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Analysis of conformational change of neck-linker of kinesin chimeric protein fused with GFP using small angle X-ray solution scattering

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Introduction

Recently, we have successfully dissolved the crystal structure of ADP bound K16 motor domain. The overall structure of the K16MD is similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequence. However, neck-linker region of K16 showed an ordered conformation in a position like that of Eg5. Previously, we have designed the K16 motor domain chimera protein fused with GFP at the neck-linker in order to monitor the conformational change of the neck-linker during ATP hydrolysis by small angle X-ray solution scattering. We determined the Radius gyration (Rg) values of K16-GFP in the presence or absence of nucleotides by X-ray solution scattering. The Rg of nucleotide-free K16-GFP was about 42 A. In the presence of ADP and ATP, the Rg values were 38 Å and 39 Å, respectively. In this study, conventional kinesin and Eg5 fused with GFP (KIF5A-GFP, Eg5-GFP) were prepared and analyzed by small-angle X-ray scattering in order to compare its neck-linker conformation with K16-GFP.

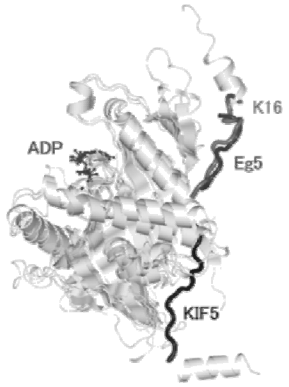

Fig. 1: Comparison of neck-linker binding site of three kinesins in ADP-state crystal structure

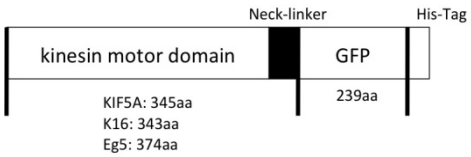

Fig. 2: Schematic diagram of kinesin-GFP fusion proteins

Results

Kinesin-GFP fusion proteins

We have prepared the three kinds of N-terminal motor domains of kinesin, K16, KIF5A, and Eg5 fused with GFP. Kinesin-GFP fusion proteins were expressed in *E. coli.* and purified using Co-chelate column and Sephacryl S-300 column. All of the fusion proteins showed almost normal microtubule-dependent ATPase activity.

Small angle scattering

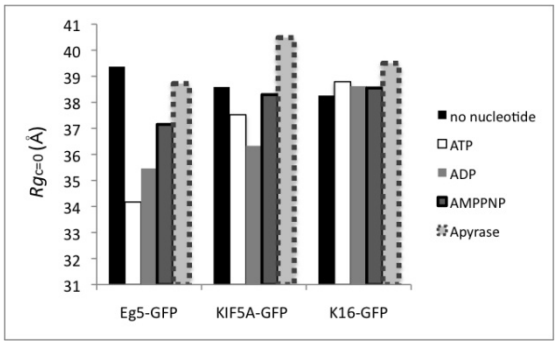

Fig. 3: *Rg* values of K16-GFP, KIF5A-GFP and Eg5-GFP in the change of nucleotide states.

The *Rg* values of three kinesin-GFP fusion proteins showed different nucleotide-state dependency, suggesting that in the physiological solution, neck-linker of these three kinesins changes their conformation in a different manner. KIF5A-GFP, Eg5-GFP showed larger Rg values in the condition of no nucleotide and apyrase than that in the presence of nucleotides reflecting the conformational change of neck-linker between docked and undocked states. On the other hand, K16 did not show nucleotide dependent alteration in Rg value. The data indicates that the neck linker of K16 forms undocked conformation as shown in crystal structure during ATP hydrolysis. The data may be consistent with the unique biochemical properties of K16 such as the K16 retains unusual stability of activity in the absence of nucleotide unbound state[1)]. Nucleotide-dependent alteration of Rg for wild-type kinesin motor domain without GFP was not observed (data not shown). It was suggested that the GFP-fused kinesin is useful not only for FRET studies but also global conformational analysis.

References

1) Umeki N, Mitsui T, Kondo K, Maruta S., **J. Biochem. 139**, 857-864 (2006).

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