Time-resolved Techniques for Muscle Diffraction with Synchrotron Radiation*

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The x-ray diffraction diagrams from native, functioning muscle can be detailed and informative, but muscle is a very weak diffractor and therefore the intense x-ray beams provided by synchrotron radiation are essential in current research on the mechanism of contraction. The central area of interest is the detailed behavior of the myosin crossbridges as they go through their cycles of interaction with actin which produce the relative sliding motion of the actin and myosin filaments past each other which leads to muscle shortening. The various steps in these cycles take place on a millisecond time scale, so the changes in x-ray patterns need to be recorded with this time resolution. Small but very important changes in filament axial periodicity also occur during muscle activity due to filament compliance, and these have a large effect on the interpretation of mechanical transient measurements which provide crucial evidence about the characteristics of the crossbridge cycle. Thus spacing changes need to be measured to an accuracy of .05% or less, again with millisecond time resolution.

We have been using imaging plates and CCD detectors (which both give the necessary spatial resolution at convenient camera lengths) in a number of different ways to achieve the required combinations of high spatial and temporal resolution. (1) For experiments in which we require a tetanic contractions of a muscle (at 2-4 min. intervals to allow time for recovery), we use either an imaging plate on which the x-ray pattern is accumulated over 50-100 cycles of contraction, or a CCD array where the whole array is read out and stored between contractions (to avoid the accumulation of dark current noise). In each case, an intermediate speed shutter allows the x-ray beam to fall on the muscle only during the required time interval. Control patterns (either rest or isometric contraction) are recorded during alternate cycles; in the case of the imaging plate by displacing the plate sideways between exposures and arraying a blanking-off plate to move alternately to one side or the other so as to record the two types of image (e.g., fast shortening vs. rest) on the same plate, side-by-side. (2) For higher resolution (say 10 msec), in a succession of up to nine time frames, a 128 pixel wide section at one side of a CCD is used (with a fiber-optic taper) to record the required region of the pattern (e.g. a meridional slice), the images are shifted sideways electronically between each time frame (separated by a rapid acting shutter) so that the nine successive images are temporarily stored on the CCD and then read out between contraction cycles. (We have also used a rotating imaging place to obtain time courses from 'streak patterns'). (3) For the highest time resolution (1-2 msec, during rapid mechanical transients) we have so far used only imaging plates plus a rapid mechanical shutter, so that only one single time point is obtained each contraction cycle. However, we hope to speed up the CCD frame-shifting in the future.

A number of new results will be discussed.

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