Assessment of the ATP Binding Properties of Hsp90*

(Ursula Jakob§, Thomas Scheibel†, Suchira Bose‡, Jochen Reinstein†, and Johannes Buchner§‡)

From the §Institut für Biophysik und Physikalische Biochemie, Universität Regensburg, 93040 Regensburg and the †Max-Planck-Institut für molekulare Physiologie, Abteilung physikalische Biochemie, Reinfelddamm 201, 44139 Dortmund, Federal Republic of Germany

Hsp90, one of the most prominent proteins in eucaryotic cells under physiological and stress conditions, chaperones protein folding reactions in an ATP-independent way. Surprisingly, ATP binding and ATPase activity of Hsp90 has been reported by several groups. To clarify this important issue, we have reinvestigated the potential ATP binding properties and ATPase activity of highly purified Hsp90 using a number of different techniques.

Hsp90 was compared to the well characterized ATP-binding chaperone Hsc70 and to two control proteins, immunoglobulin G and bovine serum albumin, that are known to not bind ATP. Hsp90 behaved very similarly to the non-ATP-binding proteins and very differently from the ATP-binding protein Hsc70. Like bovine serum albumin and immunoglobulin G, Hsp90 (i) did not bind to immobilized ATP, (ii) could not be specifically photocross-linked with azido-ATP, (iii) failed to exhibit significant changes in intrinsic protein fluorescence upon ATP addition, and (iv) did not bind to three fluorescent ADP analogues. In contrast, Hsc70 strongly bound ATP and ADP, specifically cross-linked with azido-ATP, and exhibited major shifts in fluorescence upon addition of ATP. Finally, reexamination of the amino acid sequence of Hsp90 failed to reveal any significant homologies to known ATP-binding motifs. Taken together, we conclude that highly purified Hsp90 does not bind ATP. Weak ATPase activities associated with Hsp90 preparations may be due to minor impurities or kinases copurifying with Hsp90.

Cells respond to external stresses such as a sudden increase in temperature with the synthesis of a distinct set of proteins called heat shock or stress proteins (Nover, 1991). The predominant classes of stress proteins including GroE, Hsc70, Hsp90, and small Hsps have been implicated in protein folding as molecular chaperones (Morimoto et al., 1994; Buchner, 1996). While the precise molecular mechanism of these chaperones is still under extensive investigation, the ATP dependence of chaperone-mediated protein folding is clearly the hallmark of the GroE and Hsc70 class of stress proteins. Both the ATPase activity of these proteins and its influence on assisted protein folding have been analyzed in detail (cf. Morimoto et al., 1994). In contrast, conflicting evidence exists concerning Hsp90's ATP binding properties and ATPase activity. Hsp90 is one of the most abundant proteins in the eucaryotic cell, even at physiological conditions. In complex with other effector proteins such as Hsc70 and prolyl isomerases, Hsp90 has been implicated as a molecular chaperone in the maturation of specific protein substrates such as steroid receptors and kinases in vivo (Pratt, 1993; Smith et al., 1993; Jakob and Buchner, 1994; Buchner, 1996). Interestingly, assembly of these complexes with substrate proteins has been found to be ATP-dependent (Pratt, 1993; Smith et al., 1993). However, since Hsc70 is also involved in the formation of these high molecular weight assemblies, it is not yet clear which of the Hsps is responsible for the ATP requirement. More recently, using in vitro folding and unfolding assays, it has been demonstrated that Hsp90 may be a general cytosolic chaperone under physiological (Wiech et al., 1992; Shaknovich et al., 1992; Shue and Kohlh, 1994) and heat shock conditions (Jakob et al., 1995a; Schumacher et al., 1994). These chaperone functions as well as interactions of Hsp90 with estrogen receptors (Ianno et al., 1994) were found to be ATP-independent. In contrast, binding of ATP to Hsp90 has been reported (Csermely and Kahn, 1991). Binding of ATP was suggested to result in conformational changes of Hsp90 (Csermely et al., 1993), which in turn would affect the interaction with other proteins (Kellermayer and Csermely, 1995). In addition evidence was presented suggesting that some purified Hsp90 preparations exhibit potent peptide stimulated ATPase activity with high turnover numbers (Nadeau et al., 1992; 1993). However, Hsp90 purified from other species did not show ATPase activity (Wiech et al., 1993; Nardai et al., 1995).

