their biological functions, as well as for efficient control of various technological processes. A number of recent experimental studies have observed the formation of various clusters in protein solutions in different systems (1–5). While the existence of small molecular complexes in solutions is a common phenomenon, a surprising thing in protein solutions is a variety and complexity of such aggregates. At least three different classes of protein clusters, depending on their sizes and volume fractions, have been reported in experiments (6). But the most intriguing of them are mesoscopic protein clusters, for which the mechanisms of assembly are still not well understood (4,6,7). These mesoscopic clusters are also very important for clarifying many phenomena in chemistry, biology, and medicine because they frequently serve as nucleation sites for the formation of ordered and disordered protein solid aggregates such as protein crystals, sickle cell hemoglobin polymers, and amyloid fibrils (8,9).

Mesoscopic protein clusters are relatively large: typically, the number of protein molecules in them is ~10^5–10^6, and their diameters vary from 100 to several hundreds of nanometers (4,7,8). At the same time, the number of mesoscopic clusters is relatively small and they occupy a tiny fraction of the solution volume (~10^{-6}–10^{-4}) (4,7,8). In addition, the sizes of these clusters do not depend on the overall protein concentrations in the solution, and their presence does not modify the bulk properties of the system. They are also very stable at various experimental conditions. These surprising properties stimulated multiple theoretical efforts to understand the formation and stability of the protein mesoscopic clusters (6,7). The lifetimes of such molecular aggregates significantly exceed the expectations from the equilibrium theory of colloidal aggregation (10). The chemical potential of the protein molecule is much lower in the solution than in large clusters. But experiments also indicate that mesoscopic clusters are apparently in equilibrium with the rest of the solution (11). A possible resolution of this dilemma came with the proposal that these clusters consist of a mixture of protein oligomers and monomers (7). Then the concentrations of various protein species in the mesoscopic clusters would not violate the equilibrium conditions, while clusters made of only protein monomers cannot be found in equilibrium with the rest of solution. However, these theoretical ideas have not been fully tested yet. An article by Vorontsova et al. (6) in this issue of the *Biophysical Journal* fills this gap by presenting a comprehensive experimental analysis of the various mechanisms of the mesoscopic cluster formation.

Most proteins are charged macromolecules, suggesting a crucial role of Coulomb forces in all processes where they are involved. Because of this fundamental role of electrostatics, Vorontsova et al. (6) focused on quantifying the effect of Coulomb interactions on properties of the protein mesoscopic clusters. Utilizing elegant experimental approaches, they showed that the cluster sizes were independent of the ionic strength or pH of the solution, while the cluster volume fraction decreased at the same conditions (6). This was a big surprise because the increased electrostatic screening should lower the molecular repulsion in the clusters, leading to the opposite trends. The important conclusion from these observations is that, most probably, Coulomb interactions do not participate in the assembly of the mesoscopic protein clusters. But this also raised a question about the nature of fundamental forces that govern the formation of these molecular aggregates. Vorontsova et al. (6) proposed and quantitatively tested an idea that hydrophobic forces are responsible for bringing together the protein molecules in mesoscopic clusters. Adding the denaturant urea to the protein solutions led to the decrease in the cluster sizes and the increase in the cluster volume fractions. This agrees well with the oligomeric mechanism of the mesoscopic clusters formation. Urea has exposed the peptide backbones of protein molecules, weakening the intermolecular bonds in the protein oligomers. Obviously, this should lead to smaller protein clusters. Simultaneously, more hydrophobic groups are exposed due to partial protein unfolding. But this should stabilize the cluster phase because these additional hydrophobic interactions compensate more the molecular repulsions (6). A much clearer picture of how the mesoscopic protein clusters assemble emerges from these experimental observations. Strong peptide backbone interactions between monomeric proteins lead to the formation of the protein oligomers, while the partial protein unfolding stabilizes the protein aggregates in the cluster phase.

The work of Vorontsova et al. (6) is a significant step forward in our understanding of the complex biological processes. It uncovers a unique mechanism of the formation of protein aggregates, which is driven by hydrophobic but not electrostatic interactions. This mechanism is fundamentally different from other proteins systems. However,

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despite a tremendous success of this work, many fundamental questions concerning the mechanism of the protein aggregation are still not well understood. It is unclear how such complex structure and composition of mesoscopic clusters can support the formation of macroscopic-ordered protein aggregates? Another fundamental question is related to a protein specificity in the formation of clusters and aggregates. The experiments by Vorontsova et al. (6) have been done with the protein lysozyme, while some deviations in the clustering behavior have been observed for other protein systems (5). Despite these issues, the article of Vorontsova et al. (6) is an excellent example of how to quantitatively analyze and test the fundamental theoretical ideas.

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