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STRUCTURE OF THE OLIGOMERIC ACTIN-MYOSIN HEAD COMPLEX AS STUDIED BY X-RAY SOLUTION SCATTERING

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Introduction

We made a nonpolymerizable G-actin by MBS and DHT[1)] and measured X-ray solutiuon scattering from a 1:1 acto-myosin head (S1) complex[2,3)]. The model consisted of atomic structures of actin monomer and S1 which fits the whole scattering intensities closely was examined on the basis of rigid-body rotation and translation of the two molecules. The unique orientation of the two molecules was further checked by the use of the data from a complex of DNaseI-actin and S1[3)]. In the best-fit model a tip of S1 binds to the actin subdomain 1 as expected from the reconstructed image of electron micrographs of F-actin decorated with S1. However, this model differs considerably from those of the F-acto-S1 rigor complex; S1 molecule is oriented toward the opposite direction[4)]. This may be caused by the fact that the next actin for the second binding site is missing in the monomeric complex. We have attempted to prepare the complexes of S1 and oligomeric actins and examine their solution structures by X-ray scattering.

Experimental

The dimeric and trimeric actins were prepared by cross-linking F-actin at Lys191-Cys374 by a bifunctional reagent pPDM, then deplolymerizing it and modifying it with DHT4). Nonpolymerizable oligomeric actins (dimer and trimer) were purified and collected by a column chromatography. The purity was checked by SDS-PAGE, showing more than 90%. 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) with a 1D-PSD.

Results and Discussion

The Guinier plots of the scattering data (I(S)) from monomeric, dimeric and trimeric actins gave all straight lines, indicating little aggregate in any solution. I(0)/c versus c plots were linear and their values extrapolated to zero protein concentration (c=0) were obtained. The results clearly showed that the monomeric, dimeric and trimeric actins exist as a dimer (2A) of monomer (~90kD), a dimer ($2A_2$) of dimer (~180kD) and a dimer ($2A_3$) of trimer (~270kD) at high protein concentration (c>3mg/ml). The Rg values were ~3.0nm, ~4.0nm and 4.5nm for 2A, $2A_2$ and $2A_3$, respectively. The Guinier plots of the scattering data from S1 complexes as 1:1 with monomeric actin, 1:2 with dimeric actins and 1:3 with trimeric actins gave straight lines in any solution at relatively higher ionic strengths (90-110mM KCl). Molecular weights estimated from the extrapolated values of the I(0)/c versus c plots showed that S1 bound to the monomeric, dimeric and trimeric actins, and forms A-S1(~155kD), A_2-S1(~200kD) and A_3-S1(~250kD) complexes, respectively (Fig. 1A). The Rg values extrapolated to c=0 were ~5.0nm for an A-S1 complex, ~5.2nm for an A2-S1 one and ~5.5nm for an A_3-S1 one (Fig. 1B). Thus, S1 binding made each dimer of the oligomeric actins dissociate into a monomer, forming an S1 complex with each oligomer. The detailed studies are in progress to derive the solution structure of each oligomeric actin-S1 complex.

References

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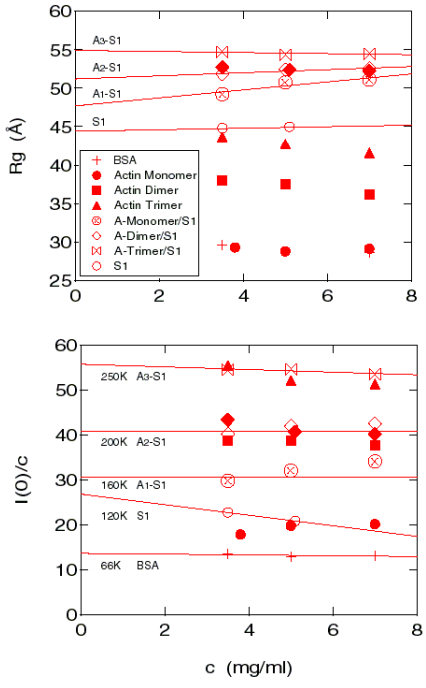

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