Molecular Chaperones and the Assembly of the Prion Ure2p in Vitro

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The protein Ure2 from Saccharomyces cerevisiae possesses prion properties at the origin of the [URE3] trait. In vivo, a high molecular weight form of inactive Ure2p is associated to [URE3]. The faithful and continued propagation of [URE3] is dependent on the expression levels of molecular chaperones from the Hsp100, -70, and -40 families; however, so far, their role is not fully documented. Here we investigate the effects of molecular chaperones from the Hsp40, Hsp70, Hsp90, and Hsp100 families and the chaperonin CCT/Tric on the assembly of full-length Ure2p. We show that Hsp104p greatly stimulates Ure2p aggregation, whereas Ssa1p, Ydj1p, Sis1p, and Hsp82p inhibit aggregation to different extents. The nature of the high molecular weight Ure2p species that forms in the presence of the different molecular chaperones and their nucleotide dependence is described. We show that Hsp104p favors the aggregation of Ure2p into non-fibrillar high molecular weight particles, whereas Ssa1p, Ydj1p, Sis1p, and Hsp82p sequester Ure2p in spherical oligomers. Using fluorescently labeled full-length Ure2p and Ure2p-(94–354) and fluorescence polarization, we show that Ssa1p binding to Ure2p is ATP-dependent, whereas that of Hsp104p is not. We also show that Ssa1p preferentially interacts with the N-terminal domain of Ure2p that is critical for prion propagation, whereas Ydj1p preferentially interacts with the C-terminal domain of the protein, and we discuss the significance of this observation. Finally, the affinities of Ssa1p, Ydj1p, and Hsp104p for Ure2p are determined. Our in vitro observations bring new insight into the mechanism by which molecular chaperones influence the propagation of [URE3].

The [URE3] trait, discovered in the early 70s (1), is an inheritable prion factor in the yeast Saccharomyces cerevisiae (2). In a manner similar to what is observed for the vertebrate prion PrP, the [URE3] prion state is associated with a change in the solubility of the protein Ure2 (3).

Similarly to other proteins with prion properties from S. cerevisiae, Ure2p is a two-domain protein. The physical boundary between the two domains of this 354-amino acid polypeptide is

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3 The abbreviations used are: CCT, cytosolic chaperonin containing TCP1; Tric, TCP1 ring complex; IAEDANS, 5-(2-iodoacetamido)ethylamino)naphthalene-1-sulfonic acid.
that Ure2p-molecular chaperone complex formation is nucleotide- and domain-dependent.

**MATERIALS AND METHODS**

**Expression and Purification of Ure2p**—Recombinant full-length Ure2p, Ure2p-(94–354), and the variants Ure2pC355 and Ure2p-(94–354)C355 were produced as previously described (4, 6, 27). Protein concentrations were determined spectrophotometrically (HP 8453 diode array spectrophotometer, Hewlett-Packard) using an extinction coefficient of 0.67 mg/cm\(^2\) at 280 nm and molecular masses of 40.2 and 30 kDa for full-length Ure2p and Ure2p-(94–354), respectively. The proteins were stored at −80 °C in buffer A (75 mM KCl, 20 mM Tris, pH 7.5, 1 mM EGTA, 1 mM dithiothreitol) at a concentration of 2.8 mg/ml.

**Expression and Purification of Other Proteins**—Sup35p, Hsp104p, Ssa1p, Ydj1p, and Sis1p were overexpressed either in *Escherichia coli* or *Saccharomyces cerevisiae* and purified and stored as described (24). CCT was isolated from rabbit reticulocyte lysate (28), and purified Hsp82p (29) was generously provided by Dr. C. Combeau (Sanofi-Aventis Pharma, Vitry, France). All monomeric protein concentrations were measured using their absorbance at 280 nm and their calculated extinction coefficients.

