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CONFORMATIONAL CHANGES OF THE BACKWARD MOVING MYOSIN MOTOR (M VI) BY SMALL-ANGLE X-RAY SCATTERING

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Introduction

The myosin motors can produce a force and movement on actin filaments using chemical energy of hydrolysis of ATP. Most myosins move toward the barbed (+) end of actin filaments, but myosin VI, one of the myosin superfamily, moves toward the pointed (-) end. In order to clarify the mechanism of the opposite directional motion, we have investigated the structural changes of the myosin VI related with an ATP hydrolysis using X-ray solution scattering techniques.

Experimental

M6WT construct was used to express myosin V1 in the baculovirus. Myosin VI subfragment 1 (M6S1) were purified and collected through a column chromatography. Purified skeletal muscle myosin subfragment 1 (M2S1) were also used as reference. The X-ray solution scattering experiments were done at 20°C at the BL15A1 using the small-angle diffractometer. All X-ray scattering data were collected as a function of scattering vector length ($S=2\sin\theta/\lambda$) with a 1D-PSD. The protein concentration (c) was varied in the range of 2mg/ml to 7mg/ml.

Results and Discussion

The Guinier plots of the scattering data from the M6S1 samples with or without nucleotide gave all straight line, indicating no aggregate in any solution. The radius of gyration (Rg) value and the zero angle scattering (I(0)) were calculated from the Guineir plots on each samples. The Rg and I(0)/c versus c plots were linear and their values extrapolated to zero protein concentration were determined. All M6S1 and M2S1 samples had almost the identical molecular weight from their I(0)/c values. Fig. 1 shows the concentration dependence of Rg from various samples. The Rg value of M6S1 was 48.8Å, very similar to that of M2S1 without nocleotide. On the other hand, the Rg of M6S1 in the MgATP solution increased by ~3Å. This change occurred oppositely to that of M2S1 in the MgATP solution where its Rg decreased by ~3Å. The Rg of M6S1 with ADP decreased a little. The changes of Rg suggest that the elongation of a M6S1 molecule is induced in the ATP solution. This result is in contrast with the fact that the skeletal muscle myosin S1 becomes to more compact in the MgATP solution. The conformational change of M6S1 observed here closely relate to a power to produce opposite directional motion.

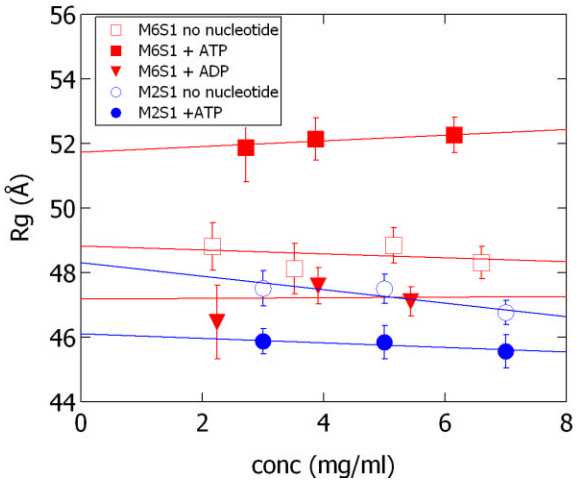

Figure 1 Concentration dependence of Rg calculated from Guinier plots of X-ray scattering intensities of myosin VI (M6S1) solution. That of Rg from skeletal myosin S1 (M2S1) solution is shown as reference.

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