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Thermally-induced structural changes in an armadillo repeat protein suggest a novel thermosensor mechanism in a molecular chaperone

Paul J. Bujalowski [a,b,1], Paul Nicholls [a,b,1], José M. Barral [a,b,c,*], Andres F. Oberhauser [a,b,c,*]

[a] Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX 77555, USA
[b] Department of Biochemistry Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555, USA
[c] Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, TX 77555, USA

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ABSTRACT

Molecular chaperones are commonly identified by their ability to suppress heat-induced protein aggregation. The muscle-specific molecular chaperone UNC-45B is known to be involved in myosin folding and is trafficked to the sarcomeres A-band during thermal stress. Here, we identify temperature-dependent structural changes in the UCS chaperone domain of UNC-45B that occur within a physiologically relevant heat-shock range. We show that distinct changes to the armadillo repeat protein topology result in exposure of hydrophobic patches, and increased flexibility of the molecule. These rearrangements suggest the existence of a novel thermosensor within the chaperone domain of UNC-45B. We propose that these changes may function to suppress aggregation under stress by allowing binding to a wide variety of aggregation prone loops on its client.

1. Introduction

UNC-45B is a canonical member of the UCS (UNC-45, Cro1, She4p) family of proteins with homologues throughout the metazoa and fungi [1,2]. The absence or reduction of UNC-45 results in a range of defects, including malformation of the heart in *Mus musculus* [3], motility defects in *Caenorhabditis elegans* [4] and myofibril defects in *Danio rerio* [5]. The precise function of the protein has been well established as a molecular chaperone [2,6–8] assisting the folding and refolding of myosin. It has also been suggested to serve as a scaffold in the sarcomere [9] and also is necessary for proper function of other client proteins namely the transcription factor GATA4 [3] and the progesterone receptor [10].

The UNC-45B protein is made up of 3 domains, the UCS, central and TPR domains (Fig. 1a). The function of the TPR domain has been established as interaction with the Hsp90 chaperone system of which it may also be an activator [11–13]. The function of the central domain is enigmatic, but the UCS domain has been identified to interact with the myosin head and is able to partially rescue the uncoordinated phenotype in *C. elegans* [14].

UCS proteins are associated with several diverse myosin families, notably the processive myosin V [15], the single headed myosin I [15] as well as the classical myosin II [2,6]. During development in *D. rerio* the chaperone protein is found in the A-band of the sarcomere and in the mature sarcomere it becomes sequestered by the Z-lines [16]. This is in contrast to its role in yeast where UCS proteins are essential cofactors for myosin activity [17,18]. However after development, in *D. rerio* sarcomeres during heat and chemical shock the UNC-45B protein departs the Z-line and is shuttled to the A-band (Fig. 1b) [16]. While this is in full agreement with its role as a molecular chaperone, the molecular mechanism of this shuttling behavior is unclear. Such a molecular mechanism would likely feature a change in the affinity of the Z-line-UNC-45B interaction, myosin-UNC-45B interaction or both.

Structurally, the UCS proteins are characterized by a conserved armadillo repeat motif [9,19,20]. These armadillo repeats consist of 3 alpha helices with an average of 42 amino acids with diverse sequences, but highly conserved structures (Fig. 1a). These helices arrange in a superhelical structure defining a protein binding groove [21]. As armadillo repeats are known to bind a wide variety of substrates, intrinsic flexibility of the motif may be essential to this property [22]. Intrinsic flexibility is not confined to the

* Corresponding authors at: Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX 77555, USA.
E-mail addresses: jmbarral@utmb.edu (J.M. Barral), afoberha@utmb.edu (A.F. Oberhauser).
[1] These authors contributed equally to this manuscript.

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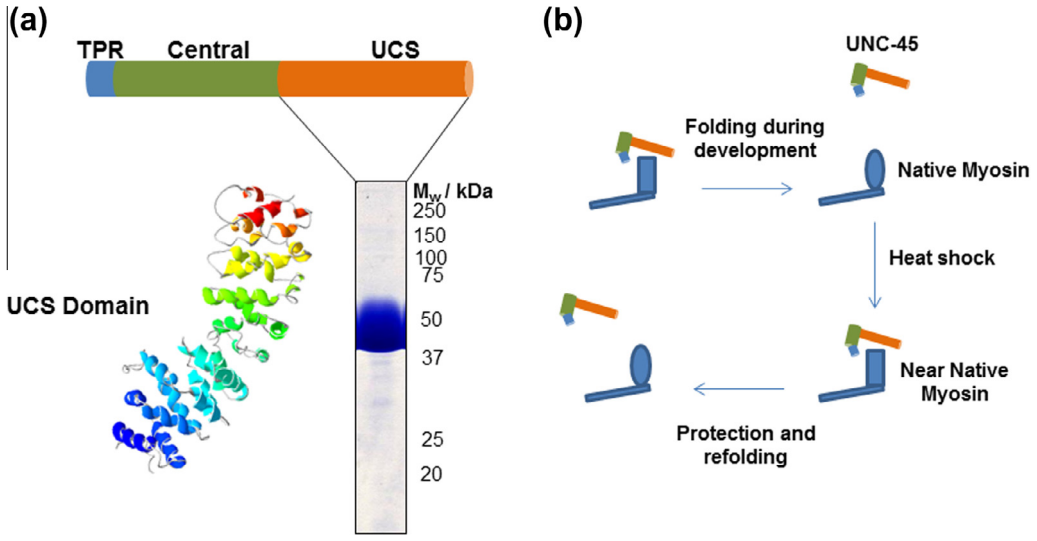

