Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602102455

The Rigor Structure of Acto-Myosin and Its Implications for Motor Function

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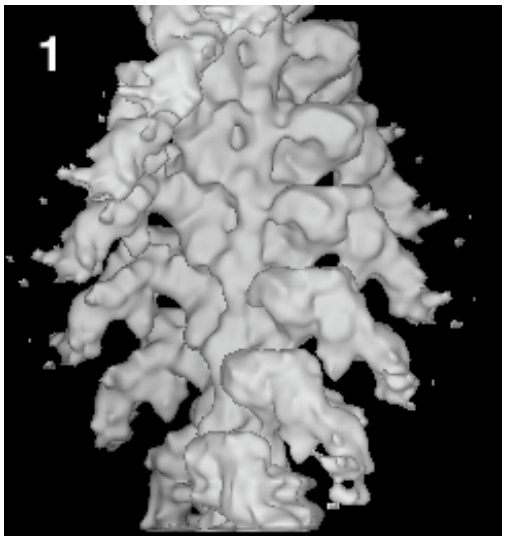
One most important question in the field of actin/myosin motor function is the structure of the strong binding state of the acto-myosin complex. Especially the difference between the initial weak binding state and the strong binding state and its coupling to the nucleotide state of myosin has not yet been revealed by high resolution X-ray structures. An alternative approach is to combine high resolution EM with known X-ray models of actin and myosin. Recently we reported the necessity of conformational changes of the upper 50k domain when docking the original chicken myosin-S1 structure [1] into our reconstructions [2]. Here we present a refined reconstructed density, which now represents the nucleotide free complex of myosin subfragment1 and F-actin at a resolution of about 17Å (5 □ FRC criterion). The density shown in FIG. 1 was calculated from defocus series of the complex embedded in ice recorded at a LEO EFTEM 912 □. The present analysis combines a new form of helical image processing with multivariate statistical analysis to increase resolution. The details of image processing are given elsewhere [3].

The fitting of the molecular X-ray models was obtained in a least squares procedure, the best fits gave an overall R-factor in the order of 15% (Gaussian kernel for the molecular models at a half maximum width of 18Å). The obvious F-actin backbone can be used to assess the quality of the reconstruction. Fitting the available model of F-actin [4] gives a very good agreement as is illustrated in FIG. 2. For fitting of myosin its original chicken myosin S1 structure was separated in "body" and "nose", the "nose" part mainly consisting of the □-helical structures of the upper 50k domain.

It has been assumed that the structure of myosin without nucleotide [1] represents myosin bound to actin in the rigor complex. However, in order to fit the atomic models the "nose" part of the 50K upper domain must be swung in by 10-15Å, representing a rotation of about 20˚ (see FIGs 3, 4). Interestingly, the body part refines perfectly well into the reconstructed density without bending of the original structure. At the current resolution it is now obvious that there is also a change of conformation at the nucleotide binding site (FIG. 4). The nucleotide binding cleft opens by about 5Å, presumably resulting in a lower nucleotide affinity in the strong acto-myosin binding state. This comes about since the switch 1 element of the nucleotide binding site is anchored in the 50K upper domain so that the strong binding to actin will lead to a 5-10Å movement of switch 1. Thus strong binding to actin opens switch 1. This movement can be clearly distinguished from the opening of switch 2, which is caused by a 5˚ rotation of the 50K lower domain with respect to the rest of the motor domain. While the opening/closing of switch 2 has been observed a number of times by X-ray crystallography, the closing of the major cleft has not yet been seen in isolated cross bridges. The realisation that switch 1 moves (opens) during actin binding opens up new scenarios for the mechanisms of ADP and phosphate release during the cross bridge cycle.