Whether Hsp90 acts in an ATP-dependent or independent way is of crucial importance for understanding the molecular mechanism of this chaperone. Therefore we have examined the ATP binding properties of Hsp90 in detail using several independent experimental approaches. By including known ATP-binding or non-ATP-binding proteins as positive and negative controls, we were able to show that several of the methods employed previously are not suited to unambiguously demonstrate ATP binding. Those methods that turned out to be reliable demonstrate that Hsp90 does not bind ATP.

MATERIALS AND METHODS

Proteins—Hsp90 and Hsc70 from bovine pancreas as well as Hsp90 from yeast and Escherichia coli were purified as described previously (Wiech et al., 1993; Jakob et al., 1995a, 1995b). In addition, heparin-Sepharose was used as a final step in the purification of Hsp90 (Olsson et al., 1995). The protein concentrations of bovine Hsp90 and Hsc70 were determined according to Bradford (1976) using bovine serum
albumin as a standard. Yeast Hsp90 concentrations were obtained using the published extinction coefficient of 0.73 for a 0.1% solution at 280 nm (J. akob et al., 1995a). Mitochondrial citrate synthase (CS) (EC 1.3.1.7), BSA, RNase A, MAK33 IgG, and the respective Fab fragment were obtained from Boehringer Mannheim GmbH.

Chemicals—C8-ATP-agarose was purchased from Sigma, 8-azido-[32P]ATP from ICN and yeast Hsp90 protein was from Boehringer Mannheim GmbH. Polyethyleneimine-cellulose was obtained from Schleicher & Schüll.

Analysis of the Nucleotide Content of Hsp90—Nucleotides were analyzed by reverse phase chromatography with a 2ml C18 column (Bishop). Detection was at 254 nm. The isolectric elution system consisted of 50 mM potassium phosphate, pH 7.0, for the analysis of AMP/ADP/ATP and 100 mM potassium phosphate, pH 6.8, 10 mM tetrabutylammonium bromide, [NC(H2)4]Br, and 8% (v/v) acetonitrile for the analysis of GMP/GTP/GTP.

Average retention times for the adenosine nucleotides were 3.3 min (ATP), 3.9 min (ADP), and 4.8 min (AMP) at a flow rate of 2 ml/min at room temperature. The retention times for the guanosine nucleotides were 4.7 min (GMP), 3.4 min (GDP), and 2.5 min (GTP) at a flow rate of 1.5 ml/min.

Prior to injection, the protein was denatured to dissociate any bound nucleotides by the addition of 2 μl of 1 M HClO4 to 20 μl of protein solution. The solution was then kept on ice for 1 min, and 28 μl of 2 M potassium acetate was added to reach neutral pH. The sample was then centrifuged at 5000 rpm for 1 min, and 10 μl of the supernatant was analyzed for nucleotide content as described above.

Chaperone Assay—The influence of Hsp90 and Hsc70 on the thermal aggregation process of CS at 43°C was monitored in the absence or presence of 1 mM MgATP as described previously (J. akob et al., 1995a).

