
Keywords: instrumentation, non-crystalline biological systems.

X-ray diffraction from single fibres of frog skeletal muscle can be used to record the conformational change in the myosin head, the molecular motor in muscle, during the quick force recovery following a step perturbation in length, which represents the mechanical manifestation of the synchronous execution of the working stroke (Irving et al. Nature 357:156, 1992). The unprecedented collimation and intensity of the X-ray beam at ID2 (ESRF, France) has allowed us to show that in isometrically contracting single muscle fibres (at 2.1 µm sarcomere length, 4 °C) the M3 reflection, which arises from the repeat of myosin heads along the myosin filament, is composed of two closely-spaced sub-peaks of comparable size, due to X-ray interference between the two arrays of actin-attached myosin heads in each myosin filament (Dobbie et al. Biophys. J. 76:A32, 1999; Linari et al. PNAS 97:7226, 2000). Because of the antiparallel arrangement of the heads in the two halves of the myosin filament, the interference distance is expected to reduce during the execution of the working stroke, as the actin filaments are pulled towards the centre of the myosin filament (M-line). We determined the change of the relative intensity of the M3 sub-peaks in the 2-ms period following completion of the working stroke elicited by imposing shortening steps during isometric tetani at sarcomere length ∼2.1 µm, 4°C. The results show that the catalytic domains of the heads become closer to the M-line during the working stroke, but the underlying motion results much smaller than that expected if all the heads had undergone the tilting motion caused by the imposed sliding between actin and myosin filaments. These results reopen the question on the nature of the working stroke.

About X-ray damage on Crystalline Biological Samples. R.B.G. Ravelli and S.M. McSweeney. EMBL Grenoble outstation, 6 Rue Jules Horowitz, BP 156, 38042 Grenoble Cedex 9, France.

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Radiation damage at cryogenic temperatures has become a problem for well-focused undulator beamlines at third generation synchrotrons for almost all crystalline biological samples. The overall quality of the data obtained for a given crystal decays as data collection proceeds. Recently1,2,3, it was shown that specific changes are introduced: disulphide bonds break and decarboxylation of acidic residues occurs. Serious non-isomorphism can be introduced, hampering MAD and SAD phasing methods. A new criterium that is more sensitive than the traditional measures of radiation damage has been introduced2.

In this presentation, we will review the generality of these observations and discuss possible mechanisms. Data will be shown on the effect of scavengers and temperature, and the possibilities of making active use of specific damage to study enzyme mechanisms4 are discussed. A new method is presented that tries to compensate for radiation damage in SAD data sets.
