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Measurement of Dynamic Fluctuation of Proteins using Small-Angle X-ray Scattering

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Introduction

Proteins are molecular machines which carry out many functions in the biological systems. The structure of the protein is characteristic of its own function, just as artificial machines, while it is influenced by the thermal vibration and random collision of water molecules. Under such condition, the structural fluctuation of the protein has important role for the mechanism of the protein. Small-angle X-ray scattering has been used to measure an averaged structure of the protein in the solution and it is not considered suitable to measure the dynamic properties. We have attempted the detection of the dynamic fluctuation occuring at overall of the molecule using small-angle X-ray scattering measurements and molecular dynamics simulations analysis.

Experiments

Myosin subfragment-1 (S1) of skeletal muscle and engineered calmodulin molecules were prepared for X-ray scattering measurements. The X-ray solution scattering experiments were done at the BL15A1 using the small-angle diffractometer at a camera length of ~2.4 m and ~1.2 m. All X-ray scattering data were collected with an X-ray image intensifier (XRII) and a CCD camera. The two-dimensional X-ray patterns were measured and the intensity data were integrated as a one-dimensional function of the scattering vector length. The protein concentration was varied in the range of 2 to 8 mg/ml. X-ray scattering was measured at the temperatures of 5°, 10°, 20° and 30°C to vary the degree of fluctuation in protein structure.

The molecular dynamics simulations of the calmodulin were executed under the various temperature conditions. The multiple structures were collected from the simulation trajectory. The scattering intensities were calculated from the collected structures and averaged, which express the experimental scattering from the fluctuated structures in the solution. The simulated intensities were compared in various temperatures.

Results and Discussion

The scattering intensities of calmodulin in the specific angular region exhibited a tendency to change monotonously with an increase in temperature. In the scattering pattern of calmodulin, the intensities around S~0.01 A^{-1} ($S=2\sin\theta/\lambda$) at 20°C was smaller than those at 5°C. The difference between 30°C and 5°C was much distinguished. From the observations, the characteristic nature of structural fluctuations of proteins in solution may be evaluated by the measurements of small-angle X-ray scattering.

The simulation models calculated from the molecular dynamics trajectory were adopted to evaluate the scattering intensities. The model intensities at the temperatures 5°, 10°, 20° and 30°C were compared and they were investigated with the observed experimental data. The difference of scattering intensities between 30°C and 5°C of simulation model was found at S ~0.012 A^{-1}, which was very similar to experimental results. The scattering profile was also compared with the intensity change which was calculated from the structural models of the collective motion of the calmodulin.

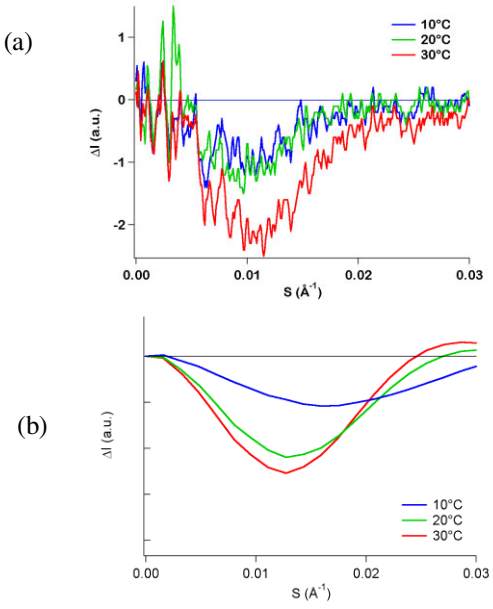

Figure 1. (a) The intensity differences of the calmodlin scattering profile at various temperatures. The differences were calculated from the profile at 5°C. (b) The differences of model intensity.

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