Analysis of critical amino acid residues in UNC-45 necessary for its interaction with myosin using site-directed mutagenesis

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Abstract

UNC-45 is an important protein for muscle contraction and heart formation. The UNC-45 protein is a chaperone for the myosin heavy chain head, and UNC-45 only binds to the UCS domain of the myosin head. In our research we introduced two crucial mutations in the protein UNC-45 that are crucial for the interaction of myosin and UNC-45. The mutation was accomplished using the multiple sequence alignment method and site-directed mutagenesis. The mutations were generated in UNC-45 of *Mus musculus* (mouse), and the specific amino acids that were mutated were: L741A and L806F. The mutant proteins were sequenced and over-expressed for analysis.

Introduction

Myosin is one of the major proteins that composes muscle and is vital for muscle contraction and heart formation.[1] Myosin is composed of six subunits with two heavy chains and four light chains.[1] The chaperone for the heavy chain myosin head is UNC-45, and only binds to the UCS domain of the myosin head. UNC-45 is composed of three main domains: a 14 kD amino terminal tetratricopeptide repeat (TPR) domain, a 42 kD central region, and a conserved carboxy terminal UCS domain.[2] The TPR domain interacts with the heat shock protein 90 (Hsp90), while the UCS domain binds the myosin head.[3] Because of this, the UCS domain is important for myosin formation, and thus muscle contraction and heart formation. Figure 1 (right) shows the domain organization of UNC-45 and Figure 2 (right) shows the carboxy termini 40 kD of the polypeptide UNC-45.[2]

For our experiments we identified two amino acid residues in the C-terminal 40 kD region of UNC-45 that are critical for its interaction with myosin head. We used point mutation on the UNC-45 gene to change these amino acids and expressed the mutant proteins. Mutations were successful as confirmed by DNA sequencing and SDS-PAGE analysis showed that the mutant proteins were expressed. In future, we shall assay for the effect of these mutations on the interaction of UNC-45 with myosin.

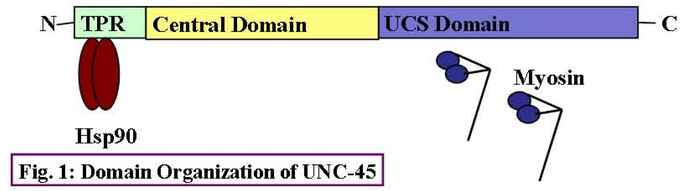

Fig. 1: Domain Organization of UNC-45

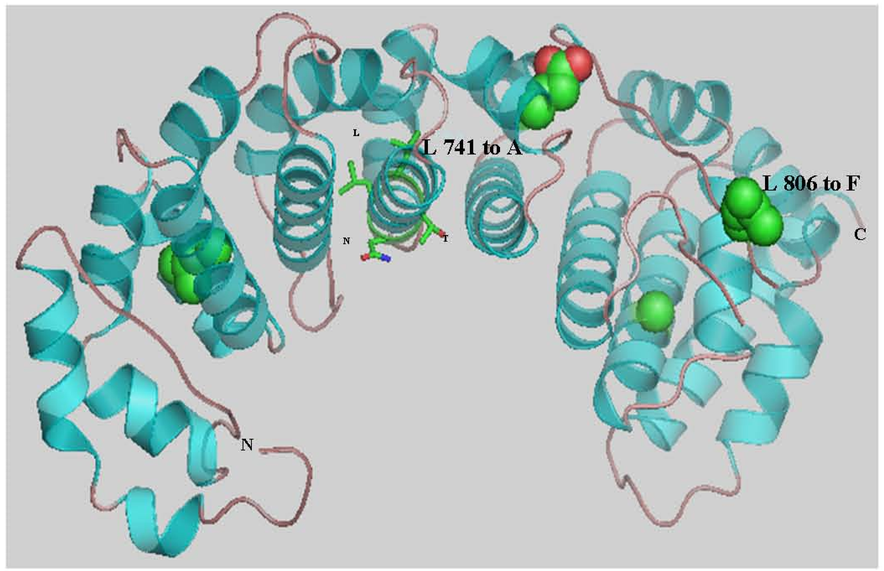

Fig. 2: UNC-45 carboxy terminal 40 kD[2] showing the two mutations. Leucine, L, at amino acid number 741 to alanine, A, (L741A) and leucine, L, amino acid number 806 to phenylalanine, F (L806F).

Fig. 3: Multiple Sequence Alignment of the Two Mutated Sequences. A) Leucine, L, at amino acid number 741 to alanine, A, (L741A) and **B)** leucine, L, amino acid number 806 to phenylalanine, F (L806F).

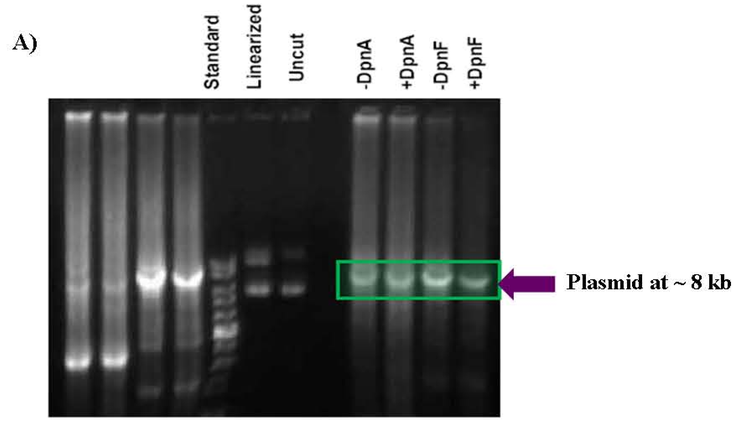

Fig. 4: A) Mutation of UNC-45 gene using linear Non-PCR based mutagenesis. An 0.8% agarose gel containing the mutated plasmids at the band ~ 8kb. **B) Expression of mutated UNC-45 protein in bacteria**. A 10% SDS-PAGE gel showing the over-expressed protein of interest at ~100 kD.

Conclusions and Future Work

- Two amino acids in UNC-45 critical for its interaction with myosin were identified by sequence alignment and successfully mutated using site-directed mutagenesis.
- The mutant proteins were successfully over-expressed in bacteria.
- The interactions of the mutant proteins with myosin will be assayed.

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Resources:
1) Nelson, David L., and Cox, Michael M. (2008) *Lenninger Principles of Biochemistry* 5th Ed, 175-176.
2) Dr. Odunuga, "Site-directed mutagenesis of mouse striated muscle UNC-45 protein"
3) Odunuga, Odutayo O., and Epstein, Henry F. "UNC-45: A Chaperone for Myosin and a Co-Chaperone for Hsp90". (2007) *Networking of Chaperones by Co-Chaperones*, 62-64.