Published in final edited form as:

J Biomol Struct Dyn. 2018 May ; 36(7): 1764–1775. doi:10.1080/07391102.2017.1334594.

Molecular dynamics simulations of Hsp40 J-domain mutants identifies disruption of the critical HPD-motif as the key factor for impaired curing *in vivo* of the yeast prion [*URE3*]

You-Lin Xue^{a,d}, Hao Wang^a, Michael Riedy^b, Brittany-Lee Roberts^b, Yuna Sun^a, Yong-Bo Song^e, Gary W. Jones^c, Daniel C. Masison^b, and Youtao Song^{a,*}

^aSchool of Environmental Science, Liaoning University, Shenyang, China; ^bLaboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA; ^cNational University of Ireland Maynooth, Maynooth, Ireland; ^dLight Industry College, Liaoning University, Shenyang, China; ^eSchool of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang, China

Abstract

Genetic screens using *Saccharomyces cerevisiae* have identified an array of Hsp40 (Ydj1p) J-domain mutants that are impaired in the ability to cure the yeast [*URE3*] prion through disrupting functional interactions with Hsp70. However, biochemical analysis of some of these Hsp40 J-domain mutants has so far failed to provide major insight into the specific functional changes in Hsp40-Hsp70 interactions. To explore the detailed structural and dynamic properties of the Hsp40 J-domain, 20 ns molecular dynamic simulations of 4 mutants (D9A, D36A, A30T, and F45S) and wild-type J-domain were performed, followed by Hsp70 docking simulations. Results demonstrated that although the Hsp70 interaction mechanism of the mutants may vary, the major structural change was targeted to the critical HPD motif of the J-domain. Our computational analysis fits well with previous yeast genetics studies regarding highlighting the importance of J-domain function in prion propagation. During the molecular dynamics simulations several important residues were identified and predicted to play an essential role in J-domain structure. Among these residues, Y26 and F45 were confirmed, using both *in silico* and *in vivo* methods, as being critical for Ydj1p function.

Keywords

heat shock protein; MD simulation; conformational change; molecular docking; Hsp40–Hsp70 interaction

*Corresponding author. ysong@lnu.edu.cn.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

The supplementary material for this article is available online at <http://dx.doi.org/10.1080/07391102.2017.1334594>

Communicated by Ramaswamy H. Sarma

Introduction

Hsp40 (heat shock protein 40 kDa) proteins have been preserved throughout evolution and are important for protein translation, folding, unfolding, translocation, and degradation, primarily by stimulating the ATPase activity of molecular chaperone Hsp70s (Gassler et al., 1998; Horne, Li, Genevaux, Georgopoulos, & Landry, 2010; Landry, 2003; Qiu, Shao, Miao, & Wang, 2006). As one of the co-chaperones of Hsp70s, Hsp40s stimulate Hsp70 ATP hydrolysis and thereby regulate Hsp70s client protein interactions (Alderson, Kim, & Markley, 2016; Hennessy, Nicoll, Zimmermann, Cheetham, & Blatch, 2005; Horne et al., 2010). Most members of the Hsp40 family of proteins possess a signature J-domain, which is an N-terminal 70-amino-acid domain believed to be the major binding site and the minimal region required for interactions between Hsp40s and Hsp70s (Greene, Maskos, & Landry, 1998; Wittung-Stafshede, Guidry, Horne, & Landry, 2003). The J-domain molecular structure is characterized by four α -helices, with a loop region containing a highly conserved tripeptide of histidine, proline, and aspartic acid (so-called HPD motif) located between helices II and III (Ajit & Sahi, 2016; Tsai & Douglas, 1996).

In line with yeast prions being considered as a protein-folding problem, altered abundance or function of a variety of molecular chaperones can affect their propagation (Jones, Song, Chung, & Masison, 2004; Kirkland, Reidy, & Masison, 2011; Masison, Kirkland, & Sharma, 2009). Hsp104, a molecular chaperone that protects cells from exposure to stress by resolubilizing denatured protein aggregates, is thought to facilitate yeast prion “seed” generation by breaking the aggregates into more numerous pieces (Hung & Masison, 2006; Kryndushkin, Alexandrov, Ter-Avanesyan, & Kushnirov, 2003; Sharma et al., 2009). Protein disaggregation by Hsp104, which requires the assistance of Hsp70, acts in prion “seed” propagation by dismantling polymers from the aggregates (Cashikar, Duennwald, & Lindquist, 2005). Altered expression of Hsp40 and Hsp70 strongly influences the dynamics of yeast prion propagation (Masison et al., 2009; Sharma, Stanley, & Masison, 2009; Summers, Douglas, & Cyr, 2009), possibly by affecting Hsp104 function.

Among prions of yeast, [*URE3*] (the self replicating form of the Ure2 protein) is highly sensitive to fluctuations in the expression level of Ydj1p, a major yeast cytosolic Hsp40 (Sharma et al., 2009, 2009). Although [*URE3*] does not require Ydj1p for its propagation, overexpression of Ydj1p weakens the [*URE3*] phenotype (ability to grow on poor nitrogen sources) and causes gradual loss of [*URE3*] from dividing cells (Higurashi, Hines, Sahi, Aron, & Craig, 2008; Moriyama, Edskes, & Wickner, 2000). *In vitro* studies demonstrated that Ydj1p binds to soluble Ure2p and inhibits the formation of Ure2p amyloid fibrils, in a concentration dependent manner. Therefore, it was postulated that direct interaction of Ydj1p with Ure2p *in vivo* may play a major role in curing cells of [*URE3*] by preventing fibril formation (Kumar, Gaur, Masison, & Sharma, 2014; Masison et al., 2009; Savistchenko, Krzewska, Fay, & Melki, 2008). However, *in vitro* once Ure2p polymerization has begun Ydj1p cannot prevent amyloid formation (Kryndushkin & Wickner, 2007; Lian et al., 2007). Yeast cells overproducing Ydj1p have larger [*URE3*] prion polymers, which suggests that *in vivo* Ydj1p seems to interfere with fragmentation of Ure2p fibrils rather than with polymer assembly (Kryndushkin et al., 2003; Masison et al., 2009).

