Protein Folding Activity of Hsp70 Is Modified Differentially by the Hsp40 Co-chaperones Sis1 and Ydj1*

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Specification of Hsp70 action in cellular protein metabolism may occur through the formation of specialized Hsp70:Hsp40 pairs. To test this model, we compared the ability of purified Sis1 and Ydj1 to regulate the ATPase and protein-folding activity of Hsp70 Ssa1 and Ssh1/2 proteins. Ydj1 and Sis1 could both functionally interact with Ssa1, but not the Ssh1/2 proteins, to refold luciferase. Interestingly, Ydj1:Ssa1 could promote up to four times more luciferase folding than Sis1:Ssa1. This functional difference was explored and could not be accounted for by differences in the ability of Sis1 and Ydj1 to regulate Ssa1 ATPase activity. Instead, differences in the chaperone function of Ydj1 and Sis1 were observed. Ydj1 was dramatically more effective than Sis1 at suppressing the thermally induced aggregation of luciferase. Paradoxically, Sis1 and Ydj1 could bind similar quantities of chemically denatured luciferase. The polypeptide binding domain of Sis1 was found to lie between residues 171–352 and correspond to its conserved carboxyl terminus. The conserved carboxyl terminus of Ydj1 is also known to participate in the binding of nonnative polypeptides. Thus, Ydj1 appears more efficient at assisting Ssa1 in folding luciferase because it contains a zinc finger-like region that is absent from Sis1. Ydj1 and Sis1 are structurally and functionally distinct Hsp40 proteins that can specify Ssa1 action by generating Hsp70:Hsp40 pairs that exhibit different chaperone activities.

Hsp70 proteins play an essential physiological role by protecting cells from stress, promoting protein folding, driving protein translocation across membranes, mediating the assembly and disassembly of macromolecular complexes, and facilitating protein degradation (1–4). Hsp70 facilitates these different types of biochemical reactions via an ATP-dependent mechanism that involves the binding and release of short segments of polypeptides that exist in extended conformations (5). Since Hsp70 has a very broad substrate specificity, a major issue concerning the mechanism of its action is what determines the cellular reactions it catalyzes. Cells contain multiple Hsp70 family members that share around 60% sequence identity with the largest degree of variation being observed in their polypeptide binding domains (5). Thus, it is plausible that structural differences in the polypeptide binding grooves of Hsp70 proteins help determine which reactions they catalyze. Such a mechanism is supported by the observation that Hsp70 proteins that are localized in the cytosol and lumen of the endoplasmic reticulum are not interchangeable (6). In addition, the yeast cytosol contains two different forms of Hsp70, Ssa1–4 and Ssb1–2 proteins, which have distinct functions and exhibit a high degree of sequence dissimilarity in regions predicated to be involved in polypeptide binding (7–9). However, swapping the polypeptide binding domains of Ssa and Ssb proteins does not alter the specificity of their action (10). Therefore, factors other than the substrate specificity of Hsp70 must influence the fidelity of the biochemical reactions it catalyzes. Likely candidates for these specificity factors are co-chaperones that have been identified as regulators of the Hsp70 action (11–16).

Hsp40 (DnaJ-related proteins) co-chaperones function as molecular chaperones and regulators of Hsp70 ATPase activity (17, 18). Two features of the Hsp40 family would enable them to direct Hsp70 to catalyze specific types of reactions in protein metabolism. First, multiple Hsp40 proteins are present in subcellular compartments, and they are often concentrated to different locations, which enables Hsp70 to catalyze localized reactions in protein metabolism. Second, Hsp40 family members have different domain structures and are therefore not functional equivalents (19, 20). Hsp40 family members contain different combinations of four conserved domains initially identified in Escherichia coli DnaJ (21), which include the J-domain, G/F-rich region, zinc finger-like domain, and conserved carboxyl terminus. The J-domain corresponds to the amino-terminal 70-amino acid residues of DnaJ and is found in all Hsp40 proteins and is responsible for regulation of the ATP hydrolytic cycle of Hsp70 (22–24). The G/F-rich region is present in approximately 50% of Hsp40 family members, appears to function as a spacer between the J-domain and other regions of Hsp40 proteins, and may directly interact with Hsp70 (23, 25). The zinc finger-like region and the conserved carboxyl terminus are also present in different combinations in nearly 50% of Hsp40 family members and are both involved in interactions with nonnative polypeptides (24, 26, 27). A subfamily of Hsp40 proteins contains only the J-domain and other specialized domains that allow them to bind specific substrates or localize them to discrete locations within the cell (18). Thus, it is possible that a single form of Hsp70 can interact with different subtypes of Hsp40 proteins to generate functionally distinct Hsp70:Hsp40 chaperone machines.

