Conformations of the Nucleotide and Polypeptide Binding Domains of a Cytosolic Hsp70 Molecular Chaperone Are Coupled*

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70-kDa heat shock protein (Hsp70) molecular chaperones are ATPases that participate in protein folding by regulating protein-protein interactions. ATP binds to the highly conserved amino-terminal domain, whereas polypeptides bind to the less conserved carboxyl-terminal domain. These domains are functionally coupled. Polypeptides were previously shown to dissociate from Hsp70s upon ATP binding and to stimulate ATPase activity. We probed the structure of the yeast cytosolic Hsp70 Ssa1p using limited proteolysis to determine whether the conformations of its nucleotide and polypeptide binding domains are also coupled. Ssa1p adopted three distinct conformations, nucleotide-free, ADP-dependent, and ATP-dependent. Complete conformational changes required K+ and Mg2+. Using amino-terminal sequencing, ATP-agarose chromatography, and a carboxyl-terminal-specific antibody, we mapped the locations of the major proteolytic fragments. Nucleotides altered the conformations of both the nucleotide and polypeptide binding domains. Similarly, a polypeptide altered the conformations of both domains. These results indicate that the conformations of the nucleotide and polypeptide binding domains are coupled.

Hsp70† molecular chaperones are highly conserved ATPases that play a role in many cellular pathways including protein folding and protein translocation into organelles (reviewed in Ref. 1). They bind to extended (2), hydrophobic regions of polypeptides (3, 4), prevent their aggregation (5, 6), and guide them to their native conformations (6–8). The ATPase activity of the Escherichia coli Hsp70 DnaK is regulated by two protein modulators, DnaJ and GrpE (9). DnaJ increases the rate of hydrolysis of bound ATP, whereas GrpE stimulates nucleotide exchange (9, 10). Members of the Saccharomyces cerevisiae Hsp70 family are found in the cytosol, endoplasmic reticulum, and mitochondria (11). Ssa1p, one of at least six cytosolic Hsp70s, and its DnaJ-like modulator Ydj1p (12) comprise a cytosolic protein folding machinery (8). The lack of a requirement for a GrpE-like factor in cytosolic protein folding systems of eukaryotes (8, 13) underscores an intriguing divergence from the DnaK-based system (5, 6).

The ATPase and polypeptide binding activities of Hsp70s are localized to distinct domains (14, 15). Nucleotides bind to the amino-terminal domain, whereas polypeptide substrates bind to the carboxyl-terminal domain. The domains are functionally coupled. ATP lowers, whereas ADP increases, the affinity of Hsp70s for polypeptide substrates (16, 17). Polypeptides that bind to Hsp70s also stimulate their ATPase activity (18). In the absence of modulators, the rate-determining step for ATP hydrolysis is cleavage of γ-phosphate (10, 19). Monovalent and divalent cations play an important role in Hsp70 activity (20, 21). K+ is required for efficient hydrolysis of ATP (22) and productive interactions with other proteins (16, 23).

The conformation of Hsp70s is influenced by nucleotides and polypeptide substrates (21, 24–27). Recently, Buchberger et al. (26) showed that DnaK, like the immunoglobulin heavy chain binding protein (BiP) (21), adopts three distinct conformations, nucleotide-free, ADP-dependent, and ATP-dependent. ATP protects Hsp70 (27) and BiP (21) from proteolysis while rendering DnaK more susceptible (25, 26). Whether binding or hydrolysis of ATP induces a conformational change remains controversial. Liberek et al. (25) proposed that ATP hydrolysis is required to alter the conformation of DnaK because ATP, but not nonhydrolyzable analogs, changed its conformation. However, nonhydrolyzable analogs of ATP bind to Hsp70s with low affinity (28) and do not release bound polypeptides upon binding as ATP does (16). A mutant form of DnaK that cannot hydrolyze ATP nevertheless adopts a different conformation upon binding ATP, further supporting the idea that ATP binding is sufficient to induce a conformational change (26).