ATP-agarose—1 ml of ATP-agarose was used in a batch binding procedure at 4°C in Eppendorf tubes. Each batch was equilibrated with buffer A (40 mM HEPES, pH 7.5, containing 20 mM KCl, 5% glycerol and 5 mM MgCl2). Equilibration was performed by resuspending the column material in 2 resin volumes of equilibration buffer, mixing it on a rotating shaker for 10 min, and then centrifuging it at 14,000 rpm. The supernatant was discarded, and the procedure was repeated five times. 200 μg yeast Hsp90 or bovine Hsc70 were mixed with 5 mg/ml IgG carrier protein and made up to 1 ml with buffer A. The protein mixtures were added to the equilibrated ATP-agarose, and these were incubated for 24 h rotating at 4°C. The samples were then centrifuged at 14,000 rpm for 10 min. The supernatant was retained on ice for further analysis. The ATP-agarose was then washed five times with buffer A. For each wash, the ATP-agarose was incubated in 1 resin volume of buffer A for 10 min on a rotating shaker and then centrifuged at 14,000 rpm. After each centrifugation, the supernatant was retained on ice (wash 1). The agarose pellet was then washed five times as described with high salt buffer (buffer A + 0.5 mM KCl), and each time the supernatant was stored on ice at 4°C (wash 2). Dissociation of specifically bound protein was initiated by the addition of buffer A supplemented with 0.5 mM KCl and 5 mM ATP. This procedure was repeated five times. Finally, the ATP-agarose was washed with 2 resin volumes of 7 M urea/1 M NaCl/5 mM MgCl2. Samples were analyzed on 10% SDS-PAGE. All gels were silver-stained.

Cross-linking with 8-Azido-[32P]ATP—Yeast Hsp90 (5 μg) or BSA (5 μg) were diluted into 40 mM HEPES-KOH, 10 mM MgCl2, 40 μM 8-azido-[32P]ATP, pH 7.5, which was supplemented with various concentrations of non-radioactive ATP. The final volume was 20 μl. After a 10-min incubation at room temperature, samples were placed on ice and cross-linking was induced by a 6 min UV radiation (256 nm) at a distance of 4 cm. After addition of 5 μl of 5% Laemmli buffer (+5% β-mercaptoethanol), the samples were incubated for 5 min at 95°C and applied to a 10% SDS-PAGE. The gels were stained with Coomassie Blue-staining.

RESULTS

Purified Hsp90 is Free of Nucleotides—Hsp90 from different sources (bovine, yeast, E. coli) was purified by column chromatography to homogeneity (>98%) as determined by densitometry of Coomassie Blue-stained gels. To examine whether purified Hsp90 contains bound nucleotide, we determined the A280/260 ratio. UV spectra of Hsp90 gave typically an A280/260 ratio of 1.8-1.9, suggesting that the protein samples were essentially nucleotide-free. To confirm this result, we analyzed the nucleotide content of purified yeast Hsp90 by reverse phase high performance liquid chromatography under denaturing conditions. Neither adenosine nor guanosine nucleotides could be detected (data not shown). Together these results demonstrate that, unlike other ATP-dependent chaperones such as GroE...
The Chaperone Activity of Hsp90 Is ATP-independent—To compare the influence of ATP on the chaperone activity of Hsp90 with that of Hsc70, a known ATP-dependent chaperone, we performed in vitro unfolding assays in the presence or absence of ATP with both proteins. As an assay system, we used the thermal unfolding of CS. We had shown previously that Hsp90 suppresses the unspecific aggregation during thermal unfolding and apparently stabilizes CS against inactivation by binding to early unfolding intermediates (Jakob et al., 1995a). As shown in Fig. 1, 1 mM MgATP has no influence on the suppression of CS aggregation by Hsp90. In contrast, suppression of aggregation by Hsc70 is strongly influenced by ATP. This suggests fundamentally different mechanisms of action.

In the absence of ATP, Hsc70 effectively prevents aggregation by binding to the nonnative protein. In the presence of ATP, however, the protective effect of Hsc70 is lost possibly due to the rapid release and subsequent aggregation of bound protein. Similar effects were observed previously for the chaperone GroE under heat shock conditions (Höfl-Neugebauer et al., 1992).

