A Low Angle Diffraction Study of the Structure of the Actomyosin Complex; Effects of ADP Binding	X9B

K. Poole, M. Lorenz, P. Ellison, G. Evans, G. Rosenbaum, P. Boesecke, K.C. Holmes, C.R. Cremo (Max Planck Inst.)

With an atomic model for the actin filament (Holmes et.al. 1990, Nature, 347: 44-49; Lorenz et.al. 1995, *J. Mol. Biol.* **246**, 108-119) and the atomic coordinates for at least one form of the myosin motor protein (Rayment et.al. 1993, *Science* **261**, 50-58) we are in a position to make use of high angle fibre diffraction data from myosin decorated actin filaments to refine a model structure of the complex. Nucleotide-free myosin subfragment-1 (S1) isolated from both chicken skeletal and smooth muscle sources was introduced into overstretched rabbit psoas muscle fibres. In both cases the protein binds tightly to actin and this results in the intensification of the actin based layer lines in the X-ray pattern. Refining an actomyosin atomic model against the nucleotide-free skeletal S1 data produced a best fit which was similar to the model suggested by Rayment et.al. (1993) based on electron microscope evidence. ADP binding had very little effect on the diffraction pattern. The nucleotide-free smooth muscle S1 decorated filaments produced a very different diffraction pattern and the binding of ADP was shown to induce a structural change in the head resulting in a pattern similar to that from skeletal S1 decorated fibres. The Kd for ADP binding was shown to be ca. 2μM. It is most interesting that the two rigor complexes are structurally different. If the rigor structure represents the end of the power stroke then the data imply that at least some part of the power stroke in these two motors is produced by different configuration changes of the actomyosin complex. Alternatively, we must question our interpretation of the rigor structures themselves. We are in the process of refining the model against the smooth muscle S1 data but it is clear that the ADP induced structural change in the myosin head is very large and certainly involves the distal part of the head. We intend to continue our investigation of the actomyosin motor mechanism by looking at the bound structures of myosin motors or parts of motors from different sources, both natural and engineered, with a range of nucleotides or their analogues in the active site.