Polypeptide substrates, such as reduced carboxymethylated lactalbumin (27, 29) and a random library of decapeptides (30), were shown to alter the overall conformation of Hsp70s. Recently, peptide C, a peptide that binds to many Hsp70s, was shown to change the ATP-dependent conformation of DnaK to one resembling the ADP conformation (26). Peptide C stimulated DnaK’s ATPase activity and increased the amount of bound ADP (26). In the absence of nucleotides, peptide C had no effect on the conformation of DnaK (26). Interestingly, a peptide derived from the light chain of clathrin altered the conformation of Hsc70 in the presence or absence of ATP (24). Amphiphilic mitochondrial presequences (31) and ATP (31, 32) were recently shown to depolymerize Ssa1p oligomers.

Although maximal ATP-dependent changes in intrinsic fluorescence of DnaK were dependent on K+ (16), the overall structures of the ATPase domain of Hsc70 bound with ATP, and either K+ or Na+ were very similar to each other (33). Two K+ ions bound at the active site. One was between the protein and the β-phosphate of ADP, whereas the other was between the protein and P, (33). In the presence of Na+, the former site was occupied by one Na+ ion, whereas the latter site was
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occupied by a Na$_2$H$_2$O pair. Ca$^{2+}$ and Mg$^{2+}$ occupied different positions in the active site of Hsc70. Mg$^{2+}$ occupied a site near the β-phosphate of ADP, whereas Ca$^{2+}$ was found in the space occupied by P$_i$ after ATP hydrolysis (34). Nevertheless, the Ca$^{2+}$- and Mg$^{2+}$-dependent structures were essentially identical to each other (34). Ca$^{2+}$-dependent autophosphorylation has been reported for several Hsp70s (35–37).

We undertook the following study to determine whether the conformations of the nucleotide and polypeptide binding domains of Ssa1p change during their interactions with nucleotides and polypeptide substrates and, if so, would these changes help explain differences between the DnaK- and Ssa1p-based protein folding machineries. We show below that Ssa1p adopts three conformations: nucleotide-free, ADP-dependent, and ATP-dependent. Nucleotide-dependent changes occurred readily in the presence of K$^+$ and Mg$^{2+}$ but not in the presence of Na$^+$ or Ca$^{2+}$. A polypeptide protected Ssa1p from digestion and altered the conformations of all three forms of Ssa1p. Using an antibody specific for the carboxyl terminus of Ssa1p, ATP-agarose chromatography, and sequence analysis of tryptic peptides, we show that conformational changes in the nucleotide and polypeptide binding domains are coupled. Efficient conformational coupling required K$^+$ and Mg$^{2+}$.

EXPERIMENTAL PROCEDURES

Proteins and Polypeptides—Ssa1p was purified from S. cerevisiae MW141 using DEAEC-ultralase and ATP-agarose affinity chromatography as described previously (8). Trypsin (sequencing grade) was purchased from Boehringer Mannheim. We designed polypeptide Pep70 (GQLQLST) so that it mimicked the substrate specificity of Hsp70s (3, 4) and lacked trypsin-sensitive sites. Polypeptide Pro (TAQPAEAVIGY) corresponds to part of the P domain of the yeast mating factor precursor prepro-α-factor (38), whose post-translational translocation into microsomes is stimulated by Ssa1p (39, 40). The sequence of polypeptide Ssa1c (AGEPTVEEVD) corresponds to the last 13 carboxy-terminal amino acid residues of Ssa1p (41). Polypeptides Pep70 and Ssa1c were synthesized by Macromolecular Resources (Fort Collins, CO). Polypeptide Pro was synthesized by the Protein Sequencing Facility at The Rockefeller University (NY). Protein concentrations were determined using the method of Bradford (42), and bovine γ-globulin was used as the standard (Bio-Rad).

Nucleotides and Nonhydrolyzable Analogues—ADP, ATP, and AMP-PNP (0.3% ADP) were obtained from Boehringer Mannheim. ATP-$\gamma$-S (8.8% ADP) was obtained from Sigma. AMP-PNP and ATP-$\gamma$-S were separated from ADP and purified using a MonoQ column (Pharmacia Biotech Inc.) as described by Horst et al. (43).

