Micrometer-nanometer scale X-ray diffraction to study the molecular mechanism of heart regulation

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The structural dynamics of the motor, regulatory and accessory proteins of the cardiac sarcomere are investigated *in situ* by X-ray diffraction from intact right ventricular trabeculae of the rat heart at the ID02 beamline of the European Synchrotron Radiation Facility. The trabecula is vertically mounted in a thermoregulated trough (27°C), perfused with oxygenated physiological solution, and electrically paced at a frequency of 0.5Hz. A FReLoN CCD detector is placed at either 1.6 m from the preparation to collect up to the 6th order of the myosin-based meridional reflections, or 30 m to measure sarcomere length (SL) during diastole-systole cycles (5-10ms time frames). SL is held constant during force development (sarcomere isometric conditions) by feeding the motor-servo system with a signal based on the changes in SL recorded in the preceding fixed-end contraction (Caremani *et al*. *PNAS*, **113**:3675–3680, 2016). In diastole the spacings of the M3 meridional reflection (SM3, associated with the myosin heads axial periodicity) and of its second order M6 (SM6, associated with the myosin-containing thick filament backbone periodicity) are 14.361 ± 0.004 (mean ± SD) and 7.195 nm respectively at SL 2.1 μm and $[Ca^{2+}]_o$ 1 mM (four trabeculae). Increase in either SL to 2.3 μm or $[Ca^{2+}]_o$ to 2.5 mM did not change significantly the spacing of the reflections. At the peak of systolic force attained with $[Ca^{2+}]_o$ 2.5 mM and SL 2.1 μm both SM3 and SM6 were ~1% larger. The M3 reflection was sampled by X-ray interference between half-sarcomeres, showing a dominant peak with two small satellites on either side in diastole, while at the peak of systolic force in sarcomere isometric conditions it was split in two peaks of comparable size. These results indicate that in diastole, independent of SL and $[Ca^{2+}]_o$, myosin heads are folded back towards the thick filament midpoint in agreement with the OFF state of the filament in skeletal muscle (Zoghbi et al. *PNAS*, **105:**2386-2390, 2008; Linari et al. *Nature*, **228**, 576-579, 2015). Consistent with the transition to the ON state described in the skeletal muscle thick filament, the development of systolic force is associated with the 1% increase of thick filament extension and the movement of the force generating motors by ca 10 nm away from the filament midpoint (Reconditi et al. *PNAS*, **108**:7236-7240, 2011).

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