MYOSIN CLEFT CLOSURE IN MUSCLE CONTRACTION

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Introduction

The myosin actin-binding cleft located between the upper and lower 50kD domains is thought to undergo changes as a function of nucleotide state and binding to actin. Cleft closure is needed to allow tight binding to actin and might be coupled to the opening of active site accompanying ATPase. To detect cleft closure directly we measured the distances between the upper- and lower-domains in various nucleotide states and in the presence of actin using conventional and pulsed dipolar EPR.

Methods

Two different dipolar EPR methods are used: 1). Conventional EPR, which measures the distances from 0 to 25 Angstrom; 2). Fairly novel pulsed ERP technique – Double electron electron resonance (DEER), which is sensitive to the distances from 16 to 60 Angstrom.

Results

We have engineered double cysteine mutants K282C in upper domain and S595C in lower domain that were labeled with spin probes and measured the distances. We found two major populations of distances at 9 and 20 Å and shifting in the populations as a function of nucleotide state and actin binding. No large changes were found between apo and ADP state. In ADP.Pi state, the fraction of short distance population (9 Å) disappeared. In presence of actin, the short distance population increased significantly and broad distance distribution was found, which suggest that the cleft is closed upon actin binding, however, multiple conformations exist.

Conclusion

In conclusion, the actin binding cleft was fully open in ADP.Pi state. The cleft was closed upon actin binding, however, multiple populations exist.