Buffers—The following buffers were used in the experiments: Buffer NaCl-50 (20 mM HEPES-NaOH, pH 7.4, 50 mM NaCl, and 2 mM dithiothreitol), Buffer KCl-50 (20 mM HEPES-KOH, pH 7.4, 50 mM KCl, and 2 mM dithiothreitol), and 2×-Sample Buffer (500 mM Tris base, 7% (w/v) SDS, 170 mM dithiothreitol, 0.025% (w/v) bromophenol blue, and 34% (v/v) glycerol).

Preparation of Nucleotide-free Ssa1p—Purified Ssa1p was centrifuged through a 1-ml Sephadex G-50 column (44) equilibrated with either Buffer NaCl-50 or Buffer KCl-50 containing 0.1 mM EDTA and then dialyzed for 40 h in the same buffer. High performance liquid chromatographic analysis revealed that Ssa1p prepared using this method was nucleotide-free.

Trypsin Digests—Nucleotide-free Ssa1p (8 µg) was digested at 25°C with trypsin (0.68 µg) in a final volume of 20 µl of either Buffer NaCl-50 or Buffer KCl-50 containing the indicated nucleotides, polypeptides, and cations. At various times, reactions were stopped by adding 20 µl of 2×-Sample Buffer, and the resulting mixtures were immediately boiled for 3 min.

Electrophoresis—Proteins were separated on 12% SDS-PAGE gels and stained with Coomassie Blue as described previously (45). The following buffers were used in the experiments: Buffer NaCl-50 or Buffer KCl-50 containing the indicated nucleotides, polypeptides, and cations and stained with Coomassie Blue as described previously (45). The sequence of trypsin-sensitive peptide bonds. Before digesting Ssa1p with trypsin, residual amounts of ADP and ATP remaining bound to Ssa1p after purification were removed using EDTA-containing buffers.

Ssa1p was digested with trypsin for various times in the absence of nucleotides (Fig. 1A, lanes 1–6) or in the presence of either ADP (Fig. 1A, lanes 7–11) or ATP (Fig. 1A, lanes 12–16). After 30 min, differences in the patterns of residual Ssa1p and the 55-, 46-, 43-, 36-, and 33-kDa fragments were most apparent (Fig. 1A, lanes 5, 10, and 15). The amount of each fragment was also quantified using densitometry (e.g. 33-kDa fragment, Fig. 1B). In the absence of nucleotides, the pattern was characterized by the presence of multiple bands. In contrast, the ATP-dependent pattern was characterized by a 46-kDa band with a 36-kDa fragment, a visible 36-kDa fragment, a prominent 33-kDa fragment (Fig. 1A, lane 5). In the presence of ADP, the 46-kDa band was also visible; however, the pattern was distinguished by 50% more residual Ssa1p, 3-fold less of the 36-kDa fragment, and 2-fold less of the 33-kDa fragment (Fig. 1A, lane 10 and Fig. 1B). In contrast, the ATP-dependent pattern was characterized by a 46-kDa band with a ratio greater than one (Fig. 1A, lane 15). Furthermore, in the presence of ATP the amount of residual Ssa1p was 3-fold more and that of the 33-kDa fragment was 14-fold less than that in the absence of nucleotides (Fig. 1A, lanes 5 and 15, and Fig. 1B). The ATP-dependent pattern was also characterized by a 55-kDa fragment that was about 7-fold more abundant (Fig. 1A, lane 15) than that produced either in the absence of nucleotides (Fig. 1A, lane 5) or in the presence of ADP (Fig. 1A, lane 10). Together, these proteolytic patterns suggest that Ssa1p adopts at least three distinct conformations, nucleotide-free, ADP-dependent, and ATP-dependent.