Recently, Sharma et al. reported that point mutations in Ydj1p, which disrupt functions such as substrate binding, dimerization, membrane association, or ability to transfer substrate to Hsp70, have little or no effect on curing [*URE3*]. Conversely, mutations located within the J-domain, which are predicted to disrupt functional interactions of Ydj1p with Hsp70, do abolish curing of [*URE3*] by both full-length Ydj1 protein or by overexpression of J-domain alone (Sharma et al., 2009). Additionally, although elevated levels of other Hsp40s including Sis1p have no effect on [*URE3*], over-expression of the J-domains alone of Sis1p and another Hsp40, Jjj1p, did efficiently cure [*URE3*], which is consistent with overlapping functions of many yeast Hsp40 J-domains (Ajit & Sahi, 2016; Masison et al., 2009; Summers et al., 2009). These findings suggest that *in vivo* the curing of [*URE3*] by Ydj1 is primarily indirect, requiring only its J-domain's ability to interact with Hsp70.

Molecular dynamics (MD) simulations can provide detailed information on structural changes at the atomic level and have been widely used in structural studies of protein molecules (Stetz & Verkhivker, 2016; Yu et al., 2010). In order to investigate further the structural and dynamic characteristics of the J-domain and how this relates to prion-curing ability, we have performed MD simulations on the crystal structure of Sis1p J-domain (PDB code 2O37), which shares 66% sequence identity (Supplementary Figure 1) with Ydj1p J-domain (structure not available). Based on the genetic results of Sharma et al. (2009), four J-domain mutants consisting of D36A (located in HPD motif, does not cure [*URE3*]), A30T (located in helix II, does not cure [*URE3*]), F45S (located in helix III, does not cure [*URE3*]), D9A (located in helix I, efficiently cures [*URE3*]), and the WT J-domain (efficiently cures [*URE3*]) were employed in this study. D36A was used as a positive control because it totally blocked [*URE3*] curing and the HPD motif is necessary for signal transmission to Hsp70. D9A was used as a negative control because it exhibited similar [*URE3*] curing levels as WT and helix I alone cannot interact with Hsp70 (Hennessy et al., 2005).

Our computational results identified significant changes in J-domain structure for mutants that are impaired in [*URE3*] curing. The major functional consequences from these changes are an impaired interaction with Hsp70, primarily through disruption or displacement of the HPD motif. Our data support the proposal that for efficient curing of [*URE3*] *in vivo* by overexpression of Hsp40, a productive and functional interaction must occur with the cognate Hsp70.

Materials and methods

Construction of J-domain mutant models

The crystal structure of Sis1p J-domain (Hsp40 cochaperone from *Saccharomyces cerevisiae*, PDB code 2O37), which shares 66% sequence identity (Supplementary Figure 1) with Ydj1p J-domain, was used as the template molecule to obtain the structure of the mutant J-domains. The mutant (D9A, D36A, A30T and F45S) models were constructed using homologous modeling method through the online service of Swiss Model (<http://swissmodel.expasy.org/>) (Arnold, Bordoli, Kopp, & Schwede, 2006).

Molecular dynamics simulations

All molecular dynamics (MD) simulations were performed using *Gromacs 4.0* program (Hess, Kutzner, van der Spoel, & Lindahl, 2008; Van Der Spoel et al., 2005) at the Supercomputer Center of Liaoning University on dual-core Pentium 2.8 G processor of a Linux cluster. *GROMOS96 43a1* (Scott et al., 1999) force field parameters were used in all simulations. The integration time steps were 2 fs throughout all simulations. A cut-off of .9 nm was used for van der Waals interactions. Longrange electrostatic interactions were calculated using the particle mesh (Darden, York, & Pedersen, 1993; Essmann et al., 1995) with a maximum FFT grid spacing of .12 nm and cubic interpolation order. Periodic boundary conditions were activated in the simulations. Bonds containing hydrogen atoms were constrained using the *SHAKE* algorithm with a relative tolerance of 10⁻⁸.

Each monomer model was solvated in a water box containing 10,124 *SPC* water molecules (Berendsen, Postma, van Gunsteren, & Hermans, 1981) and neutralized by adding Na⁺ counter-ions. The minimum distance of a protein atom to the edge of the water box was 1 nm. The solvated and neutralized systems were then energy minimized for 80 ps. Subsequently, backbone atoms of the structure were fixed, while the side chains and solvent were allowed to move unrestrained for a further 10 ps, followed by totally unrestrained equilibration for 12 ps, at a constant temperature of 300 K and 1 atm pressure within the water box using periodic boundary conditions in the *NPT* ensemble. Following equilibration, the productive run was carried out for 20 ns with the temperature kept constant at 300 K. In all simulations, the temperature was maintained close to the intended values by weak coupling to an external temperature bath with a coupling constant of .1 ps. The protein and the rest of the system were coupled separately to the temperature bath. Both atomic coordinates and atomic velocities were recorded every 1 ps for subsequent data analysis.

Analysis of the trajectories

The root mean square deviations (RMSD) were calculated for all alpha-carbon (C α) atoms with reference to the first frame of the trajectories. The secondary structure of the mutants was determined using the *DSSP* program (Kabsch & Sander, 1983) and the properties of the hydrogen bonds in the mutants were determined using the *g_hbond* program (Scott et al., 1999). The hydrophobic surfaces were generated using the *SYBYL* program (Wallace, Laskowski, & Thornton, 1995). Images were produced using the Visual Molecular Dynamics (VMD) (Humphrey, Dalke, & Schulten, 1996) and *PyMOL* program (DeLano, 2002).

Docking simulation

The *Cluspro* 2.0 protein–protein docking server (Kozakov et al., 2010) was used for the docking of the J-domain mutants following MD simulation with the ATPase domain of Hsc70 (PDB code: 1HJO), which shares 91% sequence identity with Ssa1p (structure not available).