**Assembly of Ure2p into Fibrils**—Soluble full-length wild-type Ure2p was assembled at 8 °C in buffer A supplemented with 2 mM ATP unless specified. The assembly reaction was monitored using thioflavin T binding (30) using a Quantamaster QM 2000-4 spectrofluorimeter (Photon Technology International, Inc.). Ure2p fibrils were also examined after negative staining with 1% uranyl acetate on carbon-coated grids (200 mesh) in a Philips EM 410 electron microscope (Philips Inc., The Netherlands).

**Fluorescent Labeling of Ure2p**—Soluble Ure2pC355 and Ure2p-(94–354)C355 (25 µM) were dialyzed in buffer A without dithiothreitol for 2 h at 4 °C. The proteins were then labeled by adding 250 µM IAEDANS (Molecular Probes). The proteins were incubated on ice for 15 min. The unbound dye was removed by a desalting step using Sephadex G25 column (GE Healthcare) equilibrated in buffer A after quenching the reaction with 250 µM dithiothreitol. The labeled proteins were separated into aliquots and stored at −80 °C. Before use, the protein samples were spun for 10 min at 12,500 rpm and 4 °C to eliminate any aggregated material from the solutions. Their concentration was determined spectrophotometrically before dilution in the presence of unlabeled Ure2p and Ure2p-(94–354).

**Fluorescence Polarization Measurements**—Complex formation between Ure2p or Ure2p-(94–354) and the different molecular chaperones was analyzed using fluorescence polarization. Labeled Ure2p or Ure2p-(94–354) and the different molecular chaperones dialyzed in buffer A at various concentrations were mixed in the presence of ATP or ADP and 5 mM MgCl\(_2\), and the time course of increase in fluorescence polarization upon binding of labeled Ure2p and Ure2p-(94–354) to the added molecular chaperone was measured at 20 °C in a thermostatted Quantamaster QM 2000-4 spectrofluorimeter (Photon Technology International, Inc.) equipped with autopolarizers. Monochromators were set to 337 nm (excitation) and 475 nm (emission), respectively.

**Filtration Assay, SDS-PAGE Electrophoresis, and Western Blotting**—The time courses of Ure2p assembly into fibrils in the presence of the different molecular chaperones was also followed by a filtration assay allowing the quantification of fibrillar Ure2p. Aliquots (5 µl) were withdrawn from the assembly reactions at different time intervals, diluted into 200 µl of water or SDS 2% incubated for 5 min at 37 °C, and filtered through cellulose acetate membranes (0.20-µm pore size, Millipore Corp., Bedford, MA). After filtration of the sample, 200 µl of water or SDS 2% were filtered in each slot twice. The membrane was then washed with distilled water and processed for Western blotting. The cellulose acetate membranes were incubated with 5% skimmed milk, probed with an antibody directed against full-length Ure2p, and developed with the enzyme-coupled luminescence technique (ECL, GE Healthcare) according to the recommendation of the manufacturer.

SDS-polyacrylamide gel electrophoresis were performed in 10–12% polyacrylamide gels (14 × 15 × 0.15 cm) following the standard method described by Laemmli (31). The gels were stained with Coomassie Blue, destained, imaged using a CCD camera (Sony, Inc.), and further analyzed on a MacIntosh (Apple Computer, Inc., Cupertino, CA) computer using the software NIH Image (developed at the United States National Institutes of Health and available at rsb.info.nih.gov/nih-image).