Fig. 1. The UCS domain is the chaperone component of UNC-45B. (A) The UCS chaperone domain of the muscle specific molecular chaperone UNC-45B is made of tandem repeats of armadillo repeats consisting of 3 alpha helices. The X-ray crystal structure of UCS domain from *C. elegans* UNC-45 [9] (PDB accession code 4i2z) is shown. The purified UCS protein has a molecular mass of ~46kDa as confirmed by SDS PAGE gel. (B) During biosynthesis UNC-45 aids in thick filament assembly, and then localizes to the Z-line in mature muscle. Under heat shock treatment it shuttles to the A-band to protect myosin from thermal aggregation that would damage the thick filaments.

armadillo repeats, but is found in other members of the alpha-solenoid protein class such as HEAT repeats. There, simulations have demonstrated flexibility within the hydrophobic core which is noted to resemble the behavior of a molten globule [23]. The armadillo repeats throughout proteins are not identical; indeed the repeats diverge significantly giving C- and N- terminal caps that serve to protect the hydrophobic core. Mutation of these capping repeats allowed modification of the thermal and chemical denaturant sensitivity of the protein [24].

An important factor in the activity of UNC-45B is trafficking between A-band and Z-line upon heat or chemical stress [25]. A mechanism for the reaction to heat has been demonstrated for the small heat shock protein Hsp26. This chaperone possesses a thermosensor domain which undergoes structural rearrangement with temperature increases. Hydrophobic moieties become exposed to solvent under warmer conditions, allowing oligomerization and chaperone activity to occur, shown by solution biophysics and cryo-electron microscopy studies [26,27]. A similar mechanism has been elucidated for Hsp22 using circular dichroism and intrinsic fluorescence approaches. However, in this case a broad melting pattern occurs with the majority of the secondary structure being converted to intrinsically disordered regions [28].

Partial disorder in molecular chaperones is not exclusive to a thermosensor based mechanism. Indeed, it has also been noted in the redox sensitive chaperone Hsp33. In that system oxidation of disulfide bonds generates intrinsically disordered regions that allow detection and binding to folding intermediates of the client proteins. When the chaperone transitions back to a folded state the client is then released, providing a mechanism for the non-ATP-dependent chaperones [29].

Here we propose that the UCS domain of UNC-45B, which has been suggested to bear the molecular chaperone component [14,30], acts as a thermosensor. We show that there is an array of changes to the structure of the protein which results in increased solvent exposure of hydrophobic regions.

2. Materials and methods

2.1. Protein expression and purification

The UCS domain of UNC-45B from *Homo sapiens* was codon optimized for expression in *Escherichia coli*, synthesized (GenScript, Piscataway, NJ) and subcloned into a pET-28 vector (EMD Millipore, Billerica, MA). Residues 500–944 were selected to encompass the entire UCS domain. This was transformed into in BL-21(DE3) competent cells (Life Technologies, Carlsbad, CA), cultured and then induced with 1 mM IPTG for 18 h at 14 °C. Cells were lysed by sonication in PBS, pH 7.4, with 0.5 M NaCl, 1 mM TCEP, 1 mM PMSF, 20 mM imidazole and a protease inhibitor cocktail (Roche, Mannheim, Germany). Clarified lysate was applied to HisTrap column (GE Healthcare) washed and eluted with a gradient from 20 to 500 mM over 20 column volumes.

2.2. ANS fluorescence

1 μM UCS domain was mixed and allowed to equilibrate with 10 μM 8-anilino-1-naphthalenesulfonic acid (ANS). This was then heated slowly at 1 K/min in a quartz cuvette in a Fluorolog fluorescence spectrometer (Horiba Jobin Yvon, Kyoto, Japan) equipped with a 40 W temperature controller (Wavelength Electronics, Bozeman, MT). We excited the samples at 370 nm and collected emission spectra from 400 to 600 nm at various temperatures. Control experiments with 10 μM ANS in buffer only were heated and the signals subtracted to provide corrected spectra.

2.3. Circular dichroism

The far UV CD spectra of the UNC-45B constructs were recorded on a Jasco J-815 Spectrometer. The protein concentration was 1 μM in 30 mM phosphate buffer pH 7.4, 100 mM KCl, 1 mM $MgCl_2$, 1 mM TCEP buffer. A 0.1 cm path length cuvette was used. We heated the cuvette at 1 K/min and recorded the ellipticity at 222 nm at each temperature.

2.4. Limited pulsed proteolysis

Equimolar concentrations (1 μM) of L-1-Tosylamide-2-phenylethyl chloromethyl ketone (TPCK) treated trypsin (Worthington Biochemicals, Lakewood, NJ) and purified UCS domain were rapidly mixed and incubated at the temperatures indicated in the figures for 1 min [31]. These were then rapidly quenched by mixing with SDS–PAGE sample buffer and boiling for 5 min. Samples were then analyzed by SDS–PAGE and stained with Coomassie blue. Scanned