Interparticle interactions in aqueous solutions of globular proteins

Proteins are the most abundant biomacromolecules in cells, where they perform an essential role in virtually every biological process. Proteins can function properly only if they remain in their stable (native) form. In the present work we studied the influence of interparticle interactions on the phase stability of some globular proteins, with our main focus on hen egg-white lysozyme (HEWL). In the first part we investigated the phase stability of HEWL in 10 different buffer solutions by measuring their cloud-point temperature. We found that the phase stability of HEWL solutions strongly depends on solution conditions and also on the choice of buffer, with results for buffers at *p*H=7.0 clearly indicating towards buffer-specific effects. By applying a simple model within the framework of Wertheim's perturbation theory, we could predict the liquid-liquid coexistence curves of HEWL in buffers at *p*H=7.0, which were consistent with our experimental results. We also made use of the model in combination with experimentally determined B_{22} and k_D values and ascertained the influence of buffers on protein-protein interactions at *p*H=7.0. In the second part we studied how different solution conditions affect the *in vitro* fibrillization of HEWL. The presence of amyloid fibrils was detected by highly amyloidophilic dyes thioflavin T and Congo red, as well as by measuring circular dichroism (CD) spectra. We noticed HEWL fibrillizes only under specific solution conditions. We demonstrated electrostatic charge screening plays an important, but not vital, role in the fibrillization process. In addition, by adding a sufficient amount of inert polymer, PEG12000, we arrested the formation of HEWL fibrils in a highly amyloidogenic solution. In the third part we optimized the expression of a pseudo-wild type (WT*) T4 lysozyme and its respective single point mutant L99I*. It was found that the phase stability of T4 lysozyme is higher than the one of HEWL at identical solution conditions. Additionally, we have shown the T4 WT* protein is conformationally more stable than its mutant protein. Lastly, we carried out multiple atomistic molecular dynamics simulations of aqueous solutions of HEWL, T4 WT* lysozyme and human γ-D crystallin. We calculated the protein density fluctuations, which have shown distinct protein-rich and protein-poor regions for all solutions with high protein content. The nature of protein self-assembly turned out to be partially reversible, especially in solutions close to experimentally determined conditions of phase separation. A thorough analysis of simulations and residue-residue interactions between all pairs of proteins enabled us to determine the most important residue-residue pairs, as well as individual amino acids responsible for forming initial close contacts between proteins. For both HEWL and γ-D crystallin arginine was discovered to be the predominating amino acid, with its partners being mostly residues capable of forming hydrogen bonds and salt bridges. Meanwhile for T4 WT*, this role of initiating protein self-assembly was assigned to lysine. We also investigated the diffusive properties and hydration of simulated proteins.