REPORT

Proposal Title: X-ray diffraction study of the feed-back between contraction and activation of skeletal muscle fibres

Proposal Number: LS-2380

Muscle contraction is powered by myosin molecular motors which cyclically interact with actin filaments in sarcomeres. Low angle x-ray diffraction allows one to study movement of myosin motors in contracting muscle and to simultaneously measure macroscopic mechanical parameters: tension and stiffness (Ref. 1). The actin-myosin interaction is controlled by Ca^{2+} ions, which interact with regulatory protein troponin. Troponin sits on another regulatory protein, tropomyosin (Tpm), which forms a long helix on the surface of actin filament. In the absence of Ca^{2+} troponin binds actin filaments in a way that Tpm covers myosin binding sites of actin. According to the three state model (Ref. 2) this state is called 'blocked', or B-state, and corresponds to the relaxed state of muscle. When Ca^{2+} binds troponin, it detaches from actin and Tpm moves to a position that enables binding of myosin heads to actin, the system goes to 'closed', or C-state. Strong stereospecific binding of myosin heads pushes Tpm further and opens neighbour actin sites for myosin binding. The system goes to the 'open', or O-state. As the model (Ref. 2) was based on solution studies we tried to find out whether the O- to C-state transition take place upon a reduction of the number of actin-bound myosin heads in fully activated muscle fibres contracting at a near-physiological temperature. The experiments went quite successfully according to the plan. We have recorded the diffraction patterns of isometrically contracting fibres from rabbit skeletal muscle and compared them to those during near-steady shortening under low load. During beam allocation period we collected enough data to resolve weak high-angle intensity of the A2 actin layer line that is mainly determined by tropomyosin rotation with respect to the axis of a thin actin filament [1]. It was found that a transition of muscle fibres from contraction at a constant length at a near-physiological temperature to fast shortening is not accompanied by a measurable change in the high-angle A2 intensity despite a ~2-fold reduction in the fraction of myosin heads stereo-specifically bound to actin estimated from the measurements of muscle stiffness and the intensities of other actin layer lines A1, A6 and A7. This finding suggests that the C- state of the troponin-Tpm regulatory system is not occupied in fully activated skeletal muscle. Even a small fraction of myosin heads which remain bound to actin during muscle shortening at low load is sufficient to maintain the regulatory system in the open state [2].

References

1. Bershitsky, S.Y., Ferenczi, M.A., Koubassova, N.A., and Tsaturyan, A.K. 2009. Front. Bioscience 14:3188-3213.
2. McKillop D.F., Geeves M.A. 1993. Biophys J. 65(2):693-701.

Publications based on the project results

1. Koubassova N.A., Bershitsky S.Y., Ferenczi M.A., Narayanan T., Tsaturyan A.K. (2017) Tropomyosin movement is described by a quantitative high-resolution model of X-ray diffraction of contracting muscle. Eur Biophys J. 46(4):335-342. doi: 10.1007/s00249-016-1174-6.
2. Bershitsky S.Y., Koubassova N.A., Ferenczi M.A., Kopylova G.V., Narayanan T., Tsaturyan A.K. (2017) The Closed State of the Thin Filament Is Not Occupied in Fully Activated Skeletal Muscle. Biophys J. 112(7):1455-1461. doi: 10.1016/j.bpj.2017.02.017.