Conformational Changes in the Nucleotide and Polypeptide Binding Domains Are Coupled and Require K$^+$—K$^{2+}$ was shown to promote dissociation of polypeptides in the presence of Mg$^{2+}$.
of ATP (16) and to occupy two sites in the ATP binding pocket of Hsc70 (33). In contrast, Na\(^+\) stabilized Hsp70-polypeptide complexes (16) and made fewer contacts than K\(^+\) in Hsc70’s active site (33). Surprisingly, the crystallographic analysis of the ATP binding domain revealed no large difference in structure between the Na\(^+\) and K\(^+\) forms (33). To test whether partial proteolysis of Ssa1p might reveal K\(^+\)-dependent conformational changes, we dialyzed Ssa1p against either K\(^+\) or Na\(^+\) containing buffers and then digested it with trypsin in the presence or absence of nucleotides (Fig. 2). In the presence of K\(^+\), Ssa1p adopted one of the three forms described above (Fig. 2, lanes 2–4). However, in the presence of Na\(^+\), Ssa1p was less responsive to ATP. The ADP- (lane 7) and ATP-dependent (lane 8) patterns were essentially identical to each other having a very low 46.43-kDa ratio, a negligible amount of the 55-kDa fragment, and a prominent 33-kDa fragment. This suggests that ATP is less able to alter the conformation of Ssa1p in the presence of Na\(^+\). In contrast, K\(^+\) yielded very similar ADP-dependent patterns (Fig. 2, lanes 3 and 7). The proteolytic patterns of nucleotide-free Ssa1p in the presence of either K\(^+\) or Na\(^+\) were essentially identical to each other suggesting that these monovalent cations do not independently alter the conformation (Fig. 2, lanes 2 and 6). Together, these results indicate that K\(^+\) is required for complete ATP-induced conformational changes of Ssa1p.

Ca\(^{2+}\) was previously shown to promote low levels of autophosphorylation of Hsp70s (35–37). It occupies a site in the ATP binding pocket distinct from that of Mg\(^{2+}\) without changing the overall structure of the ATPase domain (34). To test whether Ca\(^{2+}\)-dependent conformational changes might be revealed by proteolytic digestion, we treated Ssa1p with trypsin in the presence of Ca\(^{2+}\) or Mg\(^{2+}\) and different nucleotides.

To map the location of the conformational changes, we determined which fragments bind to ATP-agarose and which are recognized by a polyclonal antibody raised against a synthetic peptide comprising the last 13 amino acid residues of Ssa1p (Fig. 3). The 55-, 46-, and 43-kDa fragments, but not the 36- and 33-kDa fragments, bound to ATP-agarose (data not shown). Amino-terminal sequencing revealed that, like Ssa1p, the amino termini of the 55-, 46-, and 43-kDa fragments are blocked. The antibody recognized Ssa1p and the 36-, 33-, and 20-kDa fragments (Fig. 3, lanes 1–4). Together these results indicate that the 55-, 46-, and 43-kDa fragments are amino-terminal and contain the ATP binding domain. The 36-, 33-, and 20-kDa fragments are derived from the carboxyl-terminal domain that binds polypeptides.

![Fig. 1](http://www.jbc.org/)

**Fig. 1.** Nucleotide-dependent conformations of Ssa1p. A, Ssa1p was digested with trypsin in Buffer KCl-50 containing 2 mM MgCl\(_2\) for the indicated times in the absence of nucleotides (lanes 1–6) or in the presence of 1 mM of either MgADP (lanes 7–11) or MgATP (lanes 12–16). Reaction products were separated on 12% SDS-PAGE gels and stained with Coomassie Blue. Numbers at the right indicate the molecular masses (kDa) and migrations of standards. B, Ssa1p and its fragments at each time point were quantified using densitometry. The relative amount of the 33-kDa fragment was plotted as a function of time.

