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STRUCTURAL ANALYSIS OF PROCESSIVE MYOSIN MOTORS (MV-S1 & MVI-S1) BY X-RAY SOLUTION SCATTERING

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Introduction

The myosin motors 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 like as muscle myosins, 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 radius of gyration (Rg) values obtained from Guinier plot clearly showed that the opposite directional movements were related toreverse conformational changes. The Rg value of MV-S1IQ2 was ~48 Å and that of MV-S1IQ2 in the presence of MgATP decreased by ~ 2 Å. These changes were very similar to that of the MII-S1. In contrast, MVI-S1 had the Rg values of 48 Å and 51 Å in the condition of with and without MgATP, respectively.

The structural changes related to ATP hydrolysis steps were investigated by using ATP analogues. In the ATP solution, myosin mostly exists in the chemical state of M.ADP.Pi (M denote myosin). AlF_4 is the one of the phosphate analogues and thus ADP.AlF_4 mimics the ADP.Pi. MV-S1IQ2.ADP.AlF4 showed the Rg value similar to that of MV-S1IQ2 in ATP solution. MVI-S1.ADP.AlF_4 also had similar value of MVI-S1 in ATP solution. We confirmed that the structural changes of construcs in ATP solution occurs in the chemical state of M.ADP.Pi.

The AMPPNP is the ATP analog which is not hydrolyze. Thus MVI-S1.AMPPNP mimics the chemical state of M.ATP. The Rg of MVI-S1.AMPPNP was close to the value of MVI-S1 in ATP, implying that the structural change of MVI-S1 occurs just after the binding of ATP, This result was different from the results of skeletal muscle conventional myosin II in which the structural change occurs in the M.ADP.Pi state, not in the M.ATP state.

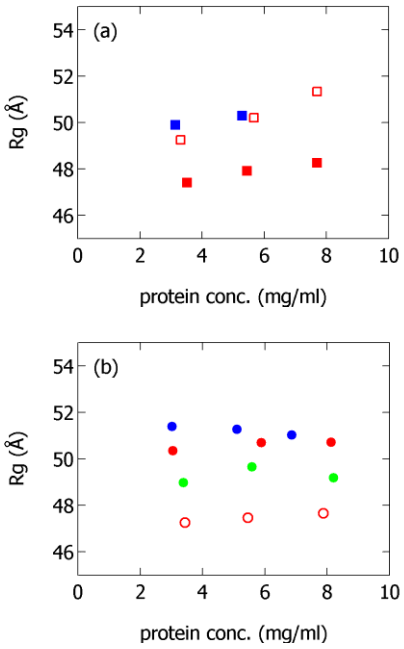

Figure 1 Protein concentration dependence of the radius of gyration (Rg) of (a) MV-S1IQ2 and (b) MVI-S1 in various nucleotide-bound states. Open symbol, myosin no nucleotide, red, in the presence of MgATP, blue, ADP.AlF_4 bound state, green, AMPPNP bound state.

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