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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 to conformational changes [1]. The Rg value of MV-S1IQ2 was ~48 Å and the Rg of MV-S1IQ2 in the presence of MgATP decreased ~ 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. These results reveal that changes in Rg come from the positional changes of their light chain-binding regions (lever arm portions). We have carried out the modeling analysis to investigate the myosin structures and Rg values during hydrolysis of ATP. Svergun and his colleagues have developed the programs that reconstruct the three-dimensional structure from the one-dimensional solution scattering profile data. We have applied this program to calculate the MVIQ2-S1 and MVI-S1 structures in the presence and absence of MgATP in their solution. The results are shown in Fig. 1. Structural model of MV-S1IQ2 becomes to more compact in the MgATP solution, because of the lever-arm swinging. It seems very similar to the structural change of MII-S1. MVI-S1 model becomes to more elongated structure, which moves a lever arm portion in opposite direction to that of MII-S1 and MV-S1IQ2 in the MgATP solution. These results indicate that the directionality of myosin motors on actin filaments closely relates to the relational structural changes of their lever arm portion.

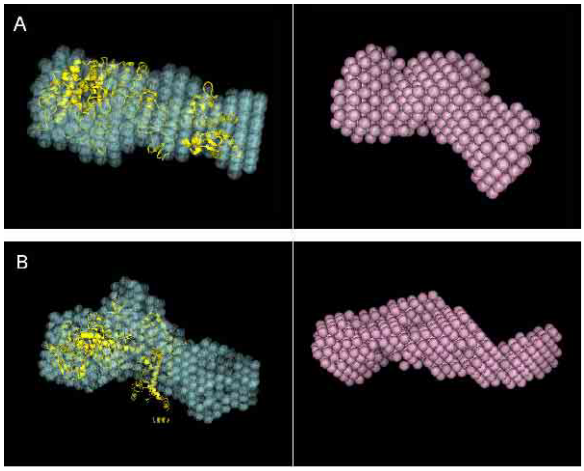


Figure 1 The Reconstructed models of processive myosin S1s. (a) Models of MV-S1IQ2 in the absence (left, blue model) and presence (right, pink model) of MgATP. (b) Models of MVI-S1 in the absence (left, blue model) and presence (right, pink model) of MgATP. Crystal structures of MV-S1IQ2 and MVI-S1 are superimposed on each solution model without MgATP.

## References

[1] Y. Sugimoto et al., PF Activity Rep. #22B, 245 (2005).

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