High-resolution structural dynamics of bifunctionally spin-labeled myosin by EPR of oriented muscle fibers

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ABSTRACT

We have performed continuous-wave electron paramagnetic resonance (CW-EPR) and double electron-electron resonance (DEER) on actin-bound myosin labeled with 3,4-Bis-(methanethiosulfonylmethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxy spin label (BSL). Our goal was to combine these two techniques to generate a reference model of the myosin II catalytic domain (CD) bound to actin. The use of BSL in our experiments greatly enhances the resolution of orientation and inter-spin distance measurements, due to the stereospecific bifunctional attachment to the protein backbone at two engineered Cys residues. We used *Dictyostelium* myosin II with Cys labeling sites as a model. Three pairs of Cys residues were engineered on our construct, which were located on the relay helix, K-helix (upper 50 kDa domain), and W-helix (lower 50 kDa domain). Skinned muscle fiber bundles (actin filaments) that were decorated with spin-labeled myosin were oriented parallel to the spectrometer's applied magnetic field. This procedure enables measurement of the orientation of BSL with respect to actin. Combining this with distances obtained from DEER, we are able to refine the structural model of *Dictyostelium* myosin II. This is a pathway to investigate structural dynamics of the system under various conditions.