Strains, growth conditions, and plasmids

Standard conditions for growing cultures and transforming cells with plasmids were used (Sherman, 2002). Yeast strain 1075 (*MATa kar1-1, SUQ5, P_{DAL5}:ADE2, his3 202, leu2 1*,

trp1 63, ura3-52[URE3]) was used for routine curing experiments. Isogenic *ydj1* strain MR502 was used for complementation by the mutant proteins. For this test, strain MR502 was transformed by single-copy plasmids encoding wild type and mutant *YDJ1* alleles. After growing transformants in liquid medium selecting for the plasmid, they were adjusted to the same concentration in water and 5 µl drops of a fivefold series of dilutions were arranged on replicate YPAD plates, which were incubated at indicated temperatures. The most stringent condition used was 34°C.

Plasmid pMR271 is pRS314 (Sikorski & Hieter, 1989) with *YDJ1* and 500 base pairs of 5' and 3' flanking DNA on a *SacI*–*SaII* fragment. It has a *Bam*HI site immediately upstream of the initiator methionine codon. Site-directed point mutations in *YDJ1* on pMR271 were made using the QuickChange Mutagenesis Kit (Agilent Technologies, Santa Clara, California) according to the manufacturer's instructions. All mutagenized alleles were verified by DNA sequencing. For galactose induction of Ydj1, the *Bam*HI–*SaII* fragments from pMR271 carrying the wild type and mutant *YDJ1* alleles were moved into pRU4, which is pRS315 (Sikorski & Hieter, 1989) with the *GAL1* promoter on a *SacI*–*Bam*HI fragment.

Comparison of curing efficiency

Wild type [*URE3*] cells were transformed by plasmids encoding the wild type and mutant *YDJ1* genes regulated by the *GAL1* promoter. Transformants were grown in dextrose medium with selection for the plasmid, washed, and then transferred to similar medium containing galactose in place of glucose. After growing for 8 cell divisions, cells were spread onto 1/2YPD plates and grown for 3 days at 30°C. Entirely red colonies were scored as having lost [*URE3*].

Results

Structural stabilities of the J-domain mutants

Molecular dynamics simulations of the fully solvated mutants and WT J-domains were performed. The total energy of the simulated systems was monitored to ensure energy conservation ($E_{tot}/E_{tot} < .002$). Due to the Berendsen temperature coupling, total energy during 20 ns in all simulations was conserved. The overall stability of each J-domain monomer was evaluated using the C_α root-mean-square deviation (RMSD) relative to the corresponding initial structure (Figure 1(A)). When the system reached a relative equilibrium region (after 15 ns), the average RMSD of WT, D9A and D36A were 0.41, 0.38, and 0.37 nm, respectively. However, the average RMSD of A30T and F45S were 0.44 and 0.45 nm, respectively, indicating that more structural alterations occurred in these two mutants. It is interesting that the RMSD of D36A (positive control) is lower than the RMSD of WT and D9A (negative control), although D36A impaired [*URE3*] curing just as A30T and F45S, suggesting a different structural mechanism that is preventing [*URE3*] curing *in vivo*.

The major structural changes in J-domain mutants is targeted to the HPD motif

According to the RMSD value, the overall stability of the A30T and F45S mutants decreased compared with WT J-domain and the other mutants. We further analyzed the residue averages of the root mean square fluctuations (RMSF) during the MD simulations to find the key region that induced the instability in these two mutants. Although the RMSF patterns for all the J-domains were quite similar, significant structural deviations were detected around the helices II–III region (from helix II to helix III), which harbors the critical HPD motif, in A30T and F45S mutants (Figure 1(B)).

For further observation of the structural changes in A30T and F45S mutants, we applied a visual analysis by superimposing alignment with VMD program. The final structures (after MD simulations) of A30T and F45S mutants were superimposed with the corresponding final structure of WT J-domain and the other mutants for the C α . As shown in Figure 1(C), a clear displacement was detected around the helices II–III loop region (HPD motif) in the A30T and F45S mutants compared with WT, D9A and D36A mutants. In addition, only minor structural deviation was found in helices II and III (slight expansion) in the A30T and F45S mutants.

To assess whether the displacement of the HPD motif of A30T and F45S was transient, we compared the conformational changes of different WT and mutant J-domain HPD motifs during the 20 ns MD simulation. As shown in Figure 2, the structural changes in WT, D9A, and D36A were concentrated within the central region and do not show major differences. However, the A30T and F45S mutants show clear displacement of the critical HPD motif and the structural changes were immediate and irreversible (started at 7 ns for A30T and 4 ns for F45S). These results suggest that there is a shared prevailing mechanism in preventing the *in vivo* curing of [URE3] common to the A30T and F45S mutants, which the data suggest is altered positioning of the HPD loop. This mechanism is distinct from changes caused by the positive control mutant D36A, which would be altered affinity of the loop for the Hsp70 binding pocket. Together our results strongly suggest the HPD motif to be the key domain for the interaction with Hsp70 and for proper signal transduction from Hsp40 to Hsp70.

Hsp70 interaction of the J-domain mutants

It is believed that J-domain interaction with Hsp70 plays a critical role in [URE3] curing *in vivo* (Hennessy et al., 2005; Horne et al., 2010). Using NMR, genetic and biochemical approaches, at least two possible J-domain/Hsp70 interaction sites were identified so far (Ahmad et al., 2011; Fan, Lee, & Cyr, 2003; Hennessy et al., 2005; Suh et al., 1998). Firstly, through a dynamic interface, the positively charged helix II of the J-domain binds to a negatively charged loop in the Hsp70 nucleotide-binding domain (NBD) (Ahmad et al., 2011); secondly, a critical salt bridge is formed by D35 (corresponding with D36 in this study) of the DnaJ J-domain HPD motif and the R167 of the DnaK ATPase domain (corresponding with R171 in this study) lower cleft (Suh et al., 1998).

