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

Masaki KOJIMA¹, Kazuki ITO², Alexander TIMCHENKO³, Yoshiyuki AMEMIYA⁴, Masaru TANOKURA⁵, Hiroshi KIHARA⁶, Kenji TAKAHASHI¹
¹School of Life Sci., Tokyo Univ. of Pharm. & Life Sci., Hachioji, Tokyo 192-0392, Japan
²Inst. of Material Sci., Univ. of Tsukuba, Tsukuba, Ibaraki 305-3857, Japan
³Inst. of Protein Res., RAS, Pushchino, Moskow region 142292, Russia
⁴Graduate School of Frontier Sci., Univ. of Tokyo, Bunkyo-ku, Tokyo 113-8656, Japan
⁵Graduate School of Agr. and Life Sci., Univ. of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan
⁶Physics Lab., Kansai Med. Univ., Hirakata, Osaka 573-1136, Japan

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 T1, 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 T1, and ribonuclease A were analyzed in 0.2 M sodium acetate (pH 5.5) at 40°C (pepsin and ribonuclease T1) 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 T1 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 including 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 T1, 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.

References
*mkojima@ls.toyaku.ac.jp