**RESULTS**

**Effects of the Different Yeast Molecular Chaperones on the Assembly of Ure2p Using Thioflavin T Fluorescence**—The effect of Hsp104p (Hsp100), Hsp82p (Hsp90), Ssa1p (Hsp70), Ydj1p (Hsp40), Sis1p (Hsp40), and CCT/Tric on Ure2p assembly was monitored in the absence or the presence of ATP using thioflavin T binding. Only CCT was found not to have a significant effect on Ure2p assembly at sub- or equimolar monomer concentrations (Fig. 1A). All the other molecular chaperones tested, namely, Hsp104p, Hsp82p, Ssa1p, Ydj1p, and Sis1p, influenced the assembly reaction at sub- or equimolar concentrations (Fig. 1B–F). Although Hsp82p (Fig. 1C), Ssa1p (Fig. 1D), Ydj1p (Fig. 1E), and Sis1p (Fig. 1F) inhibit to various extents Ure2p assembly, Hsp104p (Fig. 1B) promotes assembly. Indeed, the elongation rate increases 2.5-fold without a significant reduction in the lag phase preceding assembly. Moreover, in the presence of an equimolar concentration of Hsp104p, the thioflavin T fluorescence at steady state is 2.5-fold higher in the presence of Hsp104p than in its absence.

**Role of ATP**—Although the assembly of Ure2p is affected by Hsp104p only in the presence of ATP, the opposite effect is observed for Ssa1p. Interestingly, Hsp82p did not affect assembly in an ATP-dependent manner. We, therefore, documented the effect of the N-terminal domain of Hsp82p on Ure2p assembly.

| TABLE 1 |
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| Identity of the molecular chaperones used throughout this study |
| Molecular chaperone family | Hsp40 | Hsp70 | Hsp60 | Hsp90 | Hsp100 |
| Member used | Sis1p, Ydj1p | Ssa1p | CCT/Tric | Hsp82p | Hsp104p |

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assembly. The latter domain inhibits Ure2p assembly as efficiently as intact Hsp82p (not shown), thus confirming that Hsp82p interacts with Ure2p in an ATP-independent manner. Finally, as expected, the effects of Ydj1p and Sis1p were not ATP-dependent.

**Role of Molecular Chaperones in Ure2p Assembly**—To better characterize the high molecular weight oligomeric Ure2p species that form in the presence of Hsp104p, Hsp82p, Ssa1p, Ydj1p, and Sis1p, we used a filter trap assay derived from that first designed for polyglutamine-containing proteins by Wanker et al. (32) that allows distinguishing fibrillar and non-fibrillar Ure2p. Although fibrillar Ure2p is retained on the cellulose acetate filters in the presence of 2% SDS, none of the high molecular weight Ure2p species that forms in the presence of the different molecular chaperones used is retained on the filters under the same experimental conditions, thus indicating that unlike fibrillar Ure2p, the high molecular weight Ure2p-molecular chaperone complexes are labile in 2% SDS (Fig. 3A). We then used the filter trap assay to assess whether Ure2p fibrils are labile in the presence of Hsp104p or Ssa1p alone or together with its co-chaperone Ydj1p. Although the SDS-resistant fibrils are unaffected by the addition of Ssa1p alone (Fig. 3B) or together with its co-chaperone (Fig. 3C) in the presence of ATP, Hsp104p appears to convert Ure2p fibrils into molecular species that are no more retained on the filters (Fig. 3D).

**Characterization of the Interaction of Ssa1p and Ure2p**—A total inhibition of Ure2p assembly is observed at an Ssa1p:Ure2p ratio of 1:4 (Fig. 4A). The comparison of the assembly of Ure2p at different concentrations with that of Ure2p at a constant concentration in the presence of increasing concentrations of Ssa1p is very informative on the mode of action of Ssa1p. The time courses of Ure2p (30 μM) in the presence of Ssa1p, 0.5 and 1 μM, superimpose perfectly to that of Ure2p, 27.5 and 25 μM, respectively. This indicates that five Ure2p molecules are sequestered in an assembly-incompetent state by each molecule of Ssa1p. We previously showed that hexameric Ure2p is the precursor of the fibrillar form of the protein (27, 33). Thus, our results strongly suggest that Ssa1p binds to the hexameric form of Ure2p.

