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ATP Induces a Conformational Change of the 90-kDa Heat Shock Protein (hsp90)*

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The 90-kDa heat shock protein (hsp90) is a well conserved, abundant cytosolic protein believed to be a "chaperone" of most steroid receptors. We have recently demonstrated that hsp90 has an ATP-binding site and autophosphorylating activity (Csermely, P., and Kahn, C. R. (1991) *J. Biol. Chem.* 266, 4943–4950). Circular dichroism analysis of highly purified hsp90 from rat liver shows that ATP induces an increase of β-pleated sheet content of hsp90. Vanadate, molybdate, and heat treatment at 56 °C induce a similar change in the circular dichroism spectrum. Fourier transformed infrared spectroscopy reveals an ATP-induced increase in the interchain interactions of the 90-kDa heat shock protein due to an increase in its β-pleated sheet content. In further studies we found that ATP: 1) decreases the tryptophan fluorescence of hsp90 by 11.6 ± 1.9%; 2) increases the hydrophobic character of the protein as determined by its distribution between an aqueous phase and phenyl-Sepharose; and 3) renders hsp90 less susceptible to tryptic digestion. Our results suggest that hsp90 undergoes an "open → closed" conformational change after the addition of ATP, analogous in many respects to the similar changes of the DnaK protein, the immunoglobulin heavy chain binding protein (BiP/GRP78), and hsp70. The ATP-induced conformational change of hsp90 may be important in regulating its association with steroid receptors and other cellular proteins.

Exposure of cells to a wide variety of environmental perturbations stimulates the synthesis of a group of polypeptides known as the heat shock (stress) proteins (1–3). The heat shock proteins are usually classified on the basis of their approximate molecular masses and degrees of homology. One class is composed of proteins with molecular sizes between 105 and 80 kDa; members of the second and most highly conserved group have molecular sizes of approximately 70 kDa; the third class consists of heat shock proteins with molecular sizes around 60 kDa; and there is a fourth group of "small" heat shock proteins with molecular masses ranging between 6 and 30 kDa (4). Most stress proteins are also synthesized constitutively in significant amounts under normal, nonstressed conditions. This raises the possibility that heat shock proteins play an important role in the physiology of normal cells.

Recent studies indicate that members of the 60- and 70-kDa heat shock protein "families" cooperate in facilitating the transport of proteins across membranes of the endoplasmic reticulum and mitochondria (5, 6). The 90-kDa heat shock protein (hsp90),[1] is an abundant cytosolic protein believed to act as a "chaperone" by binding to nascent steroid receptors and preventing their premature association with DNA (2–4, 7, 8). hsp90 also modulates the activity of $pp60^{v\text{-}src}$ and the initiation factor-2 kinase (9, 10) and binds to actin and tubulin, which raises the possibility of an *in vivo* interaction with the microfilamental and microtubular network (11, 12). Despite this information, the exact function of hsp90 is not completely understood.

In an earlier study we demonstrated that hsp90 possesses and ATP-binding site and the ability to phosphorylate itself on serine residue(s) (13) analogous in many respects to the similar structure and activity of the 70-kDa heat shock proteins (14–16). Recently, an ATP-induced conformational change of the DnaK protein (17, 18) and the 70-kDa heat shock protein (19) has been reported. These findings raised the possibility that hsp90 undergoes similar changes in its secondary and tertiary structure after the addition of ATP. In the present paper, we verified this hypothesis using circular dichroism and Fourier transform infrared spectroscopies as well as the analysis of tryptophan fluorescence, hydrophobic character, and limited proteolysis of hsp90.

MATERIALS AND METHODS

Chemicals—The chemicals used for polyacrylamide gel electrophoresis were from Bio-Rad. Butyl-Sepharose 4B, DEAE-Sepharose Fast Flow, phenyl-Sepharose 4B, and Sepharose S-200 were purchased from Pharmacia LKB Biotechnology Inc. (Uppsala, Sweden). Spectragel HA hydroxyapatite resin was from Spectrum Medical (Los Angeles, CA). Anti-hsp90 antibody (AC-88) was purchased from StressGen (Victoria, B. C., Canada). TPCK-trypsin was purchased from Worthington. ATPγS was from Boehringer Mannheim. 8-Azido-[α-^{32}P]ATP (366 GBq/mmol) was from ICN Biomedicals Inc. (Irvine, CA). All other chemicals used were from Sigma.

