Nucleotides and Two Functional States of hsp90*

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Previous studies have demonstrated the ATP-dependent formation of a complex containing the heat shock protein hsp90, the unique hsp90 binding protein p23, and one of three high molecular weight immunophilins. In the present study, hsp90 and p23 are shown to form a complex that requires elevated temperature and ATP/Mg2+. Complex formation is strongly promoted by molybdate and by the nonionic detergent Nonidet P-40. ADP and the benzoquinone ansamycin, geldanamycin, are potent inhibitors of complex formation. The ATP-dependent process alters the state of hsp90, not p23, and influences the ability of hsp90 to bind to phenyl-Sepharose. Conversion of hsp90 to the ATP-bound state lowers its affinity for phenyl-Sepharose. These results show that hsp90 can exist in at least two functional states, one able to bind p23 and the other with a high affinity for hydrophobic resins. A model is presented where these states are dictated by the binding of either ATP or ADP.

The 90-kDa heat shock protein, hsp90, is an abundant cellular protein that appears to be essential for eukaryotic cell viability (1). The biochemical functions of hsp90 are still poorly understood, and most clues have come through studying its interaction with other proteins. While a variety of interactions have been described, the best understood of these is the binding of hsp90 to complexes of inactive steroid receptors (2-4). Such complexes with the progesterone receptor also include hsp70, one of three immunophilins, and a small 23-kDa protein, p23 (4, 5). The immunophilins, which are recognized by their ability to bind immunosuppressant drugs, include two FK506-binding proteins, FKBP52 and FKBP51 (FKBP54), and a protein that binds cyclosporin A called CyP-40. p23 is neither a heat shock protein nor an immunophilin. It is a ubiquitious, conserved phosphoprotein that is identified only by its association with hsp90 (6). p23 appears to be required for the in vitro assembly of receptor complexes for progesterone (7) and glucocorticoids (8).

More recently complexes of hsp90 have been isolated that contain the immunophilins FKBP52, FKBP51, and CyP-40 plus P23, indicating that these proteins also interact in the absence of steroid receptors and may represent a functional unit for the chaperoning or processing of substrate proteins (7, 9). The immunophilins appear to bind to hsp90 spontaneously (10-12) and to compete for a common site on hsp90 (13, 14).

However, binding of p23 in such complexes requires ATP, Mg2+, and elevated temperature, indicating that it is an active process (7, 9). These results indicate that hsp90 or p23 is capable of interacting with ATP in a way that influences its function. hsp90 has been reported to interact with ATP (15-18), to have an autophosphorylation activity (15, 18), and to be an ATPase (17, 18). However, these properties have not been universally observed and remain controversial (19).

Since the above studes on the binding of p23 to hsp90 were conducted in crude cell extracts, we wished to further characterize this binding using purified proteins. The present studies show that p23 does bind directly to hsp90 in a pure system through an ATP-dependent process. ATP appears to be utilized in converting hsp90 to a form capable of binding p23. Conversion of hsp90 to this form is potentiated dramatically by molybdate and is inhibited by ADP and by the hsp90 inhibitor, geldanamycin. A model is presented to explain the effects of these agents on two states of hsp90.

EXPERIMENTAL PROCEDURES

Materials—Mouse monoclonal antibody JJ3 was prepared against human p23 as described previously (6). Geldanamycin was obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, National Institutes of Health. The sources of hydrophobic resins were phenyl-Sepharose and phenyl-agarose from Sigma and agaroase containing octyl, heaxyl, pentyl, butyl, and ethyl groups from Miles-Yeda LTD. Adenosine 5’-O-(3-thiotriphosphate) (ATP'S) was from Sigma. Since this material is contaminated with ADP, it was treated with an ATP regeneration system (phosphocreatine/creatine kinase; see below) at 30 °C to convert all ADP to ATP. The creatine kinase was then removed by ultrafiltration. The nonionic detergent Nonidet P-40 was obtained from Sigma. This product is no longer available, but equivalent results were obtained using the closely related detergent Igepal CA-630 (Sigma).