The specific recognition and pairing of Hsp40 proteins with different subfamily members may also provide a mechanism to dictate Hsp70 action. For example, the chaperone action of the Ssa, but not the Ssb proteins, can be regulated by the cytosolic Hsp40 protein Ydj1 (28, 29). There is also genetic evidence to support speculation that the Ssb proteins are specifically reg-
ulated by a different cytosolic Hsp40 protein termed Sis1 with Ssa:Ydj1 and Ssb:Sis1, representing independent Hsp70 chaperone systems (30). However, the ability of Sis1 to regulate the action of the Ssb proteins has not been directly demonstrated. In addition, differences in results obtained from genetic studies may reflect the fact that Ydj1 contains all four of the conserved domains of DnaJ, whereas Sis1 lacks the zinc finger-like region.

To investigate how Hsp40 proteins specify cellular activities of Hsp70, we examined the ability of purified Sis1 and Ydj1 to regulate the protein folding activity of Ssa1 and Ssb1/2. We report that Sis1 and Ydj1 both enhance the ability Ssa1 to fold luciferase but do not interact detectably with Ssb1/2. Interestingly, the Ssa1:Ydj1 pair was found to be up to four times more efficient than the Ssa1:Sis1 pair in folding luciferase. This variance in Sis1 and Ydj1 function appeared to result from a difference in their ability to function as molecular chaperones. Thus, Ssa1 specifically interacts with at least two Hsp40 proteins, and the chaperone activity of its partner strongly influences the action of the different Hsp70:Hsp40 pairs formed.

MATERIALS AND METHODS

Purification of Hsp70 and Hsp40 Proteins—Hsp70 Ssa1 and a mixture of Ssb1/2 were purified as described previously (13, 29). The Ssb1 and Ssb2 proteins are approximately 99% identical and co-purify as a mixture (29) and are therefore referred to as Ssb1/2 in the text. Purified Ssa1 contains less than a 5% contamination with Ssb proteins, and the Ssb1/2 mixture contains a 5% contamination with Ssa2 (29). Ydj1 was tagged with 6 histidine residues at its amino terminus and purified by metal chelate chromatography (24).

To purify Sis1, a six-histidine tag was engineered onto its amino terminus by polymerase chain reaction. The His-Sis1 polymerase chain reaction fragment was subcloned into the NcoI and BsmHI sites of pETD. His-Sis1 was then expressed in BL21(DE3) via induction of cultures of 0.7 absorbance units/ml with 0.5 mM isopropyl-1-thio-b-D-galactopyranoside and growth at 37 °C for 3 h. Cell pellets from 1 liter of culture typically weighed 2 g and were resuspended in 10 ml of lysis buffer (150 mM NaCl, 20 mM Tris, pH 8.0, 3 mM KCl, 10 mM dithiothreitol, and 1 mM MgCl2, 6 mM MgCl2, 2 mM ATP, and 5 mM dithiothreitol). The denaturation reaction was allowed to proceed for 40 min at 25 °C, and then a 1-μl aliquot was removed and mixed with 125 μl of refolding buffer (25 mM Hepes, pH 7.4, 50 mM KCl, 5 mM MgCl2, 1 mM ATP) that was supplemented with the indicated chaperone proteins and incubated at 25 °C. Aliquots of 1 μl were removed from the folding reactions at the indicated times and mixed with 60 μl of luciferase assay reagent (Promega). Luciferase activity was then measured with a Turner TD-20/20 Lumimeter. The level of luciferase activity observed when Ydj1 (3.0 μM) and Ssa1 (0.8 μM) were present in reaction mixtures was typically equivalent to around 100% of the activity exhibited by the native protein purchased from Promega.