When aliquots are removed at steady state from the assembly reactions presented in Fig. 4A and are subjected to ultracentrifugation and the supernatant and pellet fractions are analyzed by SDS-PAGE, a fraction of Ure2p that increases with increasing concentrations of added Ssa1p is found in the supernatant fractions instead of being in the pellet (Fig. 4B). This further demonstrates that Ssa1p sequesters Ure2p in an assembly-incompetent soluble form.

The affinity of Ssa1p for Ure2p (Fig. 4C) was derived from measurements of the elongation rates of the fibrils assembled at a constant Ure2p and increasing Ssa1p concentrations (Fig. 4A). It is 0.5 μM.

To determine whether Ssa1p interacts with fibrillar Ure2p, Ure2p fibrils made in the absence of Ssa1p were incubated in the presence of Ssa1p for 24 h. The fibrils were pelleted, and the supernatant and pellet fractions were analyzed by SDS-PAGE. The data presented in Fig. 4D clearly indicate that Ssa1p neither binds stably to the fibrils nor disassembles them.
Functional Interplay of Molecular Chaperones during the Assembly of Ure2p—Because Ssa1p, Ydj1p, and Sis1p inhibit independently Ure2p assembly, and because size exclusion chromatography is a slow non-equilibrium method where labile complexes may irreversibly dissociate during the time course of analysis, we employed fluorescence polarization to monitor the interaction of Ure2p with Ssa1p alone or together with its co-chaperones from the Hsp40 family and in the presence of Hsp104p.

The polarization signal of a fluorophore depends on its rotational correlation time that is related to its size (34). Ure2p should display a smaller fluorescence polarization as compared with that of larger Ure2p-molecular chaperone complexes if such complexes form. To validate the method, we first meas-

FIGURE 2. High molecular weight Ure2p species that form in the presence of molecular chaperones. Negative stained electron micrographs of Ure2p (50 μM) assemblies obtained in the absence of molecular chaperones (A) and in the presence of Hsp104p (B and G), Hsp82p (C and H), Ssa1p (D and I), Ydj1p (E and J), and Sis1p (F and K) at a molecular chaperone to Ure2p molar ratio of 1:1 and 0.2:1, respectively. Bar, 0.2 μm.
ured the polarization signal of fluorescently labeled Sup35p (3 μM) in the absence or the presence of equimolar amounts of Ssa1p and Ydj1p (1 μM). We indeed recently showed that Ssa1p sequesters Sup35p in an assembly incompetent state in the presence of its co-chaperone Ydj1p. A stable

Sup35p-Ssa1p-Ydj1p complex that resists dissociation when it is subjected to size exclusion chromatography indeed forms. The fluorescence polarization of Sup35p increased very significantly in the presence of Ssa1p and Ydj1p (Fig. 5, inset) indicating that a Sup35p-Ssa1p-Ydj1p complex forms in solution. Similarly to what is observed for Sup35p, the polarization signal of AEDANS-labeled Ure2pC355 (3 μM) elongation at a constant Ure2p concentration (30 μM) and increasing Ssa1p concentrations (0.2–8 μM). SDS-PAGE analysis of the supernatant and pellet fractions of the Ure2p assembly reactions shown in panel A subjected to sedimentation at steady state. C, rate of Ure2p fibrils (μM) elongation at a constant Ure2p concentration (30 μM) and increasing Ssa1p concentrations (0.2–8 μM). SDS-PAGE analysis of the supernatant and pellet fractions of Ure2p fibrils (30 μM) incubated for 24 h in the presence of Ssa1p (8 μM). The molecular mass markers (in kilodaltons) are shown to the left of B and D.