![Fig. 2](http://www.jbc.org/)

**Fig. 2.** Conformational coupling between nucleotide and polypeptide binding domains requires K\(^+\). Ssa1p was incubated alone (lanes 1 and 5) or with trypsin (lanes 2–4 and 6–8) for 30 min in Buffer KCl-50 (lanes 1–4) or NaCl-50 (lanes 5–8) containing 2 mM MgCl\(_2\), and either no nucleotides (lanes 2 and 6), 1 mM MgADP (lanes 3 and 7), or 1 mM MgATP (lanes 4 and 8). Reaction products were separated electrophoretically as described in Fig. 1 legend.
the cleavage sites of the 36- and 33-kDa fragments reside in the region between the ATP binding site and the polypeptide binding domain, whereas that of the 20-kDa fragment resides within the polypeptide binding domain. The relative amounts of the carboxyl-terminal fragments were nucleotide-dependent. The 36- and 33-kDa fragments were most prominent in the absence of nucleotides (Fig. 3, lane 2), whereas the 20-kDa fragment was most prominent in the presence of ATP (Fig. 3, lane 4). In the presence of Na\(^{+}\) and ATP, the 20-kDa fragment was not detected (data not shown). The patterns of the carboxyl-terminal proteolytic fragments provide additional support for the idea that Ssa1p adopts at least three distinct conformations. Furthermore, nucleotide-induced changes in the polypeptide binding domain suggest that the conformations of these domains are coupled as are their functions and that the coupling requires K\(^{+}\).

Nonhydrolyzable ATP Analogs Yield an ADP-dependent Conformation—To determine whether ATP hydrolysis is required to change the conformation of Ssa1p, we digested it with trypsin in the presence of nonhydrolyzable analogs of ATP. The proteolytic patterns of Ssa1p in the presence of ATP\(\gamma\)S or AMP-PNP were very similar and more closely resembled the ADP-dependent pattern than either the nucleotide-deficient or the ATP-dependent patterns (Fig. 4, lanes 2–6). The similarities between ADP- and nonhydrolyzable ATP analog-dependent patterns included the amount of resistance of Ssa1p to proteolysis, the 46:43-kDa ratio, and the abundance of the 33-kDa fragment. Identical patterns were obtained using ATP\(\gamma\)S and AMP-PNP that were separated by ion exchange chromatography from low amounts of ADP in the commercial preparations used above (data not shown). These results suggest that Ssa1p adopts the ADP-dependent conformation in the presence of nonhydrolyzable analogs of ATP. Because nonhydrolyzable analogs bind weakly to Hsp70s (28) and do not promote release of bound polypeptides (16), our data support the suggestion (26) that these analogs do not sufficiently resemble ATP to yield ATP-dependent conformational changes.

We examined conformational changes of Ssa1p during steady-state hydrolysis of ATP. Under these conditions, Hsp70s were previously shown to be predominantly in the ATP-bound state because the rate-determining step is cleavage of \(\gamma\)-phosphate (10, 19). Thus, ATP-dependent conformational changes of Ssa1p described above most likely result from binding, not hydrolysis, of ATP.

Polypeptide Substrates Alter the Conformations of the Nucleotide and Polypeptide Binding Domains—We showed above that binding of nucleotides affects the conformations of the nucleotide and polypeptide binding domains. To test whether polypeptide binding also affects the conformations of these domains, we measured changes in the pattern of Ssa1p's tryptic fragments in the presence of nonhydrolyzable ATP analogs of ATP. Because nonhydrolyzable ATP analogs bind weakly to Hsp70s (28) and do not promote release of bound polypeptides (16), our data support the suggestion (26) that these analogs do not sufficiently resemble ATP to yield ATP-dependent conformational changes.
digestion, increased the amounts of the 55- and 33-kDa fragments 5- and 1.8-fold, respectively, and decreased the amount of the 46-kDa fragment 3.1-fold (Fig. 5, A–C, compare lanes 3 and 6). In contrast to the pattern obtained in the presence of ATP alone, the pattern obtained in the presence of both ATP and Pep70 was characterized by 20% less residual Ssa1p, 1.5-fold more of the 55-kDa fragment, a 46:43-kDa ratio of less than one, and 3.7-fold more of the 33-kDa fragment (Fig. 5, A–C, compare lanes 4 and 7). A 46:43-kDa ratio of less than one and a prominent 33-kDa fragment are features resembling those of the ADP-dependent pattern (Fig. 5, A and C, lane 3). The ability of Pep70 to stimulate Ssa1p's ATPase activity may yield more bound ADP and, therefore, a more ADP-like conformation. A control polypeptide (Pro) that poorly stimulates Ssa1p's ATPase activity does not contain tryptic-sensitive sites did not greatly alter the proteolytic patterns of Ssa1p in the absence or presence of nucleotides (Fig. 5, A–C, compare lanes 2-4 and 8-10). Together, these results suggest that Pep70 alters the conformations of both the nucleotide and polypeptide binding domains of Ssa1p in the presence or absence of nucleotides. Furthermore, they suggest the previously described functional coupling of these domains is also reflected in conformational changes in both domains.