Measurement of Complex Formation between Sis1 and Ydj1 with Unfolded Proteins—Luciferase (100 μM) was chemically denatured as described above and was then diluted 50-fold into buffer containing 150 mM KCl, 20 mM Tris, pH 8.0, 0.2 mg/ml bovine serum albumin, and the indicated concentrations of His-tagged Hsp40 proteins and incubated for 10 min at 30 °C. The 50-μl reaction mixture was then supplemented with 40 μl of a 50% slurry of Talon metal chelate resin (CLONTECH) that had been preincubated for 1 h in the above buffer to block nonspecific binding. Reaction mixtures were then extended for an additional 20 min. The proteins complexed with the metal chelate resin were then isolated by centrifugation of the reaction mixture at 14,000 rpm for 1 min at 4 °C in a microcentrifuge. The supernatant was then discarded, and the resin was washed twice with a buffer containing 150 mM KCl, 20 mM Tris, pH 8.0, and 15 mM imidazole to elute material that was bound nonspecifically. Complexes between Sis1, Ydj1, and denatured luciferase were then eluted from the washed resin by the addition of 100 μl of a buffer containing 150 mM KCl, 20 mM Tris, pH 8.0, and 125 mM imidazole followed by incubation at 25 °C for an additional 20 min. Samples were then spun in a centrifuge as described above, and the contents of the supernatants were concentrated by precipitation with 80% acetone. Resuspended pellets were analyzed on 12.5% SDS gels, and bands were stained with Brilliant Blue R-250. The quantity of luciferase bound to Sis1 and Ydj1 was then determined with a Bio-Rad model GS-700 imaging densitometer.

Identification of the Polypeptide Binding Domain of Sis1—Purified Sis1 was subjected to limited proteolysis under previously described conditions (24). Amino-terminal sequencing and molecular weight determination of the protease-resistant fragments of Sis1 liberated by this treatment was also carried out as described previously (24). Measurement of complex formation between fragments of Sis1 and luciferase was carried out as described above.

RESULTS

Sis1 and Ydj1 Exhibit Differences in Their Ability to Assist Ssa1 in Folding Denatured Luciferase—To identify functional interactions between Hsp40 and Hsp70 proteins in the yeast cytosol, we compared the ability of purified Sis1 and Ydj1 to enhance the luciferase folding activity of Ssa1 and Ssb1/2 (Fig. 1). Native luciferase was denatured with 6 M guanidine-HCL and diluted into folding buffer that contained different concentrations and combinations of chaperone proteins. Then, the kinetics of luciferase reactivation were determined. When Ssa1, Ssb1/2, Sis1, or Ydj1 were included independently, only a small amount of reactivation occurred (Fig. 1, A and B). When pairs of Sis1 and Sis1 or Ydj1 were included, we observed little increase in luciferase activity. In contrast, Sis1 and Ydj1 were both capable of enhancing the ability of Ssa1 to refold luciferase. Half-maximal folding activity for both the Ssa1:Sis1 and Ssa1:Ydj1 pairs was observed at a 1:2 Ssa1:Hsp40 ratio, where maximal activity was observed at a 1:4 ratio. Interestingly, the Ssa1:Ydj1 pair was approximately 3-fold more effective than the Ssa1:Sis1 pair in folding luciferase. Thus, Sis1 and Ydj1 appear to interact preferentially with Ssa1 instead of Ssb1/2 to promote protein metabolism in the cytosol.

Next we determined whether differences in Ssa1:Sis1 and Ssa1:Ydj1 activity are also observed when these chaperone pairs are presented with thermally denatured luciferase in-
stead of the chemically unfolded form of this protein. Because the Ssb1/2 proteins were not active in the folding of chemically denatured luciferase, they were not included in this analysis.

