

Dependence of Unfolding Fraction of Lysozyme on Temperature and Urea Concentration Revealed by SAXS

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The process of unfolding a protein from a well-known three dimensional structure to a distorted state of no biofunctions, or vice versa, has long been an interesting issue in life science. Various tools, including small-angle X-ray scattering (SAXS), X-ray absorption, fluorescence, and circular dichroism (CD), have often been used in unveiling the unfolding path ways of a protein under various denaturants or temperatures. In guiding the interpretation of the experimental results, the framework model and the hydrophobic collapse model are often employed. Nevertheless, the recent experiments suggest that the overall characteristics of the folding kinetics could be quite simple and principally related to the topology of the native state, as proposed very recently by Liang et al. in an Ising model.^[1] In this study, we adopt the Ising model to treat our SAXS data measured for the folding–unfolding thermodynamics of lysozyme in water solution, with and without the denaturant urea.

In the Ising model, the folding-unfolding is treated as a single-molecule event, and the single protein is regarded as an ensemble of a number of n and u units (say, peptide bonds) where f_n and f_u represent the fraction of fold and unfolded units in equilibrium condition. The continuous unfolding fraction value between 0 and 1 can then be determined through a Boltzman distribution governed by the free energy of Lysozyme in the solution, which is a function of solvent as well as the denaturant concentration.

Temperature-dependent (30 - 70 °C) SAXS measurement (see Figure 1) for aqueous solutions of a model-protein hen-egg white lysozyme (HEWL) in salt buffer solutions, with 0, 5, 6.5, 8 M of urea, was conducted with 15 keV photons of the BL01B beamline of the National Synchrotron Radiation Research Center (NSRRC). The SAXS result illustrates clear dependences of the unfolding fraction of lysozyme on temperature and urea concentration. An ellipsoidal form factor is used to simulate the SAXS data in an absolute scattering intensity scale. The structural parameters, including the shape, size, electron density, and radius of gyration of lysozyme as a function of urea concentration and temperature are extracted from the data fitting. The structural information obtained is then summarized into the unfolding fraction profile as a function of temperature or urea concentration, and from which curve the free energy of lysozyme in water or urea solution is extracted.

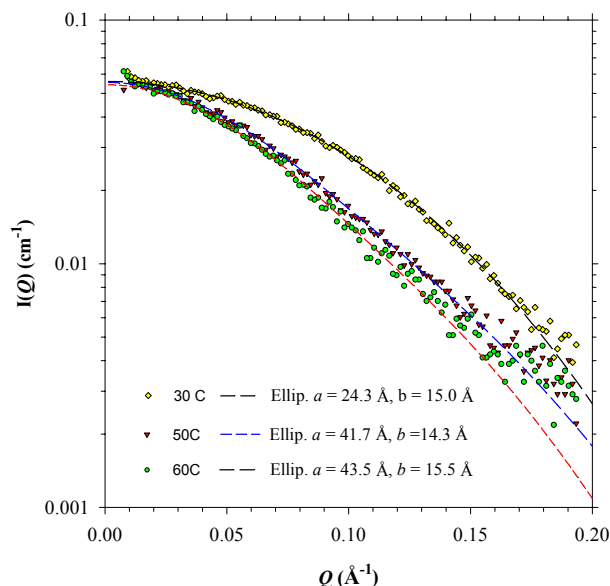


Figure 1. Represented temperature-dependent SAXS profiles for the aqueous solutions of 21 mg lysozyme, with 8M urea, 0.1 M NaCl, and 0.1 M sodium citrate for a pH value of 2.9. The data are fitted using an ellipsoidal form factor with semi-major axis a and semi-minor axis b .

References

[1] K. K. Liang, M. Hayashi, Y.-J. Shiu, Y. Mo, J. Shao, Y.-J. Yan and S. H. Lin, Phys. Chem. Chem. Phys., **5** (2003) 5300–5308.