ESRF

Experiment title: HIGH RESOLUTION X-RAY DIFFRACTION STUDIES OF CONTRACTING MUSCLES

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Report:

We have investigated up to a resolution of ca. 2.5 nm the behaviour of the actin and myosin based layer lines in X-ray diffraction patterns from "live" sartorius muscles (Rana Esculenta) contracting isometrically, against a negligible load or submitted to a sudden release sufficient to bring isometric tension (P_o) to $<0.05P_o$.

With regards to myosin, the brilliance of the ESRF allows to clearly resolve the split of ca. 1/1000 nm^{-1} present at P_o on the meridian of the third myosin layer line (3M) [1] as well as in several other higher order reflections. We conclude that these splittings can be explained in terms of an interference effect across the M-line corresponding to an interference distance of ca. 860 nm. The apparent discrepancy between this distance and the splitting between the peaks is due to the way that the interference effect samples the underlying quasi-helical transform. Time resolved experiments have shown that the intensity responses to a sudden release are different for each of the reflections on the 3M. Thus, whilst one of the reflections -at a spacing of 14.427±0.012 nm- undergoes a substantial intensity decrease simultaneously with tension loss, the other -at a spacing of 14.636±0.005 nm- has a similar intensity decrease but it is delayed by ca. 0.7-0.8 ms. Thus, it appears that the delay in the intensity response of the 3M after a quick release [1,2] is entirely due to the behaviour of one of the peaks in the doublet. The different response of the two peaks makes it unlikely that these intensity changes arise from changes in the orientation of the myosin heads and suggests that they are largely due to interference effects.

Our previous attempts to determine thick filament extensibility relied on measurements of the position of the centre of gravity of the 3M. Because the centre of gravity depends both on the positions and on the intensities of each of the peaks in the doublet and the latter have different time courses the results were ambiguous. At the ESRF it is possible to measure the spacing changes undergone by each individual peak, the ambiguity disappears and the spacing changes provide a direct measure of thick filament extensibility which the results show is ca. 0.5% for a P_0 force.

With regards the actin based X-ray diffraction features, the results show that the spacings of be 2.7, 5.1 and 5.9 nm actin layer lines increase by 0.2-0 .3% from rest to P_0 -confirming previous published work [3,4]- and, in addition, that upon a sudden release, the spacings of these layer lines decrease by 0.25-0.3% relative to their rest values, i.e. by 0.45-0.6 % relative to their P_0 values. Thus, it seems that the actin filament can undergo a total length change of ca. 0.45-0.6%, i. e. a value very similar to that deduced for thick filament extensibility. Moreover, we have found from the measurement of the absolute values of the spacing changes in the 5.9 and 5.1 nm actin layer lines that there is a change in the helical symmetry of the actin filament [4]. From the positions of the 5.9 and 5.1 nm layer lines, we calculate pitches of 73.52 ± 0.18 and 74.05 ± 0. 15 nm for the actin filament a rest and at P_0, respectively. Direct measurement of the 1st actin layer line at P_0 [5], yielded a value of 74.29 ± 0.41nm. Within error the spacings at P_0 fit the symmetry of a 54/25 helix, suggesting that in isometric contraction the actin filament acquires the symmetry needed for the formation of an actomyosin complex.

Taken together, our findings suggest that the actin and myosin length changes are purely elastic and amount to 4.5-6 nm per half sarcomere for a P_0 force. On this evidence it is conceivable that the T_1 portion of the tension curve [6] following a quick release is all due to filament elasticity and not to attached myosin heads as originally proposed. If so, it follows that the attached myosin heads must be exceedingly stiff and difficulties arise for the swinging head model which requires that the head portion of the myosin molecule should be capable of substantial elastic shape changes. Finally, if the delay in the intensity response of the 3M is indeed due to changes in the interference distance across the M-line, then interpretation of this effect in terms of the synchronous execution of a power stroke by the myosin heads will have to be revised.

References

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