Purification of p23—The bacterial expression and partial purification of human p23 have been described (7). Briefly, p23 is soluble in bacterial lysate containing 10 mM Tris, 1 mM EDTA, 10 mM thioglycerol, pH 7.5. After centrifugation, the lysate (15 ml) was fractionated on a 10-ml column of Mono Q by elution with a 0-0.5 m KCl gradient. The effluent containing p23 (approximately 90% pure) was dialyzed in 10 mM Tris, 150 mM NaCl, pH 7.5, and fractionated on phenyl-Sepharose (HP1600) using a Pharmacia FPLC system. p23 eluted as a uniform, pure protein during a gradient of descending salt concentration. The purified protein was dialyzed into 10 mM Tris, 1 mM EDTA, 100 mM KCl, and 10% glycerol, pH 7.4, and stored at -70 °C.

Purification of hsp90—hsp90 was prepared by the overexpression of human hsp90 in Sf9 cells using the system of Alnemri and Litwack (20). On the third day of infection in suspension cultures, the cells were harvested and lysed by sonication in four volumes of 20 mM Tris-HCl, pH 7.5, 20 mM KCl, 20 mM NaF, and 4 mM EDTA plus the protease inhibitors pepstatin (2 μg/ml), leupeptin (2 μg/ml), and 4-(2-aminoethyl) benzenesulfonyl fluoride (1 mM). This was fractionated on DEAE-cellulose by KCl gradient elution (0-0.5 M). Fractions containing hsp90 were identified by gel electrophoresis, pooled, dialyzed in 20 mM potassium phosphate, pH 7.5, 1 mM EDTA, 20 mM NaF, 5 mM β-mercaptoethanol, and applied to a hydroxylapatite column. Proteins were eluted with a 10-300 mM phosphate gradient. The sample from hydroxylapatite was dialyzed into 10 mM Tris, 1 mM EDTA, 10 mM NaF, 5 mM MgCl2, and stored at -70 °C. The sample from hydroxylapatite was dialyzed into 10 mM Tris, 1 mM EDTA, 10 mM NaF, 5 mM MgCl2, and stored at -70 °C.

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Fig. 1. The binding of purified p23 to hsp90. Purified p23 (~5 μg) plus hsp90 (~10 μg) were incubated together in 200 μl of buffer containing 10 mM Tris, pH 7.5, 1 mM dithiothreitol, 5 mM MgCl₂, and 50 mM KCl. Additional ingredients are indicated below. After 1 h at 30 °C, the samples were combined with 25 μl of protein A-Sepharose containing antibody JJ3 against p23 and incubated for 1 h at 4 °C. The resin pellets were washed, extracted, and analyzed by SDS gel electrophoresis. All tubes contained p23 and hsp90 with no additions (lane 1); with ATP (lane 2); with 5 mM ATP and 20 mM molybdate (lane 3); with molybdate (lane 4); with ATP and 10 mM NaF (lane 5); with ATP and 5 mM BeCl₂ plus 10 mM NaF (lane 6); with ATP, molybdate, and 0.01% Nonidet P-40 (lane 7); with 5 mM ATP and 0.01% Nonidet P-40 (lane 8); with molybdate and 0.01% Nonidet P-40 (lane 9); with ATP, molybdate, and 0.01% Nonidet P-40 without p23 (lane 10); p23 alone (lane 11); and hsp90 alone (lane 12).

RESULTS

hsp90 Binding to p23—Earlier studies showed that, in rabbit reticulocyte lysate, hsp90 and p23 form a complex that also contains immunophilins and also showed that ATP is required for the complex formation (7, 9). To test the direct interaction between hsp90 and p23, highly purified preparations of these two proteins were mixed and incubated under various conditions as shown in Fig. 1. After incubation, p23 was recovered by immune precipitation and analyzed by SDS-PAGE for the co-isolation of hsp90. No binding was observed upon simply mixing the two proteins (lane 1). The addition of 5 mM ATP promoted a slight amount of complex formation (lane 2), which was only slightly above a background level in the absence of p23.

