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An X-ray solution scattering study on the conformation of skeletal muscle myosin subfragment-1 in the presence of Mn^{2+} and ATP

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Introduction

The conformations of the skeletal muscle myosin subfragment-1 (S1, the myosin head) of the intermediate states in the ATPase cycle have extensively been investigated by solution X-ray scattering. It has been suggested biochemically that the predominant intermediates of the Mn^{2+}-dependent myosin S1 ATPase are applicable to the S1*· MgADP complex at low temperatures (< 10 °C) [1]. In order to define the magnitude and extent of the changes, the conformations of S1 in the presence of Mn^{2+} and ATP were examined by X-ray solution scattering.

Experimental

Papain-digested myosin subfragment-1 was prepared from the rabbit skeletal myosin molecules. Purified S1s through a column chromatography were centrifuged and used for X-ray experiments. Mn^{2+} and ATP were added to the S1 solution to make the final concentration of Mn^{2+} and ATP were 6 mM and 3 mM, respectively. X-ray experiments were done at 4 °C and 20 °C at the BL15A1. All scattering data were collected with a 1D-PSD.

Results and Discussion

The Guinier plots of the scattering intensity data (I(S)) from the S1 solution in the presence of Mn^{2+} and ATP gave all straight lines. The I(0)/c versus c (protein concentration) plots were also linear. Figure 1 shows the concentration dependence of the radius of gyration (Rg) values of the samples, compared with those from several reference samples. The true Rg value of S1 in the presence of Mn^{2+} and ATP was ~50 Å at 4 °C, ~2 Å larger than that of the nucleotide-free S1 while it was ~48 Å at 20 °C, comparable to that of the nucleotide-free S1. The Rg value of S1 in the MgATP solution was ~46 Å, corresponding to S1**· MgADP · Pi. The scattering intensity profile of S1 ($I(S) \cdot S^2$ versus S plot) (in the range of $S > 0.01$ Å^{-1}) in the presence of Mn^{2+} and ATP at 4 °C moved toward smaller S than those of the other samples but at 20 °C was close to that of the nucleotide-free S1 (Fig. 2). Interestingly, both of the Rg value and the whole scattering curve of S1 in the MnATP solution resembled closely to those of the pPDM-crosslinked S1 trapping MgADP (S1· MgADP-pPDM) [2].

It has been suggested that at low temperature S1*· MgADP comprises a greater proportion of the steady-state complex in the Mn^{2+}-dependent ATPase of S1 [1]. The present results provide two possibilities: (1) the conformation of the S1*· MgADP in the ATPase cycle of S1 is different from that assumed by S1 by sole addition of MgADP, (2) the conformation of S1 in Mn^{2+} and ATP solution at low temperature globally resembles to that of S1· MgADP-pPDM where the light chain-binding domain of S1 moves in the opposite direction to that in the S1**· MgADP · Pi. The results at room temperature may be applicable to an equilibrium mixture of S1*· MgADP and S1**· MgADP · Pi.

References

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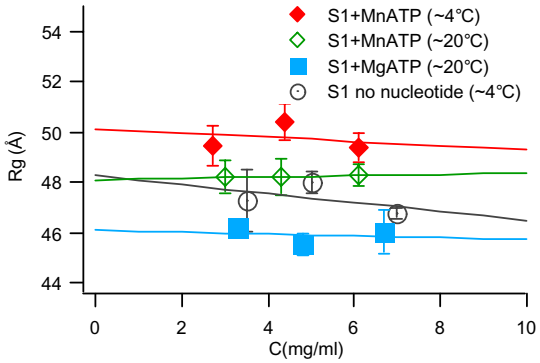

Fig.1 The concentration dependence of Rg values of S1 in the presence of Mn^{2+} and ATP solution, compared with those of other samples.

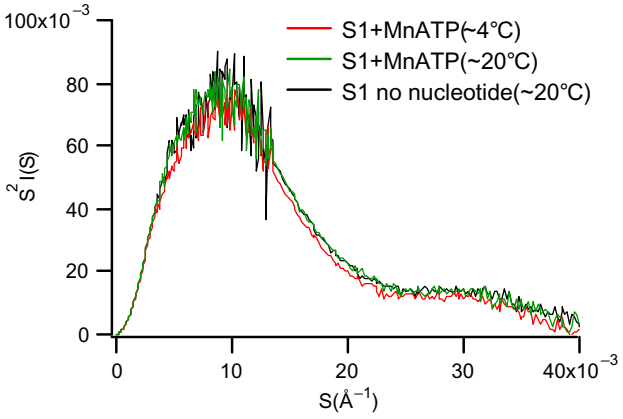

Fig. 2 The Kratky plots of scattering data of S1 in various solutions.

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