X-RAY DIFFRACTION STUDIES OF THE ACTIVATION OF MUSCLE FIBRES

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Striated muscle tissue presents a high degree of structural order at a molecular level where the structural and functional repeating unit is called the sarcomere. In particular, each muscle sarcomere contains two types of filaments: the myosin II-containing thick filaments and the thin filaments –composed mainly of actin, tropomyosin and troponin. The contractile cycle involves the interaction of both sets of filaments; and the conformational changes that the proteins within undergo during contraction can be monitored with x-ray diffraction.

Tropomyosin and troponin in the thin filaments act as a Ca^{2+} sensitive switch that controls the myosin heads ability to bind actin. According to the *steric blocking* model, at high calcium concentrations, Ca^{2+} activates the myosin-actin interactions. Calcium changes the position of the tropomyosin and troponin along the actin filament triggering a series of conformational changes in the thin filaments; the most notable is the roll of the tropomyosin over the actin groove physically unblocking the myosin head binding sites on actin. Several studies have revealed that tropomyosin can adopt three different positions over the thin filament –*three-state model: 'off', 'active', 'potentiated'*. Furthermore, tropomyosin transition between these states might be linked to the change in the myosin heads bound to actin – as they go from weak to strong binding-.

In order to determine the conformation of the tropomyosin during different steps of the contractile cycle and its relationship with the actin-bound myosin heads, we performed a set of experiments at beamline ID02, ESRF (Grenoble-France). Diffraction patterns of *Rana temporaria* sartorious muscles at different states (rest, plateau of isometric contraction –P_0, unloaded shortening and stretching at 1.5 P_0) were taken with 20 msec exposure time. The second actin layer line, known to be associated to tropomyosin position, and the first troponin meridional reflection were carefully extracted from background and analyzed.

The results show that: 1) the three-state model can explain the different conformations of tropomyosin on thin filaments in the studied states; 2) Ca^{2+} binding to troponin alone does not lead to the 'potentiated' state of tropomyosin, where all the actin binding sites are fully exposed; 3) and it is likely that myosin head transitions from weak to strong binding and the extra displacement of the tropomyosin from 'active' to 'potentiated' state are due to co-operative actions.