The binding assay typically contained about 5 μg of p23 plus 5–10 μg of hsp90 in a volume of 200 μl consisting of 10 mM Tris, 5 mM MgCl₂, 1 mM dithiothreitol, 5 mM ATP, 20 mM NaF, 0.01% Nonidet P-40, pH 7.5. An ATP regeneration system was also normally included, consisting of 10 mM phosphocreatine and 7 units of creatine phosphokinase. After incubation for 30–60 min at 30 °C, the samples were chilled in ice and combined with 25 μl pellets of protein A-agarose loaded with approximately 20 μg of monoclonal antibody JJ3 against p23 (6). The samples were incubated for 1 h on ice with frequent mixing, and the resin pellets were then washed by suspension and centrifugation in four 1-ml volumes of 10 mM Tris, 1 mM EDTA, pH 7.5 (9). The adsorbed proteins were extracted into SDS sample buffer and resolved by SDS-PAGE as described previously (9).

Binding to Phenyl-Sepharose—Samples (200 μl) of hsp90 (5–10 μg) in 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM MgCl₂, and 1 mM dithiothreitol were treated at 30 °C as described in the legend to Fig. 8. They were then combined with 25-μl pellets of phenyl-Sepharose and incubated with frequent mixing at 4 °C for 30 min. The pellets were recovered by centrifugation, washed, and analyzed as described for p23 binding.

In additional studies, not shown, we have found that p23-hsp90 binding occurs readily in both Tris and phosphate buffers with a pH optimum of about 7.0. Little or no binding occurs above pH 8 or below pH 6. The binding is sensitive to ionic strength above that of 0.1 M KCl and is decreased by 50% at about 0.25 M KCl. Mg²⁺ is required and is effective in a broad range between 0.5 and 10 mM with an optimum at or above 3 mM.

The requirements for complex formation suggest that hsp90, p23, or both are being modified or activated to a binding state. In the presence of ATP and molybdate, we pretreated hsp90 or p23 alone for 10, 20, or 30 min at 30 °C and then chilled in ice, supplemented with p23 (lanes 2–4) or hsp90 (lanes 5–7), and incubated on ice for 30 min. p23 was then immune precipitated from the samples as described in Fig. 1 (lane 10). However, the addition of 20 mM molybdate, which is known to stabilize p23-hsp90-immunophilin complexes (9), enhanced complex formation quite dramatically (lane 3), an effect that was totally dependent on the presence of ATP (compare lanes 3 and 4). Two additional compounds, tungstate and beryllium fluoride, were also effective in enhancing the presence of hsp90-p23 complexes (lanes 5 and 6). These compounds are known to interact with phosphate binding sites on proteins (21), but their mechanism of action in the present case is unknown. Further stimulation of complex formation was observed by the addition of the nonionic detergent Nonidet P-40 at 0.01% (lane 7). An enhancement can be observed with Nonidet P-40 in the absence of molybdate (lane 8), but maximal binding is observed when both agents are present at their optimal concentrations (20 mM molybdate and 0.01% Nonidet P-40). ATP is still an absolute requirement under these conditions (lane 9). Lanes 11 and 12 show the amount and purity of p23 and hsp90 that were used. These results demonstrate a direct ATP-dependent interaction between p23 and hsp90 in the absence of other proteins. However, complex formation was not very favorable in the presence of ATP/Mg²⁺ alone, and molybdate and Nonidet P-40 apparently enhanced complex formation or provided a stabilizing condition for p23-hsp90 complexes.

The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; ATP-S, adenosine 5'-O-(thiotriphosphate); AMPPNP, 5'-adenylyl β,γ-imidodiphosphate.
has been pretreated at 30 °C with ATP and molybdate is then able to bind p23 in an ice bath (lanes 2–4) to a level that is comparable with the control having both proteins at 30 °C (lanes 8–10). No gain of binding activity is achieved by preincubation of p23 (lanes 5–7). This preincubation effect on hsp90 requires both the presence of ATP/Mg$^{2+}$ and molybdate (not shown). Therefore, it appears that these agents interact with hsp90 in a temperature-sensitive step to convert it to a form capable of binding to p23.