**DISCUSSION**

We showed above that the conformation of the yeast cytosolic Hsp70 Ssa1p is sensitive to nucleotides and a polypeptide substrate. Ssa1p, like Bip (21) and DnaK (26), adopts three distinct conformations, nucleotide-free, ATP-dependent, and ADP-dependent. Complete ATP-dependent conformational changes require K\(^{+}\) and Mg\(^{2+}\). Na\(^{+}\) and Ca\(^{2+}\) cannot substitute for K\(^{+}\) and Mg\(^{2+}\), respectively. Our studies extend previous studies of nucleotide-dependent conformational changes of eukaryotic 70-kDa chaperones (21, 27) by localizing the changes to specific domains. Binding of either nucleotides or polypeptides alters the conformations of both the nucleotide and polypeptide binding domains. The results demonstrate for the first time that coupling of conformational changes between the nucleotide and polypeptide binding domains requires K\(^{+}\). Nucleotide- and polypeptide-dependent changes in the oligomeric structure of Ssa1p (31, 32)\(^3\) may also contribute to changes in its tryptic patterns.

Complete nucleotide-dependent conformational changes of Ssa1p were sensitive to monovalent and divalent cations. We showed above that K\(^{+}\) allows ATP-dependent conformational changes. K\(^{+}\), but not Na\(^{+}\), was previously shown to allow rapid dissociation of bound polypeptides upon ATP binding (16) and promote optimal activity of Hsc70 (22). Crystallographic studies revealed that two K\(^{+}\) ions bind at different positions (sites 1 and 2) in the active site of Hsc70 and make various contacts with the protein, bound nucleotide, and Mg\(^{2+}\) and its water ligands (33). In the presence of Na\(^{+}\), site 1 contains one Na\(^{+}\) ion and site 2 contains a Na\(^{+}\)-H\(_2\)O pair (33). At site 1 of Hsc70, either K\(^{+}\) or Na\(^{+}\) interacts with P\(_{\beta}\) oxygen, an oxygen of Asp-10 (Ssa1p-Asp-8), the carbonyl of Tyr-15 (Ssa1p-Tyr-13) and H\(_2\)O, but only K\(^{+}\) interacts with the P\(_{\alpha}\)-P\(_{\beta}\) and P\(_{\beta}\) oxygen. At site 2, either ion interacts with P\(_{\alpha}\) oxygen, an oxygen of Thr-204 (Ssa1p-Thr-201) and water, but only K\(^{+}\) interacts with two oxygens of Asp-199 (Ssa1p-Asp-196), an oxygen of Asp-206 (Ssa1p-Asp-203), and the carbonyl of Thr-204 (Ssa1p-Thr-201). Our results suggest that one or both K\(^{+}\) ions may transmit information to both domains about the identity of bound nucleotide. Interactions at site 2, in which the monovalent cation interacts with P\(_{\alpha}\), (33) may be particularly important in this regard. Our results showing that the proteolytic digestion of Ssa1p substrates alters the conformations of both the nucleotides and polypeptide binding domains. These results are consistent with the notion that nucleotide binding changes the conformation of the nucleotide-binding domain, and that this change is transmitted to the substrate-binding domain.

