CONFORMATIONAL CHANGES OF TnI C-TERMINUS UPON Ca^{2+} BINDING IN THIN FILAMENT

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Introduction

C-terminal region of TnI is very important to Ca^{2+} regulated muscle contraction. An NMR structure of C-terminal region of skeletal TnI was proposed [1]. However, another study shows that this region is very flexible with little secondary structure and that is not affected by Ca^{2+} binding in solution, but which becomes more structured upon actin binding [2]. It was also proposed that during Ca^{2+} activation, TnI C-terminal domain undergoes major changes in position, swinging from actin upon Ca^{2+} binding [3].

In this study, we want to clarify following questions:
1. How flexible is C-terminal region of TnI in solution?
2. Does Ca^{2+} cause any conformational change in this region in solution?
3. Does actin binding induces well defined structure ?
4. Does this region swing from actin/Tm upon Ca^{2+} binding?

Experimental

We are utilizing cw-EPR (sensitivity: 0.8-2 nm) and DEER (sensitivity: 2-8 nm) experiments to study the change of distance and its distribution between different states. Cysteine mutants in TnI C-terminal region (single cysteine mutant cTnI 170, cTnI 202 and double cysteine mutant cTnI170-202) were cloned, expressed, purified and labeled with MSL. Skeletal actin (with a native cysteine at residue 374) was labeled with MSL or MTSSL. We were monitored the distance changes of cTnI 170-202-MSL upon Ca^{2+} binding in solution or in thin filaments, and the change of distance between cTnI 170 (and cTnI 202) and actin in thin filaments.

Results and Conclusions

1. In solution, TnI 170-202 has an average distance centered at 4.8 nm and with a very wide distribution (3.4 nm). This implies that this region is flexible. The addition of Ca^{2+} has no significant effect on the distance between residue 170 and 202.
2. Binding to actin does not change the distance or its distribution between residue 170 and 202.
3. The DEER distances between TnI and actin coincides with the distances between actin monomers making the identification of the former ambiguous.

References

[1] Murakami, K., *et al., J. Mol. Biol.*, **352**, 178-201 (2005).
[2] Blumenschein, T.M., *et al.*,*Biophys J.*,**90(7)**, 2436-44 (2006).
[3] Narita, A, *et al., J Mol Biol* **308(2)**, 241-61 (2001).