In order to further investigate the interactions of the J-domain mutants with Hsp70, we carried out a docking simulation using the *Cluspro 2.0* server. Docked structures were

considered reasonable when the interaction of the J-domain positively charged helix II and the Hsp70 NBD negatively charged loop was confirmed. As a result, docked structures were obtained for WT, D9A, D36A, and F45S, no reasonable docked structure was obtained for the A30T mutant, which may reflect a greater severity of structural change in this mutant (Highest RMSD value among the J-domains) that prevents interaction with Hsp70. The most reliable docked structures of WT, D36A and F45S are shown in Figure 3, and the corresponding scores of the docked-structures are shown in Table 1. Although the position of the J-domain mutants differ from each other in the docked structures, in all reliable models, the positively charged helix II of the J-domain mutants interacted with the Hsp70 negatively charged loop, which is consistent with a previous report that the interface of the complex is dynamic (Ahmad et al., 2011). As for the interaction of R171 (Hsp70) and D36 (J-domain), close proximity (distance <8 Å) was detected from both WT and D9A (Data not shown) docked structures, but no connection was found in the cases of D36A and F45S mutants. We also performed MD simulations on the docking models and the C α RMSD of the models are shown in Supplementary Figure 2. As results, although the J-domain mutants after MD simulation shifted from their original locations, the positively charged helix II of the J-domain mutants still interacted with the Hsp70 negatively charged loop. What's more, the R171-D36 interaction was still maintained in both WT and D9A docked models after MD simulation, but not in D36A and F45S models (Supplementary Figure 3).

In summary, it seems that all J-domain mutants except A30T can bind to the NBD negatively charged loop of Hsp70 even after MD simulations, but only the D36 residue of WT and D9A can interact with the R171 of Hsp70 and form the critical salt bridge. Thus, our *in silico* results strongly support the proposal that the inability of the J-domain mutants A30T, D36A, and F45S to functionally interact with Hsp70 is the underlying reason why these mutants do not cure [URE3] obtained from *in vivo* experiments (Sharma et al., 2009).

Hydrophobic Surface is decreased in A30T and F45S J-domain mutants

Hydrophobic interactions make a major contribution to stabilizing the native structure of proteins in an aqueous environment (Pace, Shirley, McNutt, & Gajiwala, 1996; Pace et al., 2011; Prevost, Wodak, Tidor, & Karplus, 1991). Due to the fact that the amino acid substitutions A30T and F45S both reduce the hydrophobicity of the J-domain, we speculated that the reduction in hydrophobicity may influence the conformational changes of the mutants. Figure 4 demonstrates that compared with WT, the hydrophobic surface of A30T and F45S is greatly decreased. In contrast, and in agreement with the RMSD values, the hydrophobic surface of D9A and D36A did not change significantly compared to WT (data not shown).

As shown in Figure 4, the main contributing residues of the hydrophobic surface are Y26 of helix II, F45 and F52 of helix III, and the aromatic–aromatic (pi-stacking) interaction of Y26 and F52 also helps the regional stability of the structures. The reduction in hydrophobicity in both the A30T and F45S mutants is caused by the departure of F45 (located in helix III) from the hydrophobic core region of the protein, suggesting the stability around helix II and III region of A30T and F45S is reduced, which is consistent with RMSF (Figure 1(B)). The re-alignment of residue F45 may be a contributing factor to the major structural changes

observed in these mutants (Figures 1(C) and 4). Due to the reduced structure stability around the helix II and III region of A30T and F45S, the degree of freedom of the helix II–helix III loop should be increased. Furthermore, as shown in Figure 2, the displacement of the helix II–helix III loop was detected just after the start of the MD simulation and it stayed at a certain position irreversibly, suggesting there must be some other interactions fixing the flexible helices II–III loop.

Rearrangement of Helices II–III hydrogen bond network in A30T and F45S J-domain mutants

The main structure alterations were detected following completion of the MD stimulations. The average mass center distance between the residues at the bases of helix II (residues 30–32) and helix III (residues 42–45) following the 20 ns MD simulation are shown in Figure 5. The data showed that the average mass center distance is greater in the A30T and F45S mutants, while the D9A and D36A are similar to WT. Again this demonstrates that the A30T and F45S mutants are structurally very distinct from WT. Interestingly, the increase of the average mass center distance and the displacement of the critical HPD motif (Figure 2) of A30T and F45S occurred almost simultaneously (data not shown), suggesting a direct connection between the two parts of the J-domain.

In the WT structure, helices II and III are stabilized by direct hydrogen bonds formed by several highly conserved residues (Y26, Y33, F45, and S49). For D9A, although one hydrogen bond (Y26–F45) is disrupted, a new hydrogen bond (K23–N56) is formed and stabilizes the structure; additionally there is no major structural change to the HPD motif. Similarly for D36A no obvious structure alterations were found. In contrast, significant changes to the hydrogen bond network between helices II and III were detected for both the A30T and F45S mutants. Due to the steric hindrance caused by the A30T mutation, the hydrogen bond network was completely destroyed and the HPD motif was fixed to a new position by the re-orientated side chain of F45 (H34–F45) (Figure 5). As for F45S, the hydrogen bond network was also damaged and the HPD motif again was fixed in a new position, in this case by the interactions of the side chain of S45 (H34–F45S, P35–F45S).

