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).

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.


This work was supported by IHP-Contract HPRI-CT-1999-00040/2001-00140 of the E.U.

All procedures accord with current UK legislation.