Hsp90 Does Not Bind to ATP-agarose—Binding to immobilized ATP has been taken as evidence for the association of Hsp90 with nucleotides (Csermely and Kahn, 1991). We addressed the question whether purified Hsp90 binds to immobilized ATP by incubating Hsp90 with ATP-agarose. Initial experiments showed that the addition of the carrier protein IgG was necessary to prevent the unspecific interaction of Hsp90 with the agarose matrix. IgG was shown not to influence the specific binding of the control protein Hsc70 (Fig. 2). To detect specific binding of proteins to ATP-agarose, elution of the bound proteins was induced by competing with free ATP after extensive salt washes. These experiments confirmed that Hsc70 binds to immobilized ATP and can be specifically eluted by the addition of free ATP. However, specific binding of Hsp90 to ATP-agarose could not be detected. The total amount of Hsp90 applied was found in the flow-through and the wash fractions. Addition of divalent cations (Mg$^{2+}$, Ca$^{2+}$, and Mn$^{2+}$) to the incubation buffer did not change the results (data not shown). Therefore, we conclude that Hsp90 does not bind tightly to immobilized ATP.

Azido-ATP Cross-linking to Hsp90 Is Nonspecific—Previously, cross-linking of radiolabeled azido-ATP to Hsp90 has been detected (Csermely and Kahn, 1991; Shaknovich et al., 1992). To investigate whether this represents specific interactions, we compared the cross-linking behavior of Hsp90 with that of the known ATP-binding protein Hsc70 and the non-ATP-binding protein BSA. Competition with cold ATP was used to check the specificity of the cross-linking reaction.

As shown in Fig. 3, cross-linking of azido-ATP to Hsp90 was observed under the conditions used. However, prior to cross-linking we could not compete for azido-ATP binding by addition of a large excess of cold ATP. This shows that azido-ATP and ATP bind to different sites on Hsp90, or that azido-ATP binds with a much higher affinity than normal ATP, or that azido-ATP simply cross-links nonspecifically with Hsp90. Thus the photocross-linking of azido-ATP to Hsp90 cannot be taken as a reliable indicator of ATP binding. This view was reinforced by the finding that BSA (Fig. 3) and Fab fragment (data not shown) photocross-linked to azido-ATP in a manner similar to Hsp90. In contrast, the azido-ATP labeling observed with Hsc70 could be competed with micromolar concentrations of cold ATP.

Since it is known that long-lived reaction intermediates form upon the UV-activation of azido-ATP (Todd et al., 1995), the photocross-linking of azido-ATP with Hsp90 needs to be interpreted with extreme caution.

ATP Does Not Stabilize Hsp90—Substrate or cofactor binding leads to an often dramatic increase in the stabilization
energy of the respective protein. This was shown recently for the chaperone DnaK, where binding of MgATP significantly stabilized the protein as determined by monitoring GdmCl-induced unfolding transitions (Palleros et al., 1993). Using this assay system, we analyzed whether the addition of MgATP exhibits similar effects on the stability of Hsp90. In the absence of MgATP an unfolding transition identical to that observed previously (J. Jakob et al., 1993b) was obtained (Fig. 4). The presence of MgATP did not change the unfolding behavior of Hsp90, indicating that Hsp90 is not stabilized by ATP.

Addition of ATP Does Not Influence the Intrinsic Fluorescence of Hsp90—Previously it had been reported that addition of ATP decreases the intrinsic fluorescence of Hsp90 (Cserrmely et al., 1993). We readdressed this question again by comparing Hsp90 with the ATP-binding protein Hsc70 and the non-ATP-binding proteins BSA and Fab fragment concerning changes in the intrinsic fluorescence in the presence of ATP.