Fig. 1 shows that Nonidet P-40 had a positive effect on the binding of hsp90 to p23. The major effect of Nonidet P-40 appears to be on the rate of conversion of hsp90 to the active state as shown in Fig. 3. The rate of complex formation is relatively slow in the absence of detergent, taking 30–60 min at 30 °C for completion (lanes 1–6). However, in the presence of 0.01% Nonidet P-40, binding is essentially complete after 6 min at 30 °C. This dramatic influence of detergent is also observed using Triton X-100 at 0.1%. These detergents apparently act in some way to promote the conversion of hsp90 from one state to another.

Since some heat shock proteins, such as hsp70 and GroEl, have been shown to use both ATP and ADP as effectors of conformational change (22–24), we tested the effect of ADP in complex formation. Preliminary experiments showed that ADP could not replace ATP in promoting p23 binding. In fact, it is a potent inhibitor, as shown in Fig. 4. In this experiment, all samples were treated with 5 mM ATP plus 20 mM molybdate for 30 min at 30 °C, and the simultaneous additions of increasing concentrations of ADP were tested. The inhibition of hsp90-p23 complex formation is noticeable using 0.1 mM ADP and is complete when the concentration reaches 2 mM. Thus, ADP is a potent inhibitor of complex formation and appears to be effective at a 5–10-fold lower concentration than ATP.

Further tests on nucleotide specificity showed several nucleotides to be inactive, both as activators like ATP or as inhibitors like ADP. These nucleotides include AMP, GTP, CTP, and AMPPNP (results not shown). On the other hand, dATP was as effective as ATP and ATPγS was more effective than ATP, as illustrated in Fig. 5. This experiment concerns two issues: the dependence on nucleotide concentration and the influence of molybdate. The top two sets show the binding of hsp90 to p23 versus ATP concentration without or with molybdate. Only the hsp90 region of the gel is shown for convenience. As shown before, little binding occurs in the absence of molybdate, and binding is optimal with 5 mM ATP. No further binding is observed using higher ATP levels (not shown) and binding is about half-maximal at 1 mM ATP. This suggests that the affinity of hsp90 for ATP is relatively low but still in a physiological range. With ATPγS, complex formation goes to a higher maximum, and the amount of ATPγS needed for half-maximal complex formation is between 0.1 and 0.5 mM. The second important observation with this nucleotide is that it shows no requirement for molybdate. This suggests that the participation of molybdate requires a nucleotide that is readily metabolized. It should be noted that commercial ATPγS is contaminated with ADP, and the nucleotide preparation was not very effective until the ADP was converted to ATP (see “Experimental Procedures”).

Inhibition by Geldanamycin—Geldanamycin is a drug that has been shown by Whitesell et al. to bind to hsp90 and to block its interaction with pp60$^{src}$ (25). We have recently used geldanamycin to inhibit the formation of p23-hsp90 immunophilin complexes in rabbit reticulocyte lysate (9). A similar inhibition occurs in the purified system as shown in Fig. 6, where the addition of geldanamycin (GA) at 2 μg/ml completely blocked the binding of p23 to hsp90. Note that the additional protein bands observed with 1 μg/ml GA are not reproducible but are the result of incomplete washing. To gain further understanding of the effects of this drug we tested its
potency when added initially or after the conversion of hsp90 to the p23-binding state (Fig. 7). When hsp90 was first treated with ATP and molybdate at 30 °C for 3–30 min, this caused a progressive protection from the inhibitory action of GA. This indicates that GA binds preferentially to the inactive state of hsp90 and prevents the conversion of hsp90 under the influence of ATP and molybdate.