[1] I. Rayment et al., Science 261 (1993) 50.
[2] R.R. Schroeder et al. , Biophys J 78 (2000) 272a, 80 (2001) 198a
[3] The authors would like to acknowledge the collaboration with J. Frank (Howard Hughes Med. Institute, Albany), see abstract by Schroeder et al., this conference
[4] K.C. Holmes et al., Nature 347 (1990) 44



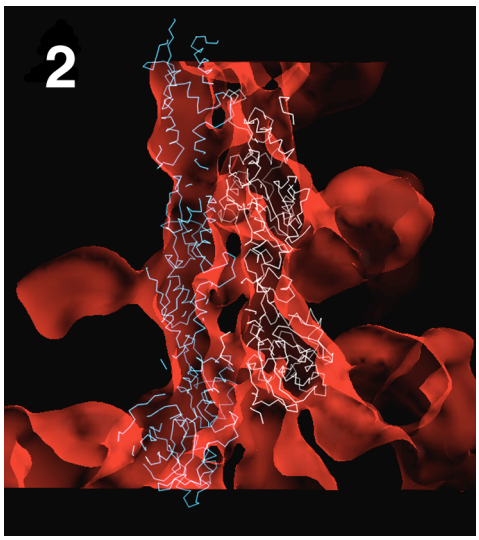
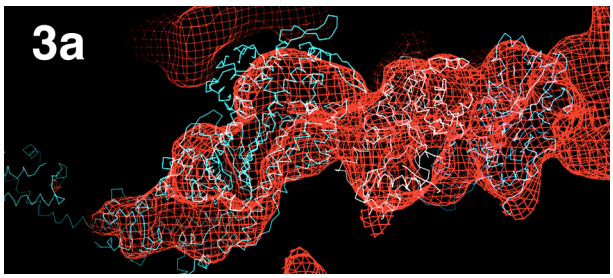


FIG. 1 Reconstructed density of the nucleotide free complex of myosin-Subfragment 1 and F-actin. The helical repeat represents a length of 35.75 nm. The resolution shown here is about 15Å (5 □ FRC criterion)

FIG. 2 Fitting of a molecular model of F-actin into the reconstructed density. The two long pitch helices and the interaction across the F-actin filament is shown



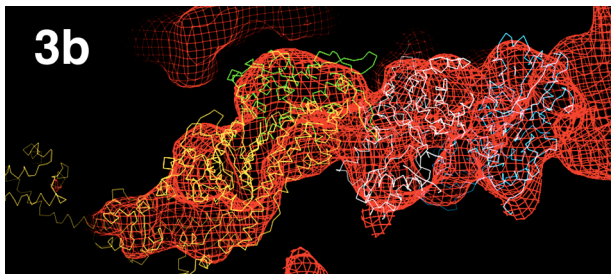
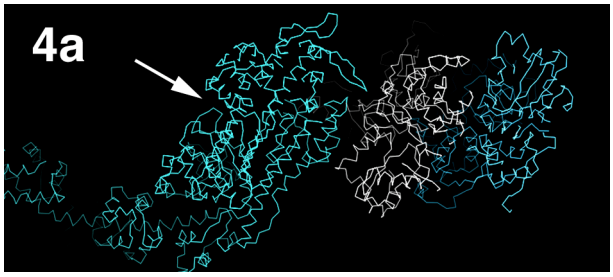


FIG. 3 Fitting of the molecular models of chicken myosin-S1 into the reconstructed density. (a) the original molecular model of the upper 50k domain conformation does not fit the density (b) independent refinement of the nose region of the 50k domain leads to improved fitting



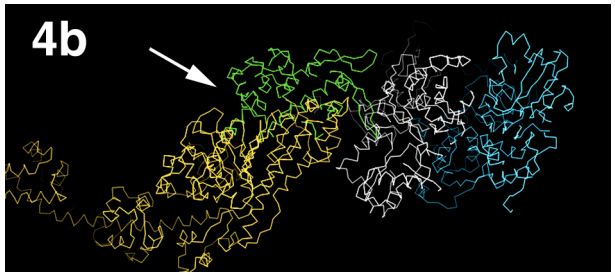


FIG. 4 Same view as in FIG. 3 without reconstructed density. Note the clear difference in conformation of the myosin model, and particularly the opening of the nucleotide binding cleft/tunnel (arrows) in the new model structure.

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