Native luciferase was inactivated by incubation at 42 °C in the presence or absence of indicated chaperone proteins, and its reactivation was then monitored upon lowering temperatures to 25 °C and extending incubation periods (Fig. 2). The Ssa1:Ydj1 pair could protect luciferase from irreversible aggregation and promote its reactivation to a level that was approximately 20-fold higher than that observed in the presence of these individual chaperones. The Ssa1:Sis1 chaperone pair routinely reactivated about 2.5-fold less luciferase than Ssa1:Ydj1 (Fig. 2). Inactivation of Hsp70 by the addition of EDTA and omission of MgATP resulted in complete inhibition of the folding action exhibited by the Ssa1:Ydj1 and Ssa1:Sis1 pairs. Thus, both Sis1 and Ydj1 enhance the ATP-dependent action of Ssa1 in folding denatured luciferase, but in this capacity the Ssa1:Ydj1 and Ssa1:Sis1 pairs are not functionally equivalent.

Sis1 and Ydj1 Regulate the ATPase Activity of Ssa1 but Not Ssb1/2—Why do Ssa1:Sis1 and Ssa1:Ydj1 chaperone pairs exhibit differences in their ability to fold luciferase? Why don’t Sis1 and Ydj1 enhance the ability of Ssb1/2 to refold luciferase? These Hsp40 proteins might exhibit differences in their ability to regulate the ATP hydrolytic cycle of Hsp70. In tests of this supposition, both Sis1 and Ydj1 were observed to stimulate the ATPase activity of Ssa1 by 8- to 10-fold less luciferase than Ssa1:Ydj1 (Fig. 2). Inactivation of Hsp70 by the addition of EDTA and omission of MgATP resulted in complete inhibition of the folding action exhibited by the Ssa1:Ydj1 and Ssa1:Sis1 pairs. Thus, both Sis1 and Ydj1 enhance the ATP-dependent action of Ssa1 in folding denatured luciferase, but in this capacity the Ssa1:Ydj1 and Ssa1:Sis1 pairs are not functionally equivalent.

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Sis1 and Ydj1 Are Not Equivalent at Maintaining Thermally Denatured Luciferase in a Folding-competent Conformation—

Different Regulation of Hsp70 Action by Sis1 and Ydj1

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FIG. 1. Luciferase refolding by cytosolic Hsp70 and Hsp40 proteins.

A, refolding of chemically denatured luciferase by Ssa1 or Ssb1/2 and Sis1.

B, refolding of chemically denatured luciferase by Ssa1 or Ssb1/2 and Ydj1. Chaperone proteins were added at the indicated concentrations before luciferase. Incubations were carried out at 25 °C for the indicated time periods. In the absence of ATP, little refolding of chemically denatured luciferase by the respective Hsp70:Hsp40 chaperone was observed (data not shown). Refolding of luciferase was quantitated by measuring rates of its reactivation over time with Turner TD 20/20 luminometer. Luciferase activity is expressed in arbitrary luminometer units.

Denatured Luciferase in a Folding-competent Conformation—

Differences in the chaperone function of Sis1 and Ydj1 may account for their differential ability to regulate Ssa1 action in luciferase folding. To investigate this possibility, the ability of Sis1 and Ydj1 to maintain luciferase in a folding-competent conformation during thermal inactivation was examined (Fig. 4A). Ssa1, Ydj1, or Sis1 were added individually during the denaturation period. Then the appropriate co-chaperone for these respective chaperones was added to a second incubation of the same reaction mixture that was carried out at 25 °C. The concentrations of chaperone proteins utilized promoted maximal refolding of thermally denatured luciferase. The maximal activity of renatured luciferase observed was nearly equivalent to the activity of native luciferase before the denaturation period.

FIG. 2. Refolding of thermally denatured luciferase by Ssa1: Sis1 and Ssa1:Ydj1 co-chaperone pairs.

A, refolding of thermally denatured luciferase by yeast Hsp70 and Hsp40 proteins. Luciferase (0.04 μM) was thermally denatured at 42 °C for 10 min in the presence or absence of the indicated concentrations of chaperones and then allowed to refold at 25 °C. The concentrations of chaperone proteins utilized promoted maximal refolding of thermally denatured luciferase. The maximal activity of renatured luciferase observed was nearly equivalent to the activity of native luciferase before the denaturation period.

