X-ray diffraction analysis of the effects of myosin chain-2 phosphorylation on the structure of fast skeletal muscle fibres

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The isometric twitch tension of a fast skeletal muscle is enhanced by a factor of about 2 following a brief tetanic stimulation (Close & Hoh, 1968). This phenomenon, known as post-tetanic potentiation (PTP), is currently thought to be due to the phosphorylation of the fast myosin light chain-2 (MLC2) by the enzyme myosin light chain kinase (MLCK), which is activated by Ca/calmodulin during the tetanus. Phosphorylation of MLC2 in permeabilized fibres enhances their Ca sensitivity, producing more force during submaximal Ca activation. Phosphorylation of MLC2 in isolated thick filaments causes the loss of the regular helical arrangement of myosin heads characteristic of normal relaxed filaments (Levine *et al.*, 1996). It was postulated that MLC2 phosphorylation increases the mobility of myosin heads, which spend more time in proximity to thin filaments, leading to force enhancement. In this work, we test this hypothesis by using X-ray diffraction to detect structural changes in muscle fibres following MLC2 phosphorylation.

The experiments were done on glycerinated rabbit psoas fibres. Muscle bundles were isolated from animals killed by stunning and exsanguination. Bundles containing 10 glycerinated fibres were prepared for X-ray diffraction after exposure to: 1) relaxing solution containing 10 mM 2,3-butanedione monoxime to dephosphorylate endogenously phosphorylated MLC2, 2) subthreshold Ca solution (pCa 6.8), 3) phosphorylating solution containing 2mM calmodulin, 0.15mM MLCK (pCa 6.8) and 10mM tautomycin to inhibit endogenous phosphatase, 4) calmodulin/MLCK solution without Ca. X-ray diffraction analyses were carried out on beam line BL45XU at the SPring-8 synchrotron facility.

Equatorial reflections 1,1 and 1,0 are due to longitudinally oriented planes in the muscle filament lattice that pass through thick and thin filaments (1,1) and thick filaments only (1,0). The 1,1/1,0 intensity ratio gives information about distribution of mass around the filaments. In the presence of relaxing solution, 1,1/1,0 ratio was low, indicating that myosin heads were mostly located near thick filaments. When fibres were exposed to pCa 6.8, the ratio was nearly doubled, indicating a movement of the myosin heads towards thin filaments even with no force development. After exposing fibres to phosphorylating solution for 20 minutes, the ratio significantly increased further. At 2 minutes after the enzyme was washed out in low Ca solution, the ratio decreased to control level. Prolonging the wash out time did not change the ratio significantly. Incubating fibres in enzyme without Ca produced no change in ratio. MLC2 phosphorylation and dephosphorylation under our experimental conditions were verified using two-dimensional polyacrylamide gel electrophoresis. Lattice spacings decreased slightly on exposure to low Ca, but no significant change was observed following phosphorylation. However, reducing the lattice spacing by increasing sarcomere length dramatically reduced the change in 1,1/1,0 ratio with phosphorylation.

The present results provide structural evidence for a movement of cross-bridges towards the thin filaments following MLC2 phosphorylation, thereby strongly supporting this as the molecular mechanism for PTP. Sarcomere length dependence of the effects of phosphorylation correlates well with earlier work showing that the phosphorylation-induced increase in Ca sensitivity was similarly reduced by increased sarcomere length, as well as by osmotic compression (Levine *et al.*, 1996). These procedures enhance Ca sensitivity in their own right by bringing cross-bridges closer to thin filaments. Thus, at long sarcomere lengths, the cross-bridges are already close to thin filaments, and phosphorylation has little further effect. We predict that in intact fibres, post-tetanic potentiation should decrease with sarcomere length. The increased 1,1/1,0 ratio at pCa 6.8 suggests that elevation of baseline Ca following a tetanus may contribute to twitch potentiation early in PTP.

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