Nucleotide Dependence of Ure2p-Molecular Chaperone Interactions and Identification of the Ure2p Region That Interacts with Molecular Chaperones—We then examined whether Ssa1p alone or in the presence of Ydj1p, on the one hand, and Hsp104p, on the other, interact with Ure2p in an ATP-dependent manner. We, therefore, incubated AEDANS-labeled Ure2p (0.3 μM) in the presence of increasing concentrations of Ssa1p, Ydj1p, Ssa1p, and Ydj1p at a Ydj1p:Ssa1p molar ratio of 1:5 and Hsp104p in the presence of 5 mM ATP or ADP. The interaction between Ssa1p and Ure2p is significantly inhibited in the absence of ADP (Fig. 6A), whereas it appears nucleotide-independent in the presence of Ydj1p (Fig. 6C). Interestingly, the
fluorescence polarization signal measured upon the interaction of Hsp104p and Ure2p was only diminished by 20% in the presence of ADP as compared with what was observed in the presence of ATP (Fig. 6D).

Ure2p is a two-domain protein. The flexible N-terminal domain that is essential for the prion properties of Ure2p spans amino acid residues 1–93, whereas the functional, glutathione S-transferase-like domain of the protein extends from amino acid residues 94–354. To identify the regions within Ure2p that interact with molecular chaperones, we compared the interaction of AEDANS-labeled full-length Ure2pC355 and Ure2p-(94–354)C355 with Ssa1p, Ydj1p, Ssa1p, and Ydj1p and Hsp104p. Ssa1p binds full-length Ure2p and Ure2p-(94–354). The dissociation constants derived from fitting the data points with Michaelis-Menten equation \( y = ax/(b + x) \) yields dissociation constants 39 and 29 nM for full-length Ure2p in the presence of ATP and ADP, respectively, and a dissociation constant of 20 nM for Ure2p-(94–354)C355. However, the extent of fluorescence polarization increase upon binding of Ssa1p to Ure2p-(94–354)C355 is significantly smaller than that observed for full-length Ure2pC355 (Fig. 6A). This suggests that the Ure2p-(94–354)C355:Ssa1p complexes are smaller than Ure2pC355:Ssa1p complexes. Ydj1p binds to Ure2p-(94–354)C355 with higher affinity than to full-length Ure2pC355 and the Ure2p-(94–354)C355 complexes are slightly larger than Ure2pC355:Ydj1p complexes (Fig. 6B).

Finally, Hsp104p interacts with similar affinities with full-length Ure2pC355 and Ure2p-(94–354)C355. The dissociation constants for hexameric Hsp104p are 62 and 54 nM for Ure2pC355 or Ure2p-(94–354)C355, respectively. Overall, these results suggest that Ssa1p binds preferentially to the flexible N-terminal domain of Ure2p, whereas Ydj1p, in agreement with the recent findings of Lian et al. (26) binds preferentially the C-terminal domain of the protein.

**DISCUSSION**

Genetic studies have revealed that molecular chaperones play a critical role in the faithful propagation of the yeast prion [URE3] (36, 37). Indeed, Hsp104p is required for the propagation of [URE3] (20). The Hsp70 family member Ssa1p and its co-chaperone Ydj1p also modulate [URE3] propagation (20, 21, 26).

Herein, we document the effects of the molecular chaperones Hsp104p (Hsp100), Hsp82p (Hsp90), Ssa1p (Hsp70), Ydj1p (Hsp40), Sis1p (Hsp40), and CCT/Tric individually and in combination in the assembly of the full-length Ure2p in vitro. Only CCT was found not to have a significant effect on Ure2p assembly. All the other molecular chaperones influenced assembly. Hsp82p, Ssa1p, Ydj1p, and Sis1p inhibited assembly in a concentration-dependent manner, whereas Hsp104p stimulated the formation of large unstructured Ure2p aggregates that bind the dye thioflavin T. Globular high molecular weight particles were observed at equimolar Ure2p:Hsp82p, Ure2p:Ssa1p, Ure2p:Ydj1p, and Ure2p:Sis1p ratios, whereas a mixture of globular particles, amorphous aggregates, and fibrils was observed at submolar Ure2p-molecular chaperone ratios. We and others (4, 16, 33) previously reported the existence of a globular species at the early stages of Ure2p assembly into protein fibrils. The presence of these particles at high molecular chaperone concentrations together with the inability of Ure2p
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to polymerize suggests that Hsp82p, Ssa1p, Ydj1p, and Sis1p interact with these potential precursors of Ure2p fibrils and inhibit assembly into fibrils through this interaction. Similar to what was observed upon assembly of Sup35p in the presence of Hsp104p, an increased thioflavin T binding is observed (24). However and in contrast with what was observed for Sup35p (24), no reduction of the lag phase preceding assembly was observed, and the high molecular weight species formed in the presence of Hsp104p were not of a fibrillar nature. To further demonstrate that the latter high molecular weight Ure2p aggregates are not of a fibrillar structure, we took advantage of the resistance of Ure2p fibrils to SDS treatment and showed using a filtration assay that the aggregates are indeed not of fibrillar nature.

