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Structure refinement from X-ray solution scattering data of proteinases by molecular dynamics calculation

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Introduction

In order to elucidate the detailed protein structure in solution, the small angle X-ray scattering (SAXS) measurements of various kinds of protein molecules including proteinases were performed with a CCD-based X-ray detector, and compared with SAXS patterns calculated from the three-dimensional structures determined experimentally. The measured samples are aspergillopepsin II, pepsin, ribonuclease T_1, ribonuclease A, and β-lactoglobulin. Among them, the last three proteins were investigated intensively, since the structures of these proteins were well studied.

Experimental

All the measurements were performed at BL-15A with a CCD-based X-ray detector[1]. Sample solutions were prepared at 5-40 mg/ml. Pepsin, ribonuclease T_1, and ribonuclease A were analyzed in 0.2 M sodium acetate (pH 5.5) at 40°C (pepsin and ribonuclease T_1) or 20°C (ribonuclease A). Aspergillopepsin II was analyzed in 0.2 M sodium acetate (pH 4.5) at 40°C. β-lactoglobulin was analyzed in 0.01 M PBS (pH 2.0) at 20 °C. Theoretical SAXS patterns were calculated in the same procedure as reported [2].

Results and Discussion

We previously measured the SAXS of ribonuclease T_1 with a CCD-based X-ray detector, and reported that the data quality was largely improved [3]. In the present study, we measured SAXS patterns for other proteins inculuding proteinases and ribonucleases. Fig. 1 shows the SAXS patterns of aspergillopepsin II and pepsin. In the case of aspergillopepsin II, the SAXS profile was almost identical with the previous one [4] measured with a PSPC detector. The three-dimensional structures had been already determined for the proteins except aspergillopepsin II. Especially, ribonucleases A and T_1, and β-lactoglobulin were well studied. Therefore, we investigated these proteins intensively. Fig. 2 shows the SAXS profiles for ribonuclease A (a) and β-lactoglobulin (b) at various protein concentrations. The radii of gyration were 16 Å (a) and 18 Å (b), and were consistent with the previous results [5,6]. At present we are calculating the SAXS pattern from the atomic coordinates in order to compare with the experimental ones.

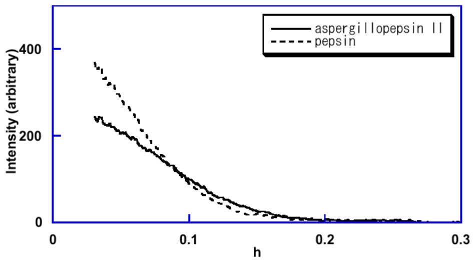

Fig.1. SAXS profiles of aspergillopepsin II and pepsin .

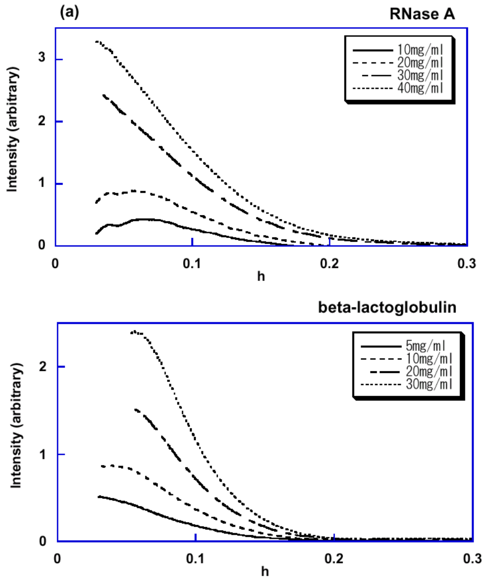

Fig.2 SAXS profiles of ribonuclease A (a) and beta-lactoglobulin (b).

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