Biological Science

15A/2003G143

CONFORMATIONAL CHANGES OF PROCESSIVE MYOSIN MOTORS (MV, MVI) BY SMALL-ANGLE X-RAY SCATTERING

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Introduction

The myosin motors can produce a force and movement on actin filaments using the chemical energy of hydrolysis of ATP. Myosin V is one of the unconventional myosins like as Myosin VI. Myosin V moves toward the barbed (+) end of actin filaments, but myosin VI moves toward the pointed (-) end. In order to clarify the mechanism of the opposite directional motion on the actin filaments, we have investigated the structural changes of the subfragmet-1 (S1) of myosin V and VI which are related with an ATP hydrolysis using X-ray solution scattering techniques.

Experimental

Recombinant myosin V S1 with two IQ motifs (MV-S1IQ2) and myosin VI S1 (MVI-S1) were obtained from Sf9 cells by baculovirus expression system. MV-S1IQ2 and MVI-S1 constructs were purified and collected through a column chromatography. Purified skeletal muscle myosin subfragment-1 (MII-S1) was also used as a 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 2 to 7mg/ml.

Results and Discussion

The Guinier plots of the scattering data from the MV-S1IQ2, MVI-S1 and MII-S1 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 samples had almost the identical molecular weight from their I(0)/c values, constitent with SDS-PAGE data. Fig. 1 shows the concentration dependence of Rg from various samples. The Rg value of MV-S1IQ2 was 48.6Å and the Rg of MV-S1IQ2 in the MgATP solution decreased increased by ~2Å (Fig.1A). These values were very similar to that of MII-S1 with and without nocleotide. On the other hand, the Rg of MVI-S1 increased about 3Å in the MgATP solution (Fig.1B). The most probable cause of the change in Rg would come from motion of lever-arm portion. These results showed that the directionality of myosin motors on actin filaments closely relates to the directional structural changes of their lever arm: MV-S1 becomes to more compact and MVI-S1 becomes more elongated in the MgATP solution. The changes of Rg of MV- and MVI-S1 with ADP were small. Experiments of several nucleotide analogues bound to MV-S1 and MVI-S1 suggested that myosin V and myosin VI changed their shape in *M.ATP* state before *M.ADP.Pi* state in the ATPase cycle.

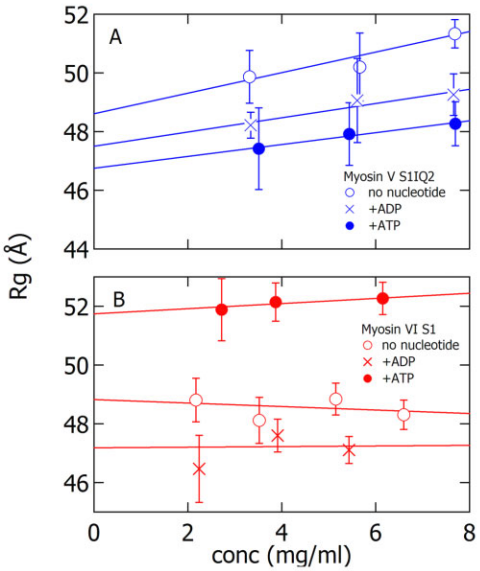

Figure 1 Concentration dependence of Rg calculated from Guinier plots of X-ray scattering intensities of myosin V and myosin VI construct solution.

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