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Cross-bridge lever arm disposition of a low ionic strength-induced actin-bound state in *Rana temporaria*

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Reduction of ionic strength promotes formation of 'weakly binding' cross-bridges in skeletal muscle, an actin-bound S1 state differing from rigor and Ca^{2+}-activated states in that S1 is unloaded, and thought to be a pre-power stroke intermediate in the cross-bridge cycle.

We induced this state in fibre bundles from sartorius muscles of *Rana temporaria* (humanely killed by decapitation, fibre bundles skinned in 1% Triton X-100 for 2–5 min) by reducing ionic strength in a relaxing solution from 130 mM (HIS) to 35 mM (LIS). Bundle stiffness increased from 6.9 ± 4.9% to 13.8 ± 6.6% (n = 12, means ± S.D.) of rigor stiffness without a rise in axial tension, consistent with the formation of low force cross-bridges.

X-ray diffraction patterns were obtained by exposure to synchrotron radiation (l = 0.15 nm, beam dimensions: 0.3 × 0.2 mm; A2 beamline, DESY, Hamburg). X-ray data were collected on a delay line linear detector or on image plates positioned 2.5 m from the preparation. The most significant effect of LIS was on the ratio of 11 to 10 equatorial intensities (I_{11}/I_{10}), which rose from 0.30 ± 0.30 (n = 49) to 0.69 ± 0.56 (n = 11). In rigor, I_{11}/I_{10} was 2.80 ± 1.99 (n = 16). The LIS rise in I_{11}/I_{10} occurred principally through an increase in I_{11}; I_{10} remained almost unchanged (Xu *et al.* 1987).

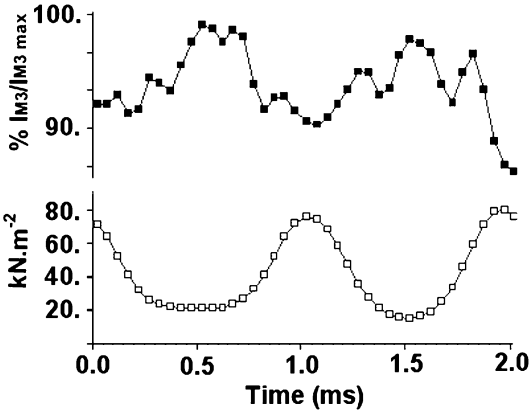

Figure 1. Force (□) and I_{M3} (■) signals during 1 kHz length oscillations in LIS. Bundle length 3.3 mm, diameter 475–575 μm. LIS composition (mM): EGTA, 1; MgATP, 1; Hepes, 10; creatine phosphate, 10; creatine kinase, 80 U ml^{-1}; Na^+, 10; K^+, 10; Mg^{2+}, 1; propionate 3. Temperature 6 °C.

In Ca^{2+}-activated fibres, 1 kHz sinusoidal length oscillations produce a sinusoidal change in meridional M3 reflection intensity (I_{M3}), maximum intensity ($I_{M3,max}$) occurring at maximum shortening. In rigor, oscillations produce a sinusoidal I_{M3} signal, but with $I_{M3,max}$ at maximum lengthening (Dobbie *et al.* 1998). This shift in $I_{M3,max}$ may indicate a change in S1 lever arm orientation. We imposed oscillations in LIS, and observed an I_{M3} change having the same phase relation to the oscillations as observed in Ca^{2+}-activated fibres (Fig.1). The intensity signal was 5–10% of $I_{M3,max}$, compared to a value of 20–30% in Ca^{2+} activation. The lever arm disposition required to account for these findings is consistent with a pre-power stroke state S1 structure in LIS.

Dobbie I *et al.* (1998). *Nature* **396**, 383–387.

Xu S *et al.* (1987). *J Muscle Res Cell Motil* **8**, 39–54.

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All procedures accord with current UK legislation.