In summary, it seems that due to the expansion in distance between helix II and III caused by the A30T and F45S mutations, the original direct hydrogen bond network cannot be formed between these helices, and the flexibility of helices II–III loops are fixed due to side chain interactions of F45 and F45S, respectively. The major structural consequence of this loss in flexibility is the irreversible displacement and fixation of the HPD motif.

in silico testing of other related J-domain mutants

Our results suggested that the hydrophobic residues Y26, F45, and F52, and the hydrogen bond forming residues Y26, F45, and S49 in the J-domain may be very important for maintaining J-domain/Hsp70 interaction. According to this proposal, J-domain mutant Y26H, which efficiently cures [URE3] (86% [ure-o]) in yeast (Sharma et al., 2009), should have similar structure and interaction to WT J-domain. On the other hand, Y26A and F45A mutants, whose DnaJ homologues totally abolished J-domain function in *E. coli* (Genevaux, Schwager, Georgopoulos, & Kelley, 2002), should have similar altered structure and

interaction to A30T or F45S mutant. To test this prediction, we further performed 20 ns MD simulations on Y26H, Y26A, and F45A J-domain mutants. As shown in Table 2, MD simulation results demonstrate that the helices II–III loop displacement, surface hydrophobicity, helices II–III mass center distance and hydrogen bond network of Y26H mutant are similar to WT J-domain, while those of Y26A and F45A mutants are similar to A30T or F45S. This finding further implies that our prediction is meaningful.

in vivo testing of J-domain mutants

To test the *in vivo* effects of residues identified from both *E. coli* homologue experiments and our *in silico* analysis in yeast, Ydj1 Y26A, F47A, and F47Y (corresponding with F45 in sis1) mutants were constructed and assessed in well characterized yeast prion curing experiments. The curing effects of over-expressed Ydj1p mutants are shown in Figure 6(A). As predicted, F47A abolished functions of Ydj1 required for it to cure prions (Figure 6(A)) and for it to act in cellular processes important for cell growth (Figure 6(B) and (C)). The more conservative substitution F47Y, which was designed to strengthen the hydrogen network between helices II and III, reduced curing, but did not affect the ability of Ydj1 to support normal cell growth. These latter results suggest that functions of Ydj1 needed to inhibit [*URE3*] propagation are more sensitive to the modest changes caused by the F47Y substitution than functions needed to support cellular processes that rely on Ydj1.

Although Y26H does not disrupt Ydj1 function (Sharma et al., 2009), Y26A, also in agreement with expectations, significantly weakened the ability of Ydj1 to cure prions (Figure 6(A)) and to support normal growth (Figure 6(C)). Both effects were greater than those caused by F47Y and less than those caused by F47A. Overall, the ability of Ydj1 to cure cells of [*URE3*] prions correlated very well with its ability to support normal cell growth.

Discussion

Ydj1 and Sis1, two important yeast cytosolic Hsp70 co-chaperones, are structurally and functionally distinct Hsp40 proteins with high sequence identity in their amino and carboxyl terminal ends (Lopez, Aron, & Craig, 2003; Lu & Cyr, 1998; Tutar & Tutar, 2008). Over-expression of Ydj1p and Sis1p J-domains alone (N-terminus of Hsp40) are effective in [*URE3*] curing *in vivo* (Higurashi et al., 2008; Masison et al., 2009; Summers et al., 2009), suggesting the important amino acid residues, such as Y26, F45, and the HPD motif, required for optimum J-domain function, are highly conserved in their structural roles.

Helix II–III expansion, which was detected in A30T, F45S, and F45A structures, seems important for the loss of J-domain function (Table 2). However, the mass center distance was reduced in Y26A structure after 20 ns simulation, which might be caused by the strong interaction between G40 (loop region) and the main chain of K32 (Helix II) (Data not shown). Thus, the loop region was fixed twice by helix II and III, respectively, in the Y26A structure. But still there are no direct hydrogen bonds found between helix II and helix III, suggesting that it is the stability of the structure, but not helix II–III expansion, that affects the HPD motif displacement, and thus J-domain function.

Hydrophobic interaction is considered the main driving force for protein folding and also plays an important role in the stability of proteins (Pace et al., 1996, 2011). The surface hydrophobic residues (Y26 of helix II, F45, and F52 of helix III) are part of the hydrophobic core of the protein (Figure 4), and the substitution of these residues changes the regional hydrophobicity of the protein, resulting in reduced stability around the helix II–III region. In addition, aromatic–aromatic interaction has been suggested important in protein folding and stability (Burley & Petsko, 1985; McGaughey, Gagné, & Rappé, 1998; Serrano, Bycroft, & Fersht, 1991), thus the pi-stacking interaction between Y26 (helix II) and F52 (helix III) assisted the stability of the helix II–III region.

Hydrogen bonds are another major factor affecting protein stability (Pace et al., 1996; Peterson, Nicholson, Thapar, Klevit, & Scholtz, 1999) and direct helix II–III hydrogen bonds were only detected in the structures without HPD motif displacement (Table 2), indicating high stability of these structures. As shown in Figure 5, Y26, F45, and S49 may be very important for the helix II–III hydrogen bond network.

Because Sis1 is essential for replication of [*URE3*] prions, a plausible explanation for the ability of Ydj1 to cure cells of [*URE3*] would be that it competes with Sis1 for interaction with Hsp70. Therefore, mutations that disrupt ability of Ydj1 to bind Hsp70 would also disrupt this competition and prevent the curing. The correlation we observed for the magnitude of the effects of the substitutions on [*URE3*] and on cell growth is consistent with this idea. For example, the degree to which the substitutions are expected to reduce affinity of Ydj1 for Hsp70 would be reflected in the degree to which they would be expected to reduce ability of Ydj1 both to perform in its cellular functions and to inhibit propagation of [*URE3*].

In conclusion, MD simulations and Hsp70 docking were performed on yeast Hsp40 and four putative important residues (Y26, F45, S49, and F52) were identified using *in silico* methods. Among these residues, it is difficult to assess the degree of importance of individual amino acids. According to a previous report, Y26 and F45 had been shown to be important in DnaJ (Ydj1p *E. coli* homologue) by genetic approaches (Genevaux et al., 2002), and we demonstrate here, using both *in silico* and *in vivo* methods, that Y26 and F45 are also very important in Ydj1p function. In addition, other important residues identified in this work such as F52 and S49 are worthy of further study. The interactions between Hsp40 and Hsp70 are transient and dynamic, a multi-faceted approach is in urgent need to explain how Hsp40 stimulates both ATP hydrolysis in Hsp70 and the process of substrate binding, refolding, and transfer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported in part by the National Natural Science Foundation of China [grant number 31570154], [grant number 31201285]; the Intramural Research Program of the NIH; National Institute of Diabetes and

Digestive and Kidney Diseases (NIDDK); the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of China [grant number 2013693]; the General Project of the Education Department of Liaoning Province, China [grant number L2014009].