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FIG. 3. ATPase activity of Ssa1 and Ssb1/2 in the presence of Sis1 and Ydj1.

A, ATPase activity of Ssa1 and Ssb1/2 in the presence of Sis1 and Ydj1. Ssa1 and Ydj1 stimulated the ATPase activity of Ssa1 and Ssb1/2 by 8- to 10-fold, but neither significantly accelerated ATP hydrolysis by Ssb1/2. On average (n = 3) Ydj1 (2 μM) stimulated the ATPase of Ssa1 and Ssb1/2 by 10.59 ± 3.15-fold and 1.62 ± 0.23-fold, respectively. Sis1 (2 μM) stimulated the ATPase of Ssa1 and Ssb1/2 by 8.38 ± 2 and 2.1 ± 0.31-fold, respectively. The minor differences in the ability of Ydj1 and Sis1 to stimulate the ATPase activity of Ssa1 were not statistically significant. In addition, the modest stimulation of Ssb1/2 ATPase by Sis1 and Ydj1 could be accounted for by minor contamination of this protein preparation with Ssa1 (29). The inability of Sis1 and Ydj1 to stimulate the ATPase activity of Ssb1/2 protein appears to explain why these co-chaperones do not assist this Hsp70 homolog in folding luciferase. However, a mechanism unrelated to regulation of ATP hydrolysis is likely to account for the large gap in the protein folding activities of Ssa1:Sis1 and Ssa1:Ydj1.

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FIG. 4. Luciferase refolding by Ssa1: Ydj1 and Ssa1:Sis1 co-chaperone pairs.

A, refolding of thermally denatured luciferase by Ssa1: Ydj1 and Ssa1:Sis1. Chaperone proteins were added before luciferase. Incubations were carried out at 25 °C for the indicated time periods. Refolding of luciferase was quantitated by measuring rates of its reactivation over time with Turner TD 20/20 luminometer. Luciferase activity is expressed in arbitrary luminometer units.
when Ydj1 was included during the denaturation period, and then Ssa1 was added in the subsequent refolding reaction. In contrast, no increase in luciferase activity was observed when Sis1 was added during the denaturation reaction, and Ssa1 was subsequently included (Fig. 4A, lane 6 versus 8).

An explanation of these results is that Ydj1 is more efficient than Sis1 at suppressing thermally induced aggregation of luciferase and therefore is more effective at maintaining luciferase in folding-competent conformation. This interpretation was tested directly by examining the ability of Sis1 and Ydj1 to suppress the aggregation of thermally denatured luciferase (Fig. 4B). When incubated at 42 °C, luciferase (0.1 μM) aggregated with linear kinetics over a 600-s incubation period. Inclusion of Sis1 at up to 10 μM had little detectable influence on luciferase aggregation (Fig. 4B). In contrast, Ydj1 was able to suppress this reaction in a dose-dependent manner with greater than 80% suppression being observed at 5 μM (Fig. 4C). Although Sis1 did not function as a chaperone when assayed individually, it was capable of interacting with Ssa1 to suppress luciferase aggregation. In fact, the Ssa1:Sis1 and Ssa1:Ydj1 pairs were both capable of acting synergistically to suppress luciferase aggregation by over 70% (Fig. 4, B and C).

Thus, Sis1 and Ssa1 can function together to suppress protein aggregation in a manner that is similar to the activity of Ssa1:Ydj1. However, Sis1 is markedly less efficient than Ydj1 at functioning independent of Ssa1 to suppress luciferase aggregation. Ydj1 and Sis1 exhibit differences in their chaperone functions.

