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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, just as artificial machines. The structure of the protein is characteristic of its own function, and is influenced by the thermal vibration and random collision of water molecules. Thus, the protein functions under the condition of structural fluctuation. Moreover, the structural fluctuation itself has important roles for the mechanism of proteins, in contrast of artificial machines. In general, small-angle X-ray scattering has been used to measure the averaged structure of proteins in the solution. We have attempted the detection of dynamic structural fluctuation using small-angle X-ray scattering.

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 Guinier plot was made from the intensity data to obtain the radius of gyration (Rg) and the zero-angle intensity (I(0)). The I(0) plots against protein concentrations showed that the molecular weights of samples were identical at various temperatures. On the other hand, the Rg values of myosin S1 showed a small increase with raising temperature but those of calmodulin showed a small decrease with raising temperature. The different changes in Rg between calmodulin and myosin S1 may reflect some feature of structural fluctuation to influence the extent of molecules. The scattering intensities 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. The scattering intensities around S~0.03 A^{-1} of myosin S1 were showed a similar behavior to calmodulin; the integrated intensity decreased as the temperature increased.

From the present observations, the characteristic nature of structural fluctuations of proteins in solution may be evaluated by the measurements of small-angle X-ray scattering.

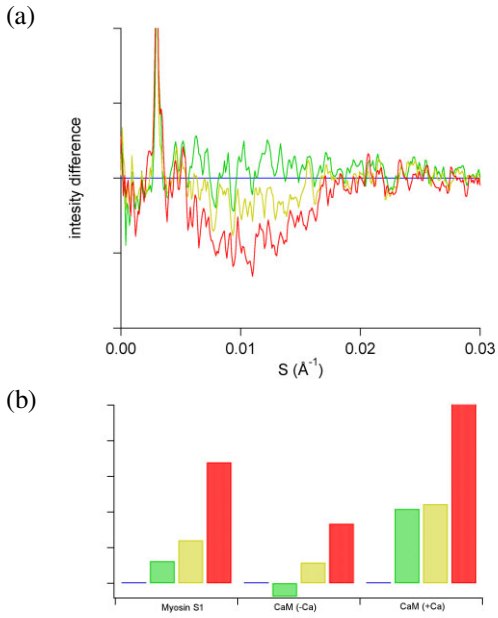

Figure 1. (a) The intensity differences of the calmodlin scattering profile at various temperatures. The difference were calculated from the profile at 5°C The color of green, yellow, red are denoted temperature of 10°C, 20°C and 30°C, respectively. (b) The histogram of intensity changes.

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