Abbreviations:

Hsp	heat-shock protein
NBD	nucleotide-binding domain
RMSD	root mean square deviation
RMSF	root mean square fluctuations
ATPase	adenosine triphosphatase
PDB	protein data bank
NMR	nuclear magnetic resonance
WT	wild-type

References

- Ahmad A, Bhattacharya A, McDonald RA, Cordes M, Ellington B, Bertelsen EB, & Zuiderweg ER (2011). Heat shock protein 70 kDa chaperone/DnaJ cochaperone complex employs an unusual dynamic interface. *Proceedings of the National Academy of Sciences*, 108, 18966–18971.
- Ajit TC, & Sahi C (2016). J domain independent functions of J proteins. *Cell Stress and Chaperones*, 21, 563–570. [PubMed: 27145962]
- Alderson TR, Kim JH, & Markley JL (2016). Dynamical structures of Hsp70 and Hsp70-Hsp40 complexes. *Structure*, 24, 1014–1030. [PubMed: 27345933]
- Arnold K, Bordoli L, Kopp J, & Schwede T (2006). The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. *Bioinformatics*, 22, 195–201. [PubMed: 16301204]
- Berendsen HJC, Postma JPM, van Gunsteren WF, & Hermans J (1981). Intermolecular forces, chapter interaction models for water in relation to protein hydration (pp. 331–342). Dordrecht: D. Reidel.
- Burley SK, & Petsko GA (1985). Aromatic-aromatic interaction: A mechanism of protein structure stabilization. *Science*, 229, 23–28. [PubMed: 3892686]
- Cashikar AG, Duennwald M, & Lindquist SL (2005). A chaperone pathway in protein disaggregation. Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104. *Journal of Biological Chemistry*, 280, 23869–23875. [PubMed: 15845535]
- Darden T, York D, & Pedersen L (1993). Particle mesh Ewald: An N log(N) method for Ewald sums in large systems. *The Journal of Chemical Physics*, 98, 10089–10092.
- DeLano WL (2002). The PyMOL molecular graphics system. San Carlos, CA: DeLano Scientific Retrieved from <http://www.pymol.org>
- Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, & Pedersen LG (1995). A smooth particle mesh Ewald method. *The Journal of Chemical Physics*, 103, 8577–8593.
- Fan CY, Lee S, & Cyr MD (2003). Mechanisms for regulation of Hsp70 function by Hsp40. *Cell Stress & Chaperones*, 8, 309–316. [PubMed: 15115283]
- Gassler CS, Buchberger A, Laufen T, Mayer MP, Schroder H, Valencia A, & Bukau B (1998). Mutations in the DnaK chaperone affecting interaction with the DnaJ cochaperone. *Proceedings of the National Academy of Sciences*, 95, 15229–15234.
- Genevaux P, Schwager F, Georgopoulos C, & Kelley WL (2002). Scanning mutagenesis identifies amino acid residues essential for the in vivo activity of the Escherichia coli DnaJ Hsp40 J domain. *Genetics*, 162, 1045–1053. [PubMed: 12454054]

- Greene M, Maskos K, & Landry SJ (1998). Role of the Jdomain in the cooperation of Hsp40 with Hsp70. *Proceedings of the National Academy of Sciences*, 95, 6108–6113.
- Hennessy F, Nicoll WS, Zimmermann R, Cheetham ME, & Blatch GL (2005). Not all J domains are created equal: Implications for the specificity of Hsp40-Hsp70 interactions. *Protein Science*, 14, 1697–1709. [PubMed: 15987899]
- Hess B, Kutzner C, van der Spoel D, & Lindahl E (2008). GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *Journal of Chemical Theory and Computation*, 4, 435–447. [PubMed: 26620784]
- Higurashi T, Hines JK, Sahi C, Aron R, & Craig EA (2008). Specificity of the J-protein Sis1 in the propagation of 3 yeast prions. *Proceedings of the National Academy of Sciences*, 105, 16596–16601.
- Horne BE, Li T, Genevaux P, Georgopoulos C, & Landry SJ (2010). The Hsp40 J-domain stimulates Hsp70 when tethered by the client to the ATPase domain. *Journal of Biological Chemistry*, 285, 21679–21688. [PubMed: 20448033]
- Humphrey W, Dalke A, & Schulten K (1996). VMD: Visual molecular dynamics. *Journal of Molecular Graphics*, 14, 33–38. [PubMed: 8744570]
- Hung GC, & Masison DC (2006). N-terminal domain of yeast hsp104 chaperone is dispensable for thermotolerance and prion propagation but necessary for curing prions by Hsp104 overexpression. *Genetics*, 173, 611–620. [PubMed: 16582428]
- Jones G, Song Y, Chung S, & Masison DC (2004). Propagation of yeast [PSI⁺] prion impaired by factors that regulate Hsp70 substrate binding. *Molecular and Cellular Biology*, 24, 3928–3937. [PubMed: 15082786]
- Kabsch W, & Sander C (1983). Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*, 22, 2577–2637. [PubMed: 6667333]
- Kirkland PA, Reidy M, & Masison DC (2011). Functions of yeast Hsp40 chaperone Sis1p dispensable for prion propagation but important for prion curing and protection from prion toxicity. *Genetics*, 188, 565–577. [PubMed: 21555396]
- Kozakov D, Hall DR, Beglov D, Brenke R, Comeau SR, Shen Y, ... Vajda S (2010). Achieving reliability and high accuracy in automated protein docking: Cluspro, PIPER, SDU, and stability analysis in CAPRI rounds 13–19. *Proteins: Structure, Function, and Bioinformatics*, 78: 3124–3130.
- Kryndushkin D, & Wickner RB (2007). Nucleotide exchange factors for Hsp70s are required for [URE3] prion propagation in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell*, 18, 2149–2154. [PubMed: 17392510]
- Kryndushkin DS, Alexandrov IM, Ter-Avanesyan MD, & Kushnirov VV (2003). Yeast [PSI⁺] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. *Journal of Biological Chemistry*, 278, 49636–49643. [PubMed: 14507919]
- Kumar N, Gaur D, Masison DC, & Sharma D (2014). The BAG homology domain of Snl1 cures yeast prion [URE3] through regulation of Hsp70 chaperones. *G3 (Bethesda)*, 4: 461–470. [PubMed: 24408033]
- Landry SJ (2003). Structure and energetics of an allele-specific genetic interaction between dnaJ and dnaK: Correlation of nuclear magnetic resonance chemical shift perturbations in the J-domain of Hsp40/DnaJ with binding affinity for the ATPase domain of Hsp70/DnaK. *Biochemistry*, 42, 4926–4936. [PubMed: 12718534]
- Lian HY, Zhang H, Zhang ZR, Looers HM, Jones GW, Rowling PJ, ... Perrett S (2007). Hsp40 interacts directly with the native state of the yeast prion protein Ure2 and inhibits formation of amyloid-like fibrils. *Journal of Biological Chemistry*, 282, 11931–11940. [PubMed: 17324933]
- Lopez N, Aron R, & Craig EA (2003). Specificity of class II Hsp40 Sis1 in maintenance of yeast prion [RNQ⁺]. *Molecular Biology of the Cell*, 14, 1172–1181. [PubMed: 12631732]
- Lu Z, & Cyr DM (1998). Protein folding activity of Hsp70 is modified differentially by the Hsp40 co-chaperones Sis1 and Ydj1. *Journal of Biological Chemistry*, 273, 27824–27830. [PubMed: 9774392]
- Masison DC, Kirkland PA, & Sharma D (2009). Influence of Hsp70s and their regulators on yeast prion propagation. *Prion*, 3, 65–73. [PubMed: 19556854]