Sis1 and Ydj1 Bind Chemically Denatured Luciferase with Similar Efficiency—Sis1 and Ydj1 may exhibit differential ability to bind unfolded polypeptides, and this might be why these proteins are not equivalent as molecular chaperones. To directly examine this possibility, we compared the ability of His-Sis1 and His-Ydj1 to form co-precipitable complexes with chemically denatured luciferase (Fig. 5). Surprisingly, Sis1 and Ydj1 were equivalent at forming complexes with unfolded luciferase (Fig. 5). Sis1 and Ydj1 both recognized unfolded luciferase but did not appear to interact with native forms of this protein (compare Fig. 5, A and C, lane 3 versus 5). Binding of denatured luciferase by Sis1 and Ydj1 was also dependent upon the concentration of the respective chaperone proteins in the binding reaction. At 5 μM, Sis1 and Ydj1 could be isolated in a complex...
Differential Regulation of Hsp70 Action by Sis1 and Ydj1

Fig. 5. Complex formation between denatured luciferase and Sis1 and Ydj1. A, the binding of native (N-Luc) and denatured luciferase (D-Luc, at 2 μM) to Sis1 (5 μM). B, dose dependence of complex formation between Sis1 (as indicated) and denatured luciferase (2 μM). C, the binding of denatured versus native luciferase (2 μM) to Ydj1 (5 μM). D, dose dependence of complex formation between Ydj1 (as indicated) and denatured luciferase (2 μM). E, quantitation of luciferase bindings to Sis1 (from panel B) or Ydj1 (from panel D) in percentage of the total luciferase. To carry out these experiments, native luciferase (100 μM) was denatured in buffer containing 6 M guanidine-HCl, 50 mM KCl, 5 mM β-mercaptoethanol, and 10 mM Tris, pH 8.0, for 1 h. Denatured luciferase was then diluted 50-fold into a similar buffer that contained either His-Sis1 or His-Ydj1 and incubated for 10 min at 25 °C. Complexes between luciferase and the respective Hsp40 proteins were then isolated with a metal chelate resin coupled to agarose beads that specifically recognizes the His-tags present on the respective Hsp40 proteins (see “Materials and Methods” for details). Pelleted protein was then solubilized in sample buffer and analyzed by 12.5% SDS-polyacrylamide gel electrophoresis. Gels were stained with Brilliant Blue R-250 to visualize proteins.

At higher Hsp40 concentrations, little increase in complex formation was observed (Fig. 5E). These results suggest that at 5 μM, Sis1 and Ydj1 bind all of the denatured luciferase (2 μM) present in reactions. However, these Hsp40:luciferase complexes appear to dissociate during isolation because complex formation is an equilibrium reaction (24, 32, 33). Sis1 can recognize and bind nonnative polypeptides and therefore can be classified as a molecular chaperone. However, these data do not explain why Sis1 is less efficient than Ydj1 at enabling Ssa1 to fold luciferase.

The Polypeptide Binding Activity of Sis1 and Ydj1 Is Contained within the Conserved Carboxy Terminus of DnaJ—Little information that describes how Hsp40 proteins bind unfolded polypeptides is available. We know that a fragment of Ydj1 that contains a portion of the zinc finger-like region and the conserved carboxyl terminus exhibits chaperone function similar to Ydj1 (24). However, Sis1 lacks the zinc finger-like region, so it is likely that the conserved carboxyl terminus of Hsp40 proteins is sufficient for substrate binding. To test this supposition and define the region of Hsp40 proteins that contains their polypeptide binding groove, the domain structure of Sis1 was probed by limited proteolysis with proteinase K (Fig. 6A). Proteinase K cleaved Sis1 into two major fragments, a 20- and a 10-kDa band. Limited proteolysis of DnaJ and Ydj1 liberated a 10-kDa protease-resistant fragment that corresponds to the J-domain (23, 24). Therefore, we assumed that the 10-kDa fragment of Sis1 was of similar origin and did not investigate its identity or function further. The 20-kDa Sis1 fragment was analyzed by amino-terminal amino acid sequencing and mass spectroscopy and was found to have a molecular weight of 20,454 and amino acid residues SSSPTYP at its amino terminus. The 20-kDa protease-resistant fragment of Sis1 therefore corresponds to residues 171–352 (31).