We found that addition of increasing concentrations of ATP results in a decrease of the intrinsic protein fluorescence independent of the protein used. This is due to the “inner filter effect” of ATP, i.e. the light absorption of ATP leading to a decreased fluorescence emission. It is possible to correct fluorescence spectra recorded in the presence of ATP for this non-specific effect of the nucleotide, as described by Birdsall et al. (1983). This allows to detect potential specific influences of ATP on the intrinsic fluorescence of proteins. After correction, as shown in Fig. 5, a slight decrease in fluorescence accounting for about 5% of the signal was observed with all proteins studied. Most importantly, however, both Hsp90 and the non-ATP-binding protein Fab gave a constant fluorescence signal at ATP concentrations, ranging from 2 μM to 2.5 mM, while the intrinsic fluorescence of Hsc70 increased by more than 10%, reflecting the specific binding of ATP. In the case of BSA, the amplitude decreased by about 25% upon increasing ATP concentrations, most likely due to unspecific interaction with ATP.

Fluorescence-labeled ADP Does Not Bind to Hsp90—Binding of ATP to proteins does not necessarily lead to changes in the intrinsic fluorescence of a protein. Therefore, we performed experiments in which we used fluorescent ATP analogues to monitor binding. Here, specific binding results in a fluorescence change of the label. Increasing amounts of Hsp90 were incubated with a fixed amount of the ligand and the fluorescence emission was recorded. The experiments showed that the fluorescence of a 0.4 μM solution of the nucleotide analogues MABA-ADP and MANT-ADP increases only slightly (by some 3% and 0%, respectively) when up to 0.85 μM Hsp90 was added (Fig. 6). In contrast, control experiments with DnaK (Hsc70 from E. coli) show the fluorescence of MABA-ADP to increase by some 250% when up to 0.8 μM of the ATP-dependent molecular chaperone was added (Fig. 6). This is indicative of a high affinity of DnaK for MABA-ATP. Furthermore, in this case cold ADP could compete for binding of the labeled analogue resulting in a constant fluorescence signal despite increasing MABA-ADP concentrations. The 3% increase in fluorescence emission of MABA-ADP observed in the presence of Hsp90 could not be competed by cold ADP. Similar experiments using the ADP analogue etheno-ADP showed only a minor fluorescence increase upon addition of Hsp90. Again, the small fluorescence change in the presence of Hsp90 could not be competed by cold ADP (data not shown).

Low ATPase Activity of Hsp90 Preparations Is Similar to Background Values Obtained for Other Proteins—In addition to ATP binding, previous reports also suggested that isolated Hsp90 may be an ATP hydrolyzing enzyme (Nadeau et al., 1991, 1992). We, however, had failed to detect any ATPase activity in highly purified bovine Hsp90 preparations (Wiech et al., 1993). Here, we have reinvestigated the ATPase activity of the yeast Hsp90 preparation, which we had been using for the ATP binding experiments. Using radiolabeled ATP we detected a low ATP hydrolyzing activity (Table I). This activity was severalfold lower than that of BiP, an endoplasmic member of the Hsp70 family, which possesses a very weak ATPase (Table I). We calculated that a potent phosphatase or kinase, such as casein kinase II present in levels as low as 0.01% in protein preparations could be responsible for this effect. To test if ATPase activity can be detected in highly purified commercially available proteins, we assayed for ATPase activity in “ultrapure RNase” (Table I). This preparation was found to contain similar levels of ATPase as our highly purified Hsp90. Furthermore, a purified DnaK mutant, which was shown to be catalytically inactive, gave values under steady state conditions that were similar to those obtained here for Hsp90. These results confirm earlier suggestions that the ATPase activity of Hsp90 from various Trypanosoma species could be due to minor impurities of the protein preparation (Shi et al., 1994).

