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

Amanda H. Nolan, Jessica Johnson, Keith Mahipala, and Oduotayo Odunuga
Chemistry Department, Stephen F. Austin State University, Nacogdoches, TX

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 compose muscle and is vital for muscle contraction and heart formation. Myosin is composed of six subunits with two heavy chains and four light chains. 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 tetrapeptide repeat (TPR) domain, a 42 kD central region, and a conserved carboxy terminal UCS domain. The TPR domain interacts with the heat shock protein 90 (Hsp90), while the UCS domain binds the myosin head. 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 poly peptide UNC-45.

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 mutations 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.

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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Fig. 1: Domain Organization of UNC-45

A) Standard
B) Ligated
C) Ligated + Hsp90
D) Plasmid at ~ 8 kb

Fig. 2: UNC-45 carboxy terminal 40 kD 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 0.88% agarose gel containing the mutated plasmids at the band ~ 8 kb. B) Expression of mutated UNC-45 protein in bacteria. A 10% SDS-PAGE gel showing the over-expressed protein of interest at ~100 kD.