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Precise SXS Analysis of Acid-unfolded Apomyoglobin Structure

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Introduction

Analysis of various intermediate states of proteins is indispensable to elucidating the mechanism of protein folding and structural stabilization. Aggregation and fibrillation of proteins are widely recognized as an important target of study in connection with amyloidosis. In relation to these problems, it is essential to study both the protein structure in nonnative states and the solvent effect responsible for it. Solution x-ray scattering (SXS) is one of the most efficient methods for obtaining global information of protein molecules as the size, shape and association state. From this viewpoint, we have studied hydration effects in SXS profiles[1)], the structure of denatured proteins and aggregates of human calcitonin combining SXS and molecular modeling methods. Here, we will report a precise study of the electrostatic effect on the SXS profile of acid-unfolded apomyoglobin.

Method

SXS experiment has been made using the PF beam line BL10C. Acid-unfolded (AU)-apoMb with 20mM HCl, pH1.7, c = 1.2, 3.3, 4.9, 7.5, 10.6, 17.6 mg/ml, and native (N)-holoMb with 10mM HEPES, pH6.0, c = 0.8, 1.8, 2.7, 5.3, 10.0 mg/ml were served for measurement. Measuring times are 90 min for AU-apoMb at c = 1.2 mg/ml and N-holoMb at c = 0.8 mg/ml, and 30 min for the other samples. To minimize damage of the sample protein by x-ray irradiation, SXS measurement was made with flowing continuously the protein solution through a sample cell. The actual x-ray irradiation time of each molecule is 0.5 s. Each of the SXS profiles for protein was obtained from the difference between a pair of SXS profiles for solution and solvent.

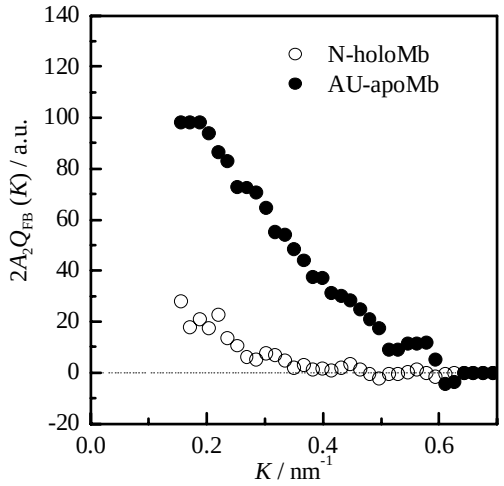
Results & Discussion

From a preliminary experiment at c = 1 mg/ml, [HCl] = 5, 10, 20, 50 mM, the solvent condition for AU-apoMb was determined as 20mM HCl, pH1.7. At lower HCl concentrations of 5 and 10mM, the intermolecular repulsion effect was so strong that the forward scattering intensity was found as low as 50-60% of that predicted from the partial specific volume (PSV) of AU-apoMb. On the other hand, at higher 50 mM HCl concentration, apo-Mb is mostly in the acid-molten globule state due to decrease in intramolecular repulsion effects.

Dependence on protein concentration of SXS profiles obtained were analyzed using the following formula:

$$\frac{c}{I_{\exp}(K,c)} = \frac{1}{I(K)} + 2A_2 Q_{\mathrm{FB}}(K)\cdot c \qquad (1)$$

Values of the coefficient $2A_2 Q_{\mathrm{FB}}(K)$ in eq.(1) for AU-apoMb and N-holoMb are shown in the figure below. The larger value of it means the larger decrease in scattering intensity due to increase in protein concentration at the scattering vector K. As seen clearly, this factor was found to decrease monotonously with increasing K. AU-apoMb yields larger values than N-holoMb. At K>0.63 nm^{-1}, it is nearly equal to zero. This shows that the intermolecular repulsion between apo-Mb molecules do not affect the SXS profiles at K>0.63 nm^{-1}.



The value of R_{sq}>4.5 nm estimated for the zero-c extrapolated SXS profile obtained from this analysis is significantly larger than the value of 3.0-3.5 nm previously reported for the 10mM HCl condition. The observed forward intensity accords within error with that predicted from the PSV of AU-apoMb. It was revealed from this work that, at low pH, the spatial distribution of protein molecules changes with both concentrations of protein and salt in solution, which is reflected on the SXS profile of the protein sensitively. The HCl concentration of 20mM is essential to obtaining information on the average structure of a single AU-apoMb from SXS measurement. In addition, the zero-c extrapolation is vital at K< 0.63 nm^{-1}. It is expected that detailed information on the structure of AU-apoMb will be obtained by combining the zero-c SXS profile and molecular modeling analysis.

References

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