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The Sructural Fluctuation of the Calmodulin Evaluated by 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 in the living cell. Under such condition, the structural fluctuation of the protein may have 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.

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 crystal structure of the calmodulin have been solved in both of the calcium present state and absent state. The scattering intensities calculated from these atomic structures were used to explain the scattering profile of the fluctuated structure. The scattering intensities of the calmodulin were calculated in the Ca++-binding state (CaCaM) and absent of the Ca++ state (apoCaM). The scattering intensity of fluctuated structure was evaluated to change the ratio of these intensities and add them. The small intensity change were derived when the ratio of CaCaM and apoCaM was altered. This intensity change was very similar to the temperature dependent experimental results, such as the intensity difference were observed in the range of S < 0.02.

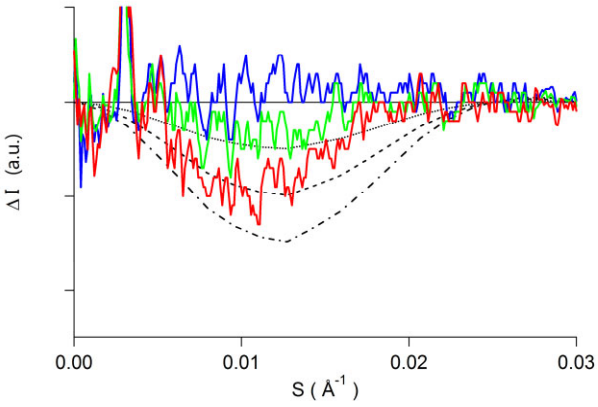

Figure 1. The intensity differences of the calmodlin scattering profile at various temperatures. The differences were calculated from the profile at 5°C. The calculated intensity differences from the two structures were also shown by the dotted line.

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