- McGaughey GB, Gagné M, & Rappé AK (1998). π -stacking interactions. Alive and well in proteins. *Journal of Biological Chemistry*, 273, 15458–15463. [PubMed: 9624131]
- Moriyama H, Edskes HK, & Wickner RB (2000). [URE3] prion propagation in *Saccharomyces cerevisiae*: Requirement for chaperone Hsp104 and curing by overexpressed chaperone Ydj1p. *Molecular and Cellular Biology*, 20, 8916–8922. [PubMed: 11073991]
- Pace CN, Shirley BA, McNutt M, & Gajiwala K (1996). Forces contributing to the conformational stability of proteins. *FASEB J*, 10, 75–83. [PubMed: 8566551]
- Pace CN, Fu H, Fryar KL, Landua J, Trevino SR, Shirley BA, ... Grimsley GR (2011). Contribution of hydrophobic interactions to protein stability. *Journal of Molecular Biology*, 408, 514–528. [PubMed: 21377472]
- Peterson RW, Nicholson EM, Thapar R, Klevit RE, & Scholtz JM (1999). Increased helix and protein stability through the introduction of a new tertiary hydrogen bond. *Journal of Molecular Biology*, 286, 1609–1619. [PubMed: 10064718]
- Prevost M, Wodak SJ, Tidor B, & Karplus M (1991). Contribution of the hydrophobic effect to protein stability: analysis based on simulations of the Ile-96—Ala mutation in barnase. *Proceedings of the National Academy of Sciences*, 88, 10880–10884.
- Qiu XB, Shao YM, Miao S, & Wang L (2006). The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. *Cellular and Molecular Life Sciences*, 63, 2560–2570. [PubMed: 16952052]
- Savitschenko J, Krzewska J, Fay N, & Melki R (2008). Molecular chaperones and the assembly of the prion Ure2p *in Vitro*. *Journal of Biological Chemistry*, 283, 15732–15739. [PubMed: 18400756]
- Scott WRP, Hünenberger PH, Tironi IG, Mark AE, Billeter SR, Fennel J, ... van Gunsteren WF (1999). The GROMOS biomolecular simulation program package. *The Journal of Physical Chemistry A*, 103, 3596–3607.
- Serrano L, Bycroft M, & Fersht AR (1991). Aromatic-aromatic interactions and protein stability: investigation by double-mutant cycles. *Journal of Molecular Biology*, 218, 465–475. [PubMed: 2010920]
- Sharma D, Martineau CN, Le Dall MT, Reidy M, Masison DC, & Kabani M (2009). Function of SSA subfamily of Hsp70 within and across species varies widely in complementing *Saccharomyces cerevisiae* cell growth and prion propagation. *PLoS ONE*, 4, e6644. [PubMed: 19680550]
- Sharma D, Stanley RF, & Masison DC (2009). Curing of yeast [URE3] prion by the Hsp40 cochaperone Ydj1p is mediated by Hsp70. *Genetics*, 181, 129–137. [PubMed: 19015537]
- Sherman F (2002). Getting started with yeast. *Methods Enzymol*, 350, 3–41. [PubMed: 12073320]
- Sikorski RS, & Hieter P (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*, 122, 19–27. [PubMed: 2659436]
- Stetz G, & Verkhivker GM (2016). Probing allosteric inhibition mechanisms of the Hsp70 chaperone proteins using molecular dynamics simulations and analysis of the residue interaction networks. *Journal of Chemical Information and Modeling*, 56, 1490–1517. [PubMed: 27447295]
- Suh WC, Burkholder WF, Lu CZ, Zhao X, Gottesman ME, & Gross CA (1998). Interaction of the Hsp70 molecular chaperone, DnaK, with its cochaperone DnaJ. *Proceedings of the National Academy of Sciences*, 95, 15223–15228.
- Summers DW, Douglas PM, & Cyr DM (2009). Prion propagation by Hsp40 molecular chaperones. *Prion*, 3, 59–64. [PubMed: 19535913]
- Tsai J, & Douglas MG (1996). A conserved HPD sequence of the J-domain is necessary for YDJ1 stimulation of Hsp70 ATPase activity at a site distinct from substrate binding. *The Journal of Biological Chemistry*, 271, 9347–9354. [PubMed: 8621599]
- Tutar L, & Tutar Y (2008). Ydj1 but not Sis1 stabilizes Hsp70 protein under prolonged stress *in vitro*. *Biopolymers*, 89, 171–174. [PubMed: 17985367]
- Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, & Berendsen HJC (2005). GROMACS: Fast, flexible, and free. *Journal of Computational Chemistry*, 26, 1701–1718. [PubMed: 16211538]
- Wallace AC, Laskowski RA, & Thornton JM (1995). LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Engineering, Design and Selection*, 8, 127–134.