**ATP Affects a Hydrophobic Site on hsp90**—Previous studies have shown that hsp90 has a high affinity for hydrophobic resins (26–28). It seemed possible that this property might reflect a structural feature of hsp90 that may also be influenced by nucleotides. We first tested the binding of hsp90 to various hydrophobic resins at relatively low ionic strength (50 mM KCl), as used for p23 binding (Fig. 8A). For this assay, hsp90 was incubated with small resin pellets for 30 min at 30 °C. The pellets were washed, and the bound hsp90 was extracted and observed by gel electrophoresis. Nearly quantitative binding of hsp90 occurred to phenyl- or octyl-resins when compared with the total amount of hsp90 in the preparation (not shown). However, very little bound to hexyl-, pentyl-, butyl-, or ethylagarose. We next tested the binding to phenyl-Sepharose under the conditions used to promote hsp90 binding to p23 (Fig. 8B). When hsp90 was first incubated at 30 °C for 30 min in the presence of p23, 5 mM ATP, 20 mM molybdate, and 0.01% Nonidet P-40, only a minor portion bound to phenyl-Sepharose during a subsequent incubation (lane 1). However, when the ATP was replaced with 5 mM ADP, the binding to phenyl-Sepharose was nearly quantitative (lane 2). ADP was not essential, since binding to a similar extent occurred in the absence of any nucleotide (lane 4). The suppressive effect of ATP on phenyl-Sepharose binding required neither an ATP regeneration system (lane 5) nor the presence of p23 (lane 6). However, p23 appeared to cause a small, but reproducible, enhancement of the action of ATP (compare lanes 1 and 3). Lanes 6 and 7 show that both molybdate and Nonidet P-40 are needed to observe the ATP-dependent suppression of phenyl-Sepharose binding. The presence of geldanamycin also blocks the action of ATP in this regard (lane 8). These results show that the ATP-dependent form of hsp90 that can bind p23 has a low affinity for hydrophobic resins compared with hsp90 with ADP or without nucleotide.

![Fig. 6. Inhibition of p23 binding to hsp90 by geldanamycin.](image)

Samples containing purified p23 plus hsp90 were prepared under standard conditions with 20 mM molybdate, 5 mM ATP, and an ATP regeneration system with the addition of geldanamycin as indicated. After incubation for 30 min at 30 °C, the samples were immune precipitated and analyzed by SDS-PAGE.

![Fig. 7. Geldanamycin inhibition before and after ATP treatment.](image)

Samples were prepared as for Fig. 6, and geldanamycin (2 µg/ml) was added before incubation (lane 1) or after 3, 10, or 30 min at 30 °C as indicated. The total incubating time at 30 °C was 60 min. The samples were then chilled and processed as described previously.

![Fig. 8. Binding of hsp90 to hydrophobic resins.](image)

A, samples (200 µl) contained hsp90 (~10 µg) in 10 mM Tris-HCl, pH 7.5, plus 50 mM KCl, 5 mM MgCl₂, and 1 mM dithiothreitol. These were mixed with hydrophobic resin (25-µl packed volume) containing phenyl (lane 1), octyl (lane 2), hexyl (lane 3), pentyl (lane 4), butyl (lane 5), or ethyl groups (lane 6). After incubation for 30 min at 30 °C, the samples were chilled, washed, extracted, and analyzed by SDS-PAGE. B, samples (200 µl) were prepared to contain (when complete) hsp90, p23, 5 mM ATP, and an ATP regeneration system or 5 mM ADP, 20 mM molybdate, and 0.01% Nonidet P-40. Variations are as follows. Lane 1, complete with ATP; lane 2, complete with ADP; lane 3, with ATP, molybdate, Nonidet P-40 and without p23; lane 4, with p23, molybdate, Nonidet P-40 and without nucleotide; lane 5, as lane 1 without regeneration system; lane 6, as lane 1 without molybdate; lane 7, as lane 1 without Nonidet P-40; lane 8, as lane 1 plus geldanamycin (10 µg/ml). After 30 min at 30 °C, the samples were combined with 25 µl of phenyl-Sepharose and incubated at 4 °C for 30 min. They were then washed, extracted, and analyzed by SDS-PAGE.