The trait [URE3] is cured upon overexpression of Hsp104p, Ssa1p, and Ydj1p (20, 21, 26) in a dividing yeast cell. To determine whether this is the direct consequence of fibrils depolymerization by molecular chaperones or to the unfaithful segregation between mother and daughter cells of Ure2p aggregates associated with [URE3], we incubated preformed fibrils with the different molecular chaperones and assayed their resistance to SDS treatment. Ure2p fibrils resist Ssa1p and Ydj1p, whereas they are labile in the presence of Hsp104p. Thus, the curing of [URE3] by the two molecular chaperones systems must be the consequence of two different modes of action. One is passive, whereas the other is active. The first curing mechanism, exemplified by Ssa1p and Ydj1p, is the consequence of the dilution of the fibrillar form of Ure2p upon cell division, whereas newly synthesized Ure2p is sequestered in a globular high molecular weight assembly-incompetent form by the overexpression of molecular chaperones in the cell. The second [URE3]-curing mechanism, exemplified by Hsp104p, is an active process. Here, Hsp104p actively dissociates in an ATP-dependent manner, fibrillar Ure2p. Hsp104p and its bacterial Clp analogues have been shown to resolubilize protein aggregates in an ATP-dependent manner (38–41). Our observations suggest that it is through a double activity, an active depolymerizing, and a passive sequestering activities that Hsp104p cures [URE3].

No evidence for a severing activity of Hsp104p upon incubation of preformed Ure2p fibrils with up to equimolar amounts of Hsp104p and 5 mM ATP was observed either from electron microscopy observations (not shown) or from our quantitative filter assay. Because Ure2p assembly into protein fibrils is irreversible (27) if Ure2p fibrils were indeed severed by Hsp104p, one would expect to generate shorter fibrils, but the overall amount of fibrils should not change. Fibrillar material should be, therefore, detected trapped to the cellulose acetate filters. No SDS-resistant high molecular weight Ure2p oligomers remain after incubation of preformed Ure2p fibrils in the presence of Hsp104p, indicating that Hsp104p fully dissociates Ure2p fibrils within 48 h in the presence of ATP. To reconcile the findings reported here with those reported in another study (25), one needs to envisage that the absence of material trapped on the filters is the consequence of an Hsp104p-severing activity that generates oligomers indistinguishable from the soluble high molecular weight Ure2p oligomers that form in the early stages of assembly.

In a manner similar to what has been shown for Sup35p (42, 43), Ure2p fibrils assembled in vitro induce the appearance of [URE3] when reintroduced within the cell (44). The finding that no Ure2p fibrils can form in the presence of high Ssa1p, Ydj1p, and Sis1p concentrations and that the fibrils are labile in the presence of Hsp104p together with the observations that the overexpression of these molecular chaperones cure [URE3] further support the view that the fibrillar form of Ure2p is the prion form of the protein that propagates [URE3].

