DEER MEASUREMENTS ON THE ACTOMYOSIN COMPLEX

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Introduction

Our project focuses on the molecular dynamics of the interaction between actin and myosin. Muscle contraction occurs when the actin-myosin complex undergoes a transition from a disordered weak-binding state (W), characterized by ionic interactions, to an ordered strong-binding state (S) that is characterized by hydrophobic interactions. Current actomyosin interface models have been derived by fitting individual crystallographic structures, of actin and myosin, into low-resolution actomyosin cryo-EM data (Rayment 1993, Schroder 1993, Mendelson 1997,Volkmann 2000, Holmes 2003, Volkmann 2003). In addition to testing and refining these models by making DEER measurements between spin labels on actin and myosin when they are in the actomyosin complex we are also interest in how the structure of the actin filament is affect by the binding of myosin, tropomyosin and cofilin.

Experimental

Our DEER experiments, we used F-actin MSL alone and with myosin S-1, tropomyosin and cofilin. The samples (approximately 110 μL in a 3 mm i.d. quartz capillary) were made with 10% glycerol in 10 mM Tris pH 7.5 and flash frozen in liquid N_2 to prevent ice crystals.

The spectra were acquired using a Bruker 680 Fourier transform spectrometer using a 4 pulse sequence (90°-τ_1-180°–τ_2-180°) with the MD5 cavity (Pannier M *et al.* 2000). The experiments were performed at 65 K. The data was then fitted using the fitting program developed by Dr. Fajer's lab. This program uses a Monte Carlo approach to select various distance distributions and simulate their spectra, which then compared to experimental spectrum. The Chi square value was used to determine the goodness of fit. All of the data was fitted to a two distance distribution model. The results of these fit are shown below.

Results and Discussion

Table 1 Results of the best fits to a two distance model

Sample	Dis. 1 (D_1, Å)	Pop. 1	Dis. 2 (D_2, Å)	Pop. 2
200 μM F-actin 8/05	51 ± 15	56 %	77 ± 20	44 %
170 μM F-actin	50 ± 19	74 %	77 ± 12	26 %
94 μM F-actin	51 ± 21	82 %	77 ± 3	18 %
65 μM F-actin -phalloidin	51 ± 21	36 %	77 ± 45	64 %
158 μM F-actin + 30 μM S1	49 ± 17	59 %	77 ± 28	41 %
121 μM F-actin + 140 μM S1	48 ± 15	51 %	76 ± 32	49 %
36 μM F-actin +36 μM Cofilin	52 ± 50	48 %	17 ± 2	52 %
F-actin + Tm	50 ± 19	68 %	77 ± 17	32 %
F-actin	49 ± 23	82 %	77 ± 11	18 %

The F-actin model predicts that the distances adjacent spin labels on Cys 374 is between 56 and 60Å (Lorenz et al. 1993). It is possible that the distance population could reflect two spin label conformations although the longer distance of 77 Å could arise from an incomplete subtraction of the background signal. More data analysis is needed to see if non-specific interactions can be filtered out from the DEER data. The wide distance distribution could be due probe mobility or to actin bundling. Experiments were done in the presence of actin binding proteins to see how their binding to actin affect the distance measurements. The addition of myosin S1 has been show to decrease spin label mobility (Thomas 1979), while tropomyosin has been show to prevent actin bundling (Korman 2000). Molecular modeling and simulation will also be needed to determine the how molecular dynamics will affect the position and motion of the labels and thus the distance measurements between them.

References

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