\(^3\) N. Wang and W. Chirico, unpublished observations.
patterns of nucleotide-free Ssa1p in the presence of K⁺ and Na⁺ are virtually identical to each other suggest that monovalent cations alone do not alter the conformation but contribute to ATP-dependent conformational changes. Complete ATP-dependent changes required Mg²⁺; however, neither Mg²⁺ nor other divalent cations tested independently altered Ssa1p's conformation. These results suggest that Mg²⁺ also mediates nucleotide-dependent conformational changes. ATP-dependent conformational changes of Ssa1p in the presence of Ca²⁺ were incomplete and suggest that partial conformational changes may accompany Ca²⁺-dependent autophosphorylation of serine/threonine residues in the active sites of other Hsp70s (35–37).

Nonhydrolyzable analogs of ATP, such as ATPγS and AMP-PNP, yielded an Ssa1p conformation essentially identical to the ADP-dependent conformation. Similarly, nonhydrolyzable analogs induced an ADP-dependent conformation of DnaK (26). Although this tends to support the idea that hydrolysis, not simply binding, of ATP is required for ATP-dependent conformational changes, recent reports indicate that Hsp70s may not recognize these compounds as good ATP analogs. They bind to Hsp70s with low affinity (28) and do not promote dissociation of bound polypeptides (16). During steady-state hydrolysis of ATP, Hsp70s are predominantly in the ATP-bound state because γ-phosphate cleavage is rate-limiting (10, 19). In the context of these reports, conformational changes of Ssa1p described above during steady-state hydrolysis of ATP are most likely mediated by binding, not hydrolysis, of ATP.

Comparing the time courses of tryptic digestion of various Hsp70s in the absence of polypeptide substrates reveals that ATP protects Ssa1p and other Hsp70s located in the cytosol (27) and endoplasmic reticulum (21), but renders DnaK (25, 26) more susceptible to proteolysis. Such a difference in susceptibility to proteolysis in the presence of ATP suggests at least one conformational difference between these two classes of Hsp70 chaperones. However, the overall conformations of their nucleotide-dependent forms are strikingly similar as exemplified by those of Ssa1p and DnaK. The size, domain position, and change in abundance in response to nucleotides of the 55-, 46-, 43-, 36-, 33-, and 20-kDa fragments of Ssa1p are nearly identical to those of DnaK (25, 26). Of primary importance is that binding of ATP to Ssa1p changes the conformation of its polypeptide binding domain as recently reported for DnaK (26). This indicates that conformational coupling of the nucleotide and polypeptide domains is conserved in the DnaK and Ssa1p polypeptides. The increase in the amount of Ssa1p's 20-kDa (DnaK-21 kDa) carboxyl-terminal fragment in the presence of ATP suggests that the polypeptide binding domain is more accessible to trypsin and, therefore, in a more open conformation. Such a conformation is consistent with the finding that ATP accelerates binding and release of polypeptides (17).

The amount of this 20-kDa fragment produced in the presence of ADP was negligible. Decreased exposure of this domain in the presence of ADP may contribute to slower release of bound polypeptides.

Although nucleotide-dependent changes in tryptic patterns of Ssa1p and DnaK are nearly identical to each other in the absence of polypeptide substrates, significant differences emerge in the presence of polypeptides. We showed that Pep70 alters the conformations of both the nucleotide and polypeptide binding domains of all three forms of Ssa1p, nucleotide-free, ADP-dependent, and ATP-dependent. In contrast, DnaK's conformation in the absence of nucleotides was unaffected by peptide C (26) even though they formed a stable complex (10). Furthermore, DnaK's conformation in the presence of ATP and peptide C resembled the ADP-dependent conformation (26).

The ADP-like conformation may have resulted from more bound ADP caused by peptide C-dependent increase in either DnaK's ATPase activity or the level of protection of trypsin-sensitive sites (26). We cannot exclude the possibility that the differences in polypeptide-dependent conformational changes between Ssa1p and DnaK are peptide-specific. Together, conformational changes of Ssa1p described above and those of DnaK previously described (26) indicate that both domains of these Hsp70 chaperones are coupled but that their interactions may be regulated differently. The lack of a requirement for a GrpE-like factor in the Hsp70-based protein folding system in the euakaryotic cytosol further supports the idea that its reaction cycle differs from that of the DnaK-based system of E. coli (8, 13).

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