To better understand the functional interplay of molecular chaperones during the assembly of Ure2p and prioritize their action, the affinity of each molecular chaperone for Ure2p was measured using fluorescence polarization. All the dissociation constants we determined were in the micromolar to nanomolar range. The molecular chaperone that has the lowest affinity for full-length Ure2p is Ydj1p, whereas that with the highest affinity is Ssa1p. Hsp104p has an intermediate affinity for soluble Ure2p. Although the nature of nucleotide bound to Ssa1p influences very significantly the interaction between soluble Ure2p and Ssa1p, a limited dependence is observed for Hsp104p. Molecular chaperones are expected to recognize and interact with nonnative polypeptides or partially folded intermediates (45–47). Although Ydj1p was reported recently to interact equally well with full-length Ure2p and its compactly folded C-terminal domain (27), we were not expecting Ssa1p and Hsp104p to bind with high affinity Ure2p-(94–354), which lacks the flexible N-terminal domain that is critical for prion propagation. Interestingly, although Ssa1p binds with high affinity full-length Ure2p and its C-terminal domain, the high molecular weight, assembly incompetent Ure2p-Ssa1p and Ure2p-(94–354)-Ssa1p complexes are of similar sizes, and a dependence on the nature of nucleotide bound to Ssa1p was observed. The Ure2p-Ssa1p oligomers are larger in the presence of ATP than in the presence of ADP, where they appear to be similar in size to the Ure2p-(94–354)-Ssa1p complexes. Given that hexameric Ure2p is detected at the early stages of assembly (33, 48) and that we find here that one Ssa1p binds 5 Ure2p molecules, we can reasonably conclude that in the presence of ATP, Ssa1p binds an assembly-competent Ure2p hexamer and inhibits assembly. In the presence of ADP, Ssa1p sequesters Ure2p in a different form, probably a dimeric form.

In our hands, Ydj1p binds Ure2p-(94–354) with slightly higher affinity than full-length Ure2p, indicating it has better affinity for the compactly folded domain of the protein. Because the truncated form of the protein only differs from full-length Ure2p by the absence of the N-terminal domain, one plausible explanation for the difference we see in the binding of Ydj1p is that the molecular chaperone has a lower affinity for assembly competent Ure2p, where the flexible N-terminal domain interacts with the C-terminal domain of the protein (48). Another possibility is that the region that is recognized with the highest affinity by Ydj1p that must lie within the C-terminal domain of Ure2p is masked by the interaction between the N- and C-terminal domains of the protein. Alternatively, the preferential binding of Ydj1p to the C-terminal domain we observe may be due to differences in the conformations of full-length Ure2p and Ure2p-(94–354). Ydj1p mainly modulates the activity of Ssa1p in vivo. In the presence of its co-chaperone Ydj1p, the
nucleotide-dependent difference in Ure2p binding capacity of Ssa1p is abolished. Most interesting, however, is the finding that Ssa1p in the presence of Ydj1p binds Ure2p-(94–354) and full-length Ure2p with the same affinity and the Ssa1p-Ydj1p-Ure2p and Ssa1p-Ydj1p-Ure2p-(94–354) complexes are similar in size. The effect we observe cannot be accounted for by summing up the fluorescence polarization signal measured upon Ssa1p-Ure2p-(94–354) and Ydj1p-Ure2p-(94–354) complex formations as the amount of Ydj1p used in the presence of Ssa1p is 5-fold lower than that used to document Ydj1p-Ure2p-(94–354) complex formation. This suggests that the specificity of Ssa1p binding to Ure2p is significantly changed by Ydj1p. From binding preferentially the N-terminal domain of Ure2p either when it is expanded in solution or docked to the C-terminal domain of the protein, Ssa1p becomes capable of interacting with high affinity with Ure2p-(94–354) and form with it complexes that are similar in size to the Ssa1p-Ydj1p-Ure2p complexes.

Finally, we show here that Hsp104p interacts with similar affinities with full-length Ure2p and Ure2p-(94–354). The differences in affinities of the Hsp70/40 system and Hsp104p for Ure2p suggest that molecular chaperones expression levels in a Ure2p suggest that molecular chaperones expression levels in a different molecular chaperone

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