**DISCUSSION**

Previous studies on complexes with hsp90 and p23 involved the formation of multiprotein complexes in rabbit reticulocyte lysate (7, 9). Thus, it was not possible to determine the actual protein-protein interactions or the targets for agents that alter interactions. The present study shows that p23 binds directly to hsp90 and can do so in the absence of other proteins. This binding requires a high level of ATP, and it is potentiated by both molybdate and by Nonidet P-40. All of these agents appear to act directly on hsp90.

Perhaps the most significant conclusion from these studies is that hsp90 can exist in at least two different functional states as measured either by its interaction with p23 or its ability to bind hydrophobic resins. We have attempted to explain our results by the model illustrated in Fig. 9. Dimeric hsp90 is shown to exist in one conformational state bound to ADP and a
with either \([35S]\)ATP activity in addition to the kinase activity. We have treated hsp90 this would require the presence of a potent phosphatase activ-
lybdate are removed by dialysis or immune precipitation, and
phorylated states. Although hsp90 is a phosphoprotein (29, 30) study, and it is possible that additional protein interactions are
apparent affinity of hsp90 for ATP indicated in the present
arisen in previous studies may be explained in part by the low
detected in their hsp90 preparations. The problems that have
explained why the poorly metabolized analog ATP
might also facilitate an exchange of ADP for ATP on hsp90 or
reduce an ATPase activity to enhance the proportion of hsp90 in the ATP state. In an earlier report, Lanks (35) observed a detergent-dependent oligomerization of hsp90 that occurred at elevated temperatures. We have not observed any oligomerization of the hsp90 dimer under the conditions used in this study, as tested by gel filtration (results not shown).

The specific inhibition of hsp90 by the benzoquinone ansa-
mycin, geldanamycin, was first shown by Whitesell et al. (25). This drug binds hsp90 and blocks its interaction with pp60\(^{src}\). It has also been used to block the chaperoning function of hsp90 in association with glucocorticoid receptor (36), progesterone receptor (37), Raf (38), mutant p53 (39), and the reverse transcriptase of hepatitis B virus (40). Our results show that geldanamycin blocks the conversion of hsp90 to the ATP state and, thus, its association with p23. The drug does not appear to compete with p23 for a site on hsp90, since hsp90 in the ATP state binds p23 and is insensitive to geldanamycin. We propose in Fig. 9 that geldanamycin binds to a distinct site on hsp90 that locks it in the ADP state.

The binding of hsp90 to phenyl-Sepharose provides an interesting parameter for our studies in that it appears to be a measure of hsp90 in the ADP state. Phenyl-Sepharose has been used as a method to purify hsp90 (28), and it has been suggested that this binding represents the site on hsp90 that interacts with protein substrates (26, 27). This is an interesting possibility but one that remains to be substantiated. If this were true, the model would suggest that it is the ADP form of hsp90 that initially binds substrates. This would be consistent with studies by Jakob et al. (41) and Freeman et al. (42), who have shown that hsp90 can prevent the aggregation of denatured proteins and maintain them in a state capable of refolding. No nucleotide is required for this activity of hsp90. On the other hand, the assembly of hsp90 complexes with steroid receptors requires ATP and p23, and it seems likely that this process involves the interconversion of hsp90 between its con-
formational states. It should be noted that p23 also has the capacity to hold denatured proteins in a refoldable state, suggesting that it can also interact with protein substrates under certain conditions (43, 44).

These results reveal several new questions regarding the functioning of hsp90. Other cellular proteins are normally associated with hsp90 and may function as co-chaperones that could modify its conformational or functional state. These include, in addition to p23, a 60-kDa stress-related protein, p60, that can bind both hsp90 and hsp70 (45, 46) and the three immunophilins, FKBP52, FKBP51, and CyP-40 (10–12). Any of these proteins might alter the conformation of hsp90, its nu-
cleotide affinity, or its ATPase activity. These parameters would probably also be modified by interaction of hsp90 with protein substrates. Thus, it will be important to first establish more completely the concepts of the model in Fig. 9 and then to apply this information toward an understanding of the multi-
protein systems that involve hsp90.

