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Wide-Angle Scattering Revealing Internal Structure of Proteins

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Introduction

Proteins ordinary form hierarchical structures in native state, which is deeply involved in appearance of various biological functions of proteins. On the other hand, solution X-ray has been exclusively used to determine protein structures in solutions, such as rough shapes, radii of gyration, or some large-scale intramolecular inhomogeneities, mainly by using data sets in small-angle scattering regions. In many cases, solution scattering experiments of proteins are carried out at low protein concentrations to avoid some modulation on small-angle scattering data regions caused by an aggregative or repulsive intermolecular interaction. Because of this reason, high-angle scattering data turn out to be low statistics for detailed analyses, and those data have been paid less attention compared with small-angle scattering data. Previously, by using small- and medium-angle X-ray scattering data, we successfully revealed a thermal structural transition multiplicity of a globular protein strongly depending on the protein structure hierarchy [1], indicating that solution X-ray scatterings still serve us further fruitful information on protein structures. In the present study, to elucidate a further availability of the wide-angle X-ray scattering method of solutions, we have carried out further scattering experiments of several globular proteins which are classified as different structural types of proteins.

Sample Preparation

Proteins used for measurements are myoglobin from horse skeletal muscle, hemoglobin from bovine, α-chymotrypsin from bovine pancreas, lysozyme from chicken egg white, ribonuclease A from bovine pancreas, and α-lactalbumin from bovine milk, which were all purchased from Sigma Chemical Co. These proteins belong to different types of protein structure categories, namely, to all-α, all-β, and α+β proteins, respectively. The protein solutions prepared were 5 % w/v myoglobin at pH 6.4, 5 % w/v hemoglobin at pH 5.0, 5 % w/v α-lactalbumin at pH 7.0, 5 % w/v lysozyme at pH 5.0, 5 % w/v ribonuclease A at pH 7.0, and 1% w/v α-chymotrypsin at pH 4. Solution X-ray scattering experiments were carried out by using X-ray scattering spectrometers installed at BL-10C and BL-15A of PF, Tsukuba, Japan.

Results and Discussion

Figure 1 shows the wide-angle scattering curves of the different types of proteins in solutions. To compare the experimental scattering curves over the full q range with the theoretical ones, we used the program named CRYSOL [2]. uses the multipole expansion method and calculates scattering curves of proteins based on the atomic coordinates entried in the Protein Data Bank. This program is known to explain very well experimental scattering curves of proteins in solutions, especially for the q region below ~0.5 Å^{-1}, by considering the excess average scattering density (so-called "contrast") of the hydration shell [2]. The present results show a promissing ability of wide-angle scattering method of proteins in solutions for studying those functional structures under various conditions.

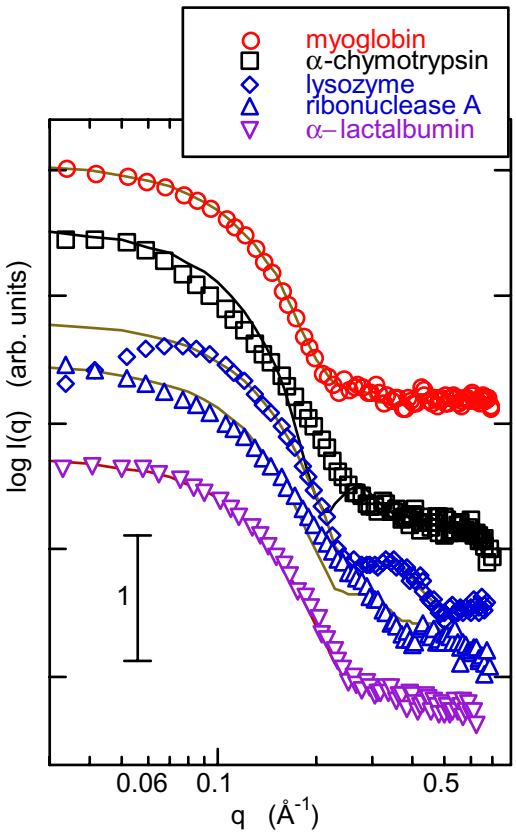

Figure 1. Wide-angle X-ray scattering curves of several globular proteins in solutions. Solid curves are theoretical ones.

References

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