Sis1 (171–352) comprises the region in Sis1 that corresponds to the conserved carboxyl terminus of Hsp40 proteins. Therefore, we wanted to compare its ability to bind polypeptides with Sis1. However, to utilize Sis1 (171–352) in our luciferase binding assays, we needed to produce it with a His-tag on its carboxyl terminus. Therefore Sis1-His was constructed, purified, and tested for its ability to regulate Hsp70 ATPase activity and cooperate with Ssa1 to suppress protein aggregation. Results from these tests demonstrated that Sis1-His exhibited the same activity as His-Sis1 and Sis1 (data not shown). Next, Sis1-His was digested with protease to determine whether a fragment similar to Sis1 (171–352) could be liberated (Fig. 6B). Sis1-His appeared to be properly folded, since proteinase K digestion liberated a 20-kDa protease-resistant fragment that migrated with a slightly slower mobility on SDS-polyacrylamide gel electrophoresis than Sis1 (171–352). Mass spectroscopy indicated that this Sis1-His fragment had a molecular weight of 21,260, which was nearly identical to the molecular weight of 21,258 predicted for Sis1 (171–352)-His. The 21,260 fragment liberated from Sis-His therefore corresponds to Sis1 (171–352) with six histidine residues attached to its carboxyl terminus.

Next, the ability of Sis1 (171–352)-His to bind denatured luciferase was compared with Sis1-His. Sis1-His and Sis1 (171–352)-His could bind quantities of luciferase that were similar to His-Sis1 (compare Fig. 5A, lane 5 versus Fig. 6B, lanes 7 and 10). Binding of luciferase by Sis1-His and Sis1 (171–352)-His was dependent on the concentration of luciferase in reactions, and both proteins appeared to have affinities for unfolded proteins in the μM range. The polypeptide binding domain of Sis1 corresponds to a region within its conserved carboxyl terminus. Therefore, similar regions within Ydj1 and Sis1 are implicated in polypeptide binding. These data also demonstrate for the first time that the conserved carboxyl...
Sis1-His (0.7 mg/ml) were suspended in 50 mM KCl, 10 mM Tris, pH 8.0, and 5 mM β-mercaptoethanol and then incubated at 30 °C for 1 h with the indicated concentrations of PK. Proteolytic digestions were halted by the addition of 0.5 mM phenylmethylsulfonyl fluoride. Samples were then run on 12.5% SDS-polyacrylamide gel electrophoresis and stained with Brilliant Blue R-250. Although recognition of Sis1 is functionally important in substrate binding or plays a structural role in stabilizing the polypeptide binding groove of Hsp40. Additional information that defines the kinetic parameters for polypeptide binding and release by Ydj1 and Sis1 are now required to explain why Ssa1:Ydj1 and Ssa1:Sis1 chaperone pairs are not functionally equivalent.

The observed differences in the biochemical activity of purified Ydj1 and Sis1 are consistent with genetic studies that demonstrate that these co-chaperones do not play equivalent roles in cell physiology (37). SIS1 is an essential gene, whereas YDJ1 is essential only for growth at elevated temperatures (31, 37). Expression of Sis1 can complement the slow growth phenotype of Δydj1 strains, but Ydj1 cannot complement the lethal phenotype of Δsis1 strains (31, 37). Because Ydj1 appears superior to Sis1 as a chaperone, it is surprising that YDJ1 cannot complement the loss of SIS1. However, Ydj1 is a farnesylated protein in vitro (19, 38), and this post-translational modification may limit its ability to substitute for Sis1 in the catalysis of certain essential cellular reactions.

Ssa1:Ydj1 and Ssb1/2:Sis1 co-chaperone pairs have been proposed to represent independent chaperone systems (30). Because Sis1 was incapable of regulating Ssb ATPase and luciferase folding activity, our data do not support the above proposal. However, the genetic evidence that suggests that Sis1 and Ssb act together in protein biogenic pathways independent of Ssa or Ydj1 should not immediately be discounted (30). It is possible that our assays with purified components lack additional co-chaperone(s) required of the Ssb proteins to functionally interact with Sis1 and/or Ydj1. Additional investigation of the mechanism for Ssb function is now required to clarify this issue.

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