A Fibre Diffraction and Atomic Modeling Study of the Actomyosin Complex

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The molecular mechanism by which motor proteins convert chemical energy into mechanical force or motion is a central question in protein biology. In muscle, it is the globular heads of myosin (subfragment-1 or S1) projecting from the thick filaments that split ATP and interact cyclically with actin filaments to pull them along. Atomic coordinates are now available for the individual proteins actin and myosin-S1 (Kabsch *et al.* Nature 347: 37-44, 1990 & Rayment *et al.* Science 261: 50-58, 1993) but, unfortunately, not for a complex of the two. Cryo-electronmicroscopic studies to date yield 20-30 Å resolution density maps of the nucleotide-free skeletal S1-decorated actin (Milligan *et al.* J. Cell Biol. 105: 29-39, 1987 & Whittaker *et al.* Nature 378: 748-751, 1995), and the current standard model of the actomyosin complex represents a fit of the S1 crystal structure and the Holmes *et al.* atomic model of F-actin (Nature 347:44-49, 1990) into such a density map (Rayment *et al.* Science, 261:58-65, 1993). We are using high-angle fibre diffraction data from myosin-decorated actin arrays to further refine this model and to probe different attached states. The experiment is to decorate the thin filaments of stretched rabbit striated muscle fibres with myosin motor fragments and to measure the associated intensity changes in the actin-based fibre diffraction patterns out to 8 Å and better. The diffracting power of the specimen is low, and the experiment is only possible with a "clean," finely focused synchrotron x-ray beam. We used a ~60 cm specimen-to-detector distance, wavelengths of 1-1.3 Å and a 20 x 25 cm Fuji imaging plate on the NSLS beamline X9B or on the ESRF beamline 4, ID2. So far we have looked at nucleotide-free chicken skeletal chymotryptic and papain S1 fragments, chicken smooth muscle S1 (phosphorylated and unphosphorylated), scallop fast adductor S1 and Dictyostelium catalytic domain (761). We have refined a model structure against the data from skeletal chymotryptic S1 decorated filaments by allowing the S1 to move with respect to the actin monomer and to bend at appropriate domain junctions until the computed diffraction pattern best matched the data. The result is a structure similar but not identical to the Rayment model. All S1 fragments looked at so far show characteristic x-ray decoration patterns indicating that there are different types of bound head structures in rigor. X-ray patterns from all but the striated muscle chymotryptic S1 decorated fibres show reversible changes on ADP binding. The effect was most striking in the smooth S1 and very slight in the skeletal papain-S1. Like Whittaker *et al.* 1995, we believe that the large structural change in the case of smooth S1 comes primarily from a movement of the lever arm. Third-generation synchrotron beamlines now deliver sufficient x-ray intensity to allow us the fascinating possibility of monitoring the kinetics of such structural changes in bound motor fragments, although the availability of rapid, high-count-rate area detectors is still a problem. The immediate way forward is to use nucleotide analogues and specifically engineered motor fragments in steady-state experiments to search for alternative bound head structures, which may represent different stages of the powerstroke we seek to understand.