Biological Science

15A/2008G050

Analysis of Conformational Changes of Novel Rice Kinesin K16 using Small-angle X-ray Solution Scattering

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Introduction

We have previously revealed that rice kinesin K16 has several unique enzymatic characteristics as compared with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain is very stable, contrast to conventional kinesin that is very labile in ADP-free state[1,2]. Recently, we have successfully solved the crystal structure of ADP bound K16 motor domain. The overall structure of the K16 motor domain was similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequences. However, the neck-linker region showed an ordered conformation in a position very different from conventional kinesin. In this study, we 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 region during ATP hydrolysis by small-angle X-ray solution scattering.

Experimental

The cDNA coding K16-GFP fusion protein was constructed by PCR. The constructs were expressed in *E. coli* and purified by Ni-NTA agarose affinity chromatography. X-ray solution experiments were carried out at the beamline 15A1. The scattering data from the specimens of kinesin-GFP fusion protein were recorded at 19°C with a 1D-PSD at a camera length of 2.33 m. The solution conditions were; 2, 3 or 5 mg/ml K16-GFP protein, 40 mM ADP, AMPPNP or ATP, 30 mM Tris-HCl (pH 7.5), 120 mM NaCl, 2 mM $MgCl_2$, 0.5 mM DTT.

Results and Discussion

We 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 region during ATP hydrolysis by small-angle X-ray solution scattering. We determined the radius of gyration (R_g) values of K16-GFP in the presence and absence of nucleotides from the Guinier plots of X-ray scattering data. The R_g of nucleotide-free K16-GFP was about 42Å. In the presence of ADP and ATP, the R_g values decreased to 38Å and 39Å, respectively. These results suggest that the neck-linker of nucleotide-free K16 is in the docked conformation while the neck-linker of nucleotide bound states is in the novel conformation which is observed in crystal structure (see Fig. 1). The recent EPR experiments on K16 mutants which have single cysteine at 331, 335 or 340 indicated that the neck-linker of the K16 was flexible even in the presence of ADP and ATP. Together with this, the results suggest that the neck-linker of K16 does not form a stable docked state during ATPase.

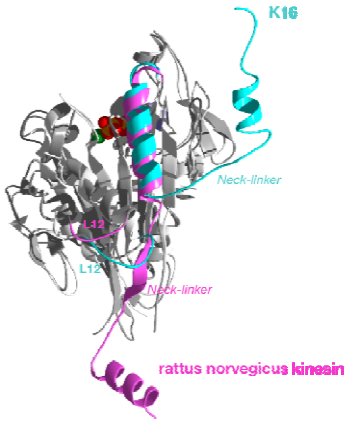

Fig. 1. A novel conformation of the neck-linker of rice kinesin K16. The crystal structure of the K16 motor domain with Mg-ADP bound shows an novel ordered neck-linker conformation in a position apparently different from that of conventional kinesin.

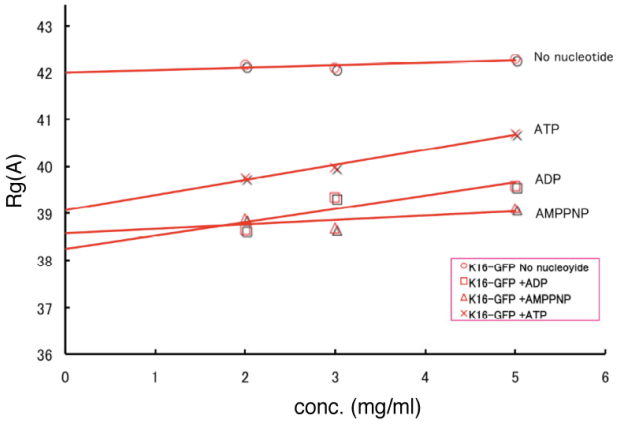

Fig. 2. Protein concentration dependence of the Rg values of K16-GFP in the various nucleotide-bound states.

References

1) Umeki et al.,J. Biochem. 139, 645-654 (2006).
2) Umeki et al.,J. Biochem. 139, 857-864 (2006).