Sequence Analysis of Hsp90 Does Not Reveal ATP Binding Motifs—Previous analysis of the sequence of murine Hsp90 showed the presence of a shortened version of the conserved

3 J. Reinstein, personal communication.
Walker type ATP binding motifs A and B (Csermely and Kahn, 1991). We addressed this question by searching the complete SwissProt data base for type A and B consensus sequences of ATP binding motifs (Walker et al., 1982; Chin et al., 1988). The search pattern used identified 622 sequences containing the complete type A motif. Most of these proteins are known ATPases or kinases. Hsp90 was not found using this search pattern. Chaperones identified in this search revealed a consensus sequence, which is slightly modified compared to that of kinases or ATP synthases (Table II, part A). Hsp90, unlike ATPases and kinases but like IgG or lysozyme, contained only the G-K-(T/S) motif, which is only part of the complete type A motif. In total, 8937 sequences (20.6% of all proteins in SwissProt data base) revealed this tripeptide sequence, reflecting the high statistical probability for the occurrence of this tripeptide sequence.

A selected set of proteins possessing either the correct type A consensus sequence or just the G-K-(T/S) tripeptide were screened for the type B consensus sequence. This motif is quite unspecific and thus is only a good indication for the presence of an ATP binding site if found together with a conserved type A sequence (Chin et al., 1988). Table II (part B) compares the presence of type A and type B binding motifs in proteins with ATP binding properties. While proteins containing both motifs are unambiguously binding ATP, those lacking type A sequences exhibit no known ATP binding activity. Hsp90 belongs to this group of proteins, thus making it rather unlikely that Hsp90 contains an ATP binding site of the Walker type.
non-ATP-binding proteins. We performed cross-linking experiments of the respective proteins with azido-ATP. Specific association of MgATP could only be detected for the ATP-dependent chaperone Hsc70. The observation that cross-linking of proteins with azido-ATP can also occur in a nonspecific way is in good agreement with a previous report by Todd and co-workers (1995). Nonspecific cross-linking is based on the formation of proteins with azido-ATP. We performed cross-linking experiments of the respective proteins with azido-ATP. Specific association of MgATP could only be detected for the ATP-dependent chaperone Hsc70. The observation that cross-linking of proteins with azido-ATP can also occur in a nonspecific way is in good agreement with a previous report by Todd and co-workers (1995). Nonspecific cross-linking is based on the formation of proteins with azido-ATP.

### Table II: Comparison of ATP-binding sequences

| Organism | Protein | Start | Sequence |
|----------|---------|-------|----------|
| A | Type A-consensus | A | G-X-X-X-X-X- G-K-T/S-X-X-X-X-X-V/I |
| B | Type B-consensus | H/R/K- | O-X-O-O-D/E |

**Type A and type B sequences denote the putative triphosphate- and adenine-binding sequences, respectively** (Walker et al., 1982; Chin et al., 1988). O represents hydrophobic residues. The data base SwissProt was used for alignment of protein sequences using FINDPATTERNS. The total number of examined sequences was 43,470. A total of 8937 sequences contain the GKT/S motif and 622 sequences a type A-consensus sequence. The selected sequences were then analyzed for the presence of a type B-consensus sequences using FINDPATTERNS.

**ATP Binding of Hsp90**

| Organism | Protein | Type A | Type B |
|----------|---------|--------|--------|
| H. sapiens | Serine/threonine-protein kinase | + | + |
| B. taurus | ATP-synthese α chain | + | + |
| S. cerevisiae | Hsp104 | +/- | + |
| A. thaliana | Cpn60 | +/- | + |
| B. taurus | PPI | +/- | + |
| E. electricus | Hemoglobin α chain | +/- | + |
| M. musculus | Ig heavy chain | - | - |
| H. sapiens | Hsp90 α | - | ? |
| S. scrofa | lysozyme c-1 | - | + |
| B. taurus | Hsc70 | +/ | + |