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**Fig. 9. A model illustrating two states of hsp90.** hsp90 is shown to exist in two conformational states; one with bound ADP and one with bound ATP. The states capable of binding phenyl-Sepharose (phe-S) or p23 are indicated as well as the proposed actions of geldanamycin (GA) and molybdate.

second state with bound ATP. The ADP state has a binding site for phenyl-Sepharose, and the ATP state can bind p23. In analogy with many other proteins that use nucleotide binding as a conformational signal, we propose that the interconversion of hsp90 states relies on nucleotide exchange in the one direc-
tion and ATP hydrolysis in the other direction. Nucleotide binding is of relatively low affinity in this system, and persist-
ent binding of ADP may not be necessary to maintain the ADP state, since this appears to be the state that exists in the absence of nucleotide. Thus, additional factors to promote nu-
cleotide exchange may not be necessary. That hsp90 has an ATPase activity has been reported (17, 18); however, this result has been refuted in a recent study by Jakob et al. (19). These investigators were unable to bind hsp90 to immobilized ATP or to detect the binding of ATP or fluorescent analogs to hsp90. Only very low ATPase activity of questionable significance was detected in their hsp90 preparations. The problems that have arisen in previous studies may be explained in part by the low apparent affinity of hsp90 for ATP indicated in the present study, and it is possible that additional protein interactions are needed to enhance nucleotide affinity or ATPase activity.

Our results might also be explained by an alternative model where hsp90 is converted between phosphorylated and dephos-
phorylated states. Although hsp90 is a phosphoprotein (29, 30) and has been reported to undergo autophosphorylation (18), we find this model to be less attractive. We have found that the p23-binding state of hsp90 is readily lost when ATP and molybdate are removed by dialysis or immune precipitation, and this would require the presence of a potent phosphatase activity in addition to the kinase activity. We have treated hsp90 with either \([32P]ATP\) or \([\gamma\text{-}32P]ATP\) and have not detected any labeling of hsp90 under the conditions used to generate the p23-binding state. Also, the inhibition by ADP is more compat-
ible with the model in Fig. 9.

Molybdate was first shown to be effective in regard to hsp90 through studies on steroid receptor complexes, where it is a very useful stabilizing agent (31). Similar effects have been obtained using tungstate, vanadate, and aluminum fluoride (32–34). These compounds and berilium fluoride have been used to study a variety of ATPases where they are believed to interact with phosphate binding sites in the catalytic site (21). In the present case, we propose that molybdate binds to hsp90 at the position of the \(\gamma\)-phosphate of ATP subsequent to ATP hydrolysis to ADP. This would maintain hsp90 in the “ATP” conformational state, able to interact with p23. This would explain why the poorly metabolized analog ATP-\(\gamma\)S shows no requirement for molybdate in its action.

The other important ingredient that enhances the ATP state of hsp90 is the detergent Nonidet P-40. It appears to greatly facilitate the rate of conversion to this form. This conversion of hsp90 is very slow in the absence of detergent, and we believe it is likely to be accompanied by quite significant confor-

mational changes. It is possible that the detergent interaction in some way allows these conformational changes to occur more rapidly in the in vitro experimental system. Nonidet P-40 might also facilitate an exchange of ADP for ATP on hsp90 or reduce an ATPase activity to enhance the proportion of hsp90 in the ATP state. In an earlier report, Lanks (35) observed a detergent-dependent oligomerization of hsp90 that occurred at elevated temperatures. We have not observed any oligomeriza-
tion of the hsp90 dimer under the conditions used in this study, as tested by gel filtration (results not shown).

A monomeric state that locks it in the ADP state.

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discussions on hsp90 modeling. SF9 cell growth, treatment, and harvesting were conducted by Dean Edwards and Kurt Christenson at the University of Colorado Cancer Center Tissue Culture Core.

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