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AN X-RAY SOLUTION SCATTERING STUDY ON THE CONFORMATION OF pPDM-CROSSLINKED MYOSIN SUBFRAGMENT-1 TRAPPING MgADP

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Introduction

The nucleotide-induced conformational changes of the myosin head (subfragment 1, S1) have been well documented in the crystalline states and in solution. To understand their role in energy transduction, it is important to define the magnitude and extent of the changes in the S1 ATPase cycle. MgADP was trapped at the active site of S1 by crosslinking two reactive thiols, SH1 and SH2, with the bifunctional thiol reagent pPDM. The previous X-ray scattering experiment showed that the radius of gyration (Rg) of this S1.ADP-pPDM sample tended to be slightly larger than control nucleotide-free S1, although little weight was put on the data1). We reinvestigated the conformation of S1.ADP-pPDM in solution by X-ray scattering.

Experimental

Papain-digested S1 was used. S1.ADP-pPDM sample was prepared by trapping MgADP at the active site of S1 by crosslinking SH1 and SH2 with the bifunctional thiol reagent p-phenylenedimaleimide (pPDM) and showed very low values of K+(EDTA)-ATPase and Ca^{2+}-ATPase of control S1. The S1.ADP-NEM2 sample was prepared by treating individual SH1 and SH2 of S1 with the monofunctional thiol reagent N-ethylmaleimide (NEM) as a control. Nucleotide free S1 and S1 in ATP solution were also measured as a reference.

Solution scattering experiments were done at 20°C at the BL15A1 using a small-angle diffractometer and all scattering data were collected with a 1D-PSD.

Results and Discussion

The Guinier plots of the scattering intensity data (I(S)) from S1, S1.ADP-pPDM, S1.ADP-NEM_2, S1 in ATP solution gave all straight lines. The I(0)/c versus c (c, protein concentration) plots were also linear and their values extrapolated to c=0 corresponded to a molecular weight of S1. Fig. 1 shows the radius of gyration (Rg) values of various S1 samples. The Rg value of S1.ADP-pPDM was ~5.2nm, about 10% larger than that of nucleotide free S1. Rg of S1.ADP-NEM2 was almost the same as that of nucleotide free S1. Thus, the change in Rg of the S1.ADP-pPDM sample was not caused by thiol treatment of SH1 and SH2. Fig. 2 shows scattering intensity profiles of S1.ADP-pPDM and the other samples. The profile of S1.ADP-pPDM extended toward larger SRg than those in the other samples. That of S1.ADP-NEM2 was nearly the same as the nucleotide free S1 profile. The data of nucleotide free S1 and S1 in ATP solution were almost identical to the previous ones[1,2]. The results indicate that the structure of crosslinked S1 with pPDM trapping ADP elongates, suggesting a reverse movement of its light-chain binding domain. S1.ADP-pPDM may be a state analog between the S1.ATP state and the S1.ADP.Pi state in an S1 ATPase cycle.

References

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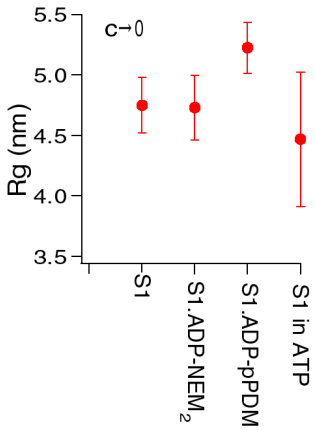

Fig. 1. The Rg values of various papain-treated S1 sapmles with and without a nucleotide.

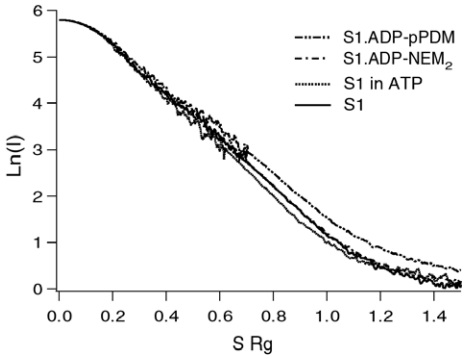

Fig. 2. The solution scattering curves of various S1 samples in the ln[I(S)] versus SRg plot. Intensity was normalized to SRg=0 where $S=2\sin\theta/\lambda$ (2θ, the scattering; λ, the wavelength of X-rays) and Rg, the radius of gyration.

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