The Dynamics of Tropomyosin in Muscle Fibers

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Introduction

Muscle contraction is a process that involves many proteins. In addition to the key proteins, actin and myosin, there is also the troponin (Tn) complex, which mainly regulates the contraction in a calcium dependent manner, and tropomyosin (Tm) that plays a major role in the modulation of actin-myosin interaction. Tm is an alpha-helical coiled-coil protein. To date, little is known about the extent of Tm flexibility and the role of Tm dynamics in muscle regulation. Moreover, little is known about the conformation of Tn and Tm and how the conformation changes during muscle contraction.

Experimental

Bruker ECS-106 is the spectrometer of choice to perform Saturation Transfer EPR (ST-EPR) in order to study dynamics of Tm in the muscle fiber. Second harmonic out-of phase (V'2) spectra will be analyzed from which the correlation time is extracted (described in Fajer PG 2000). Bruker ELEXYS 680 spectrometer equipped with a Bruker dielectric ring resonator (model MD-5) is used for the study of distances between Tm and TnI using cw and DEER.

Results and Discussion

The dynamics of tropomyosin was measured using Saturation Transfer EPR at four different points along the molecule. To limit the motion of the label we have used a bi-functional spin label (di-methane-thiosulfonate spin label, HO1944) synthesized by Prof. K. Hideg which is attached at two ends to the i, i+4 double cysteine mutants. In the reconstituted 'ghost' fibers, the rotational correlation times of 13-17, 188-192, 268-272 were in the range of 30-45 microseconds while the middle of the molecule the correlation time was 3-fold faster at 13 microseconds. These results imply that Tm is a flexible molecule with the gradient of mobility consistent with the diminished mobility of the N-C overlap regions and regions close to the binding sites of troponin complex.

In order to orient troponin complex with respect to Tm we have measured distances between the spin labeled troponin I and spin labeled tropomyosin using pulsed DEER method. The distances between TnI-203 and Tm-153 as well TnI-186 and Tm-153 seemed to be ~ 40-50A, however they are somewhat obscured by the strong 27A distance population originating from the two chains of tropomyosin.

Reference

[1] Fajer, P.G., Encyclopedia of Analytical Chemistry, 5725-5761 (2000).