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**ATP Binding of Hsp90**

| Organism | Protein | Consensus | Type A | Type B | ATP binding |
|----------|---------|-----------|--------|--------|-------------|
| H. sapiens | Serine/threonine-protein kinase | + | + | + |
| B. taurus | ATP-synthese α chain | + | + | + |
| S. cerevisiae | Hsp104 | +/- | + | + |
| A. thaliana | Cpn60 | +/- | + | + |
| B. taurus | PPI | +/- | + | + |
| E. electricus | Hemoglobin α chain | +/- | + | + |
| M. musculus | Ig heavy chain | - | - | - |
| H. sapiens | Hsp90 α | - | ? | ? |
| S. scrofa | lysozyme c-1 | - | + | + |
| B. taurus | Hsc70 | +/- | + | + |

is an inactive DnaK variant, mutated so to eliminate its ATPase active site, which, even when purified according to different protocols, gave low ATPase activities similar to the ones obtained for Hsp90. Rates of <0.7 pmol·min⁻¹·mg⁻¹ apparently represent typical background values for ATPase activities in protein preparations. Thus extreme care is needed before low ATPase activities can be attributed to any particular purified protein.

Based on these results and considering the analysis of the amino acid sequence, which did not reveal significant similarity with known ATP-binding motifs, we can certainly exclude the proposed tight binding of ATP to Hsp90 (cf. Table III) as well as the high levels of ATPase activity of Hsp90, previously reported (Nadeau et al., 1992, 1993). Whether nucleotides can bind with very low affinity to Hsp90 cannot be completely ruled out based on negative results but in the light of the consistent data obtained with different assay systems this seems rather unlikely. In addition, we cannot completely exclude the possibility that binding of additional cofactors (partner proteins, peptides, transition state metals) may modulate the structure of Hsp90 to induce ATP binding. Addressing this question will require a more detailed understanding of the interaction of Hsp90 with these potential modulators.

In this context, the general question whether ATP hydrolysis is a necessary prerequisite for efficiently chaperoning protein folding arises. The increasing number of chaperones, whose
function does not require ATP such as small Hsps, DnaJ, SecB, PapD, and calnexin/calreticulin argues strongly against this notion. The functional mechanism seems to vary between different members of these ATP-independent chaperones; however, all of them recognize nonnative polypeptides and are able to bind and release them in the absence of ATP. Although ATP and the co-chaperone GroES are required for the efficient release of nonnative proteins from the ATP-dependent chaperone GroEL under non-permissive folding conditions, it was recently demonstrated that the underlying cycles of binding and release are independent of ATP (Schmidt et al., 1994; Sparrer et al., 1996). Specifically, ATP decreases mainly the microscopic on-rate of GroEL for nonnative protein, which results in an efficient release of GroEL-bound protein because rebinding is prevented (Sparrer et al., 1996).

In the case of Hsp90, binding cycles for nonnative proteins similar to the observed ATP-independent binding cycles of GroEL have been postulated (Jakob et al., 1995a). It was further suggested that Hsp90 interacts preferentially with structurally nonnative proteins. The conversion to the native form can be induced either by ligand binding (in the case of receptors), by myristylation (in the case of kinases) or by a folding reaction (in the case of folding and unfolding intermediates). Since external factors seem to drive the final conversion of the reaction (in the case of folding and unfolding intermediates), the functional mechanism seems to vary between different members of the so-called “super chaperone complex,” what is then the role of Hsc70 and the partner proteins? Little is known about their function, but it was suggested that Hsc70 together with p60 mediates the formation of the Hsp90-Hsp56p23 steroid receptor complex. The mechanism of this ATP-driven complex formation is not yet understood, but Hsc70 seems to be the likely candidate responsible for the observed ATP requirement of this process. Modulators and cofactors like molybdate may also be involved (Johnson and Toft, 1995). Additionally, the idea has been proposed that Hsp90 and the Hsc70/DnaJ system share functions in the folding of proteins. In this scenario, the role of the ATP-independent Hsp90 would be to “hold” the nonnative protein, while Hsc70 in cooperation with DnaJ would “fold” the protein in an ATP-dependent way (Bohen et al., 1995).