- Wittung-Strafshede P, Guidry J, Horne BE, & Landry SJ (2003). The J-domain of Hsp40 couples ATP hydrolysis to substrate capture in Hsp70. *Biochemistry*, 42, 4937–4944. [PubMed: 12718535]
- Yu Y, Wang Y, He J, Liu Y, Li H, Zhang H, & Song Y (2010). Structural and dynamic properties of a new amyloidogenic chicken cystatin Mutant I108T. *Journal of Biomolecular Structure and Dynamics*, 27, 641–649. [PubMed: 20085381]

Highlights:

- The mechanism of J-domain mutants disrupting interactions with Hsp70 is unknown;
- Single mutations of the J-domain modulate the structure and dynamics of the protein;
- Conformational changes of the HPD motif disrupt the important role of J-domain;
- The study elucidates the mechanistic role of Hsp40 J-domain in Hsp70 interaction.

Figure 1.

(A) The Ca RMSD of the Hsp40 J-domains as a function of simulation time. (B) The residue averages of the RMSF for the Ca atoms of the Hsp40 J-domains during the 20 ns MD simulations. (C) Superposition of the Ca atoms of the Hsp40 J-domain structures at the end of the 20 ns MD simulation. WT, D9A, D36A, A30T, and F45S are shown in black, red, blue, cyan, and magenta, respectively.

Figure 2. Superimpositions of the HPD motif snapshots during the 20 ns simulation. H34, P35 and D36 are shown in black, red and blue, respectively, and the original positions are shown as thick lines.

Figure 3.

Hsc70 (yellow, PDB code: 1HJO) docking model of WT, D36A and F45S J-domains (gray). The HPD motifs are shown in green, Hsc70-R171 (salt bridge forming with HPD motif) are shown in magenta, the positive charged residues in helix II are shown in blue, and the relevant negative charged residues in Hsc70 are shown in red.

Figure 4.

The surface hydrophobicity of WT, A30T, and F45S J-domains. The hydrophobicity is shown from green (low hydrophobicity) to brown (high hydrophobicity). The main high hydrophobic regions and the corresponding amino acids are marked with red circles.

Figure 5.

The J-domain structures after 20 ns MD stimulation and the 20 ns average mass center distance between the bottom of residues in helices II (30–32) and the bottom of residues in helices III (42–45). The HPD motifs are shown in cyan, the amino acid residues focused in this study are shown in green (magenta for mutations), and other important residues are shown as gray sticks.

Figure 6.

(A) Curing effect of over-expressed Ydj1 mutant proteins. Wild type (wt) and indicated mutant Ydj1 proteins were overexpressed by galactose induction in [*URE3*] cells (strain 1075) and then cells were spread onto YPD plates and grown to colonies (ev is empty vector). Panels are representative 2 cm square portions of the plates. [*URE3*] cells are white and [*ure-o*] cells are red. Values in parentheses are quantified frequencies of prion loss shown as average percentage of [*ure-o*] colonies for three experiments. (B) Western blot of Ydj1 proteins from aliquots of cells used in panel (A) taken at the time of plating. Pgk1 was used as a loading control (C) A drop test of *ydj1* cells (strain MR502) carrying single-copy plasmids expressing the indicated Ydj1 proteins. Cells from medium selecting for the plasmids were diluted in into water and spotted onto 1/2 YPD plates, which were incubated at the indicated temperatures for 3 days. Mutants marked by an asterisk were assessed for curing previously (Sharma et al., 2009). Of these, only D9A cured [*URE3*] effectively.

Table 1.

Scores of the J-domain-Hsc70 docked structures.

System	Cluster no.	Member	Representative	Weighted score
WT	0	193	Center	-420.3
			Lowest energy	-522.7
D9A	0	120	Center	-438.6
			Lowest energy	-468.2
D36A	7	58	Center	-403.5
			Lowest energy	-446.1
F45S	1	105	Center	-377.8
			Lowest energy	-429.3
A30T	N.F. ^a	-	-	-

^aNot found.

Table 2.

Properties of different J-domain mutations after 20 ns MD simulations.

	WT	D9A	D36A	A30T	F45S	F45A	Y26H	Y26A
HPD motif displacement (Å)	1.2	2.5	2.6	7.1	7.6	6.9	1.9	5.2
Surface hydrophobic region residues	Y26:F45 F52	Y26:F45 F52	Y26:F45 F52	Y26 F52	Y26 F52	Y26 F52	Y26:F45 F52	F52
Mass center distance (Å)	10.47	10.34	10.54	11.21	11.39	11.36	10.37	9.31
Direct hydrogen bonds (between helix II and III)	Y26:S49	K23:N56	Y26:S49	–	–	–	H26:S49 Y33:F45	–
Loop fixation interaction	–	–	–	H34: F45	H34: S45	H34: A45	–	H34: L31 H34: K32 G40: K32
Reasonable docking model	+	+	+	–	+	+	+	+
Salt bridge forming	+	+	–	–	–	–	+	–
% [ure-o]	94	91	<0.1	5	0.2	ND ^a	86	ND ^a

^aNot detected.