Isolation of hsp90—The 90-kDa heat shock protein was isolated

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[1] The abbreviations used are: hsp90, 90-kDa heat shock protein; DnaK, the hsp70 homolog of *E. coli*; FT-IR, Fourier transform infrared; grp78, the immunoglobulin heavy chain binding protein (BiP); hsp56/59, 56–59-kDa heat shock protein; hsp70, 70-kDa heat shock protein and its constitutive homolog, hsc70; PAGE, polyacrylamide gel electrophoresis; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; ATPγS, adenosine 5′-*O*-(thiotriphosphate).

from livers of 2–3-month-old male Sprague-Dawley rats using the method of Yonezawa *et al.* (20) as described earlier (13). The purity of this preparation was higher than 95% (usually higher than 98%) as judged by densitometry of Coomassie Blue-stained SDS slab gels (21). Protein concentrations were determined using the methods of Lowry *et al.* (22), Bradford (23), and Udenfriend *et al.* (24) with bovine serum albumin and globulin as standards. We got the highest value with the Bradford/serum globulin method/standard pair, whereas the protein concentration using the Udenfriend method with bovine serum albumin was five times lower. Finally, we calculated and used the mean of all protein determinations getting a factor of 0.57 ± 0.18 with respect to the values of the Bradford/serum globulin method/standard pair.

Covalent Labeling of hsp90 with 8-Azido-[α-^{32}P]ATP—Covalent labeling of hsp90 was performed as described earlier (13). 5 μg of hsp90 was preincubated with 4 μM (1 μCi) 8-azido-[α-^{32}P]ATP in the dark in separate wells of a 96-well microtiter plate at 4 °C for 15 min in a buffer containing 50 mM Hepes pH 7.4 and 10 mM $CaCl_2$. The reaction mixture was irradiated with a 100-watt long wavelength UV lamp (Black Ray, UVP Inc., San Gabriel, CA) for 5 min at 4 °C from a distance of 10 cm. Samples were transferred to Eppendorf microcentrifuge tubes containing 30 μl of Laemmli buffer (21) supplemented with 10 mM EDTA and 20 mM dithiothreitol, boiled for 5 min, and analyzed by SDS-PAGE (21) and autoradiography. The radioactivity of the hsp90 bands was quantitated by densitometry of the autoradiograms.

Circular Dichroism Measurements—Circular dichroism (CD) spectra were recorded on a Jobin Yvon VI dichrograph. Measurements were made at room temperature in a 0.01-cm pathlength cylindrical quartz cell. The concentration of the samples was 0.3–0.5 mg of hsp90/ml in 50 mM Hepes pH 7.4 buffer. Base lines were obtained using protein-free buffer solution with the appropriate additions. The ligands were introduced from stock solution (200× concentrated), and their equilibration was facilitated with gentle agitation for approximately 1 min. Longer incubation did not cause any further difference in the CD spectra. Mean residue ellipticities were calculated based on a mean residue molecular mass of 110 kDa. The CD spectra were analyzed by the method of Provencher (25, 26) as a linear combination of the CD spectra (from 195 to 240 nm) of 16 proteins whose secondary structures are known from x-ray crystallography. Secondary structure of hsp90 was also estimated by the predictive methods of Chou and Fasman (27) and Garnier *et al.* (28) using the amino acid sequence of murine hsp90 (29).

Fourier Transform Infrared Spectroscopy—Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet 170SX spectrometer (MCT detector, Ge/KBr beam splitter) using a demountable cell with a spacing of ~50 μm. For each spectrum, 1024 interferograms were averaged and Fourier transformed to yield FT-IR spectra with a resolution of 4 cm^{-1}. The spectrum of the buffered D_2O medium was digitally subtracted, and absorptions due to water vapor were removed in the same manner. In order to separate instrumentally unresolvable infrared band contours, Fourier self-deconvolution was applied to the spectra using 15 cm^{-1} bandwidth and a resolution enhancement factor of 2.0.

Fluorescence Measurements—Fluorescence measurements were carried out in a Perkin-Elmer Cetus Instruments LS 50 spectrofluorometer at 37 °C. The tryptophan fluorescence of hsp90 was measured by using an excitation wavelength of 295 nm and an emission wavelength of 330 nm, with 5-nm slit widths. Samples were measured in 1-cm rectangular quartz cuvettes in 2 ml of 100 mM Tris·HCl, pH 7.4, at a protein concentration of 0.1 mg/ml. Fluorescence values are expressed as percentage of total hsp90 fluorescence obtained after subtracting the background light intensity measured with the Tris buffer alone. Changes of tryptophan fluorescence have been corrected to the absorption of various ligands at 295 and 330 nm using the formula,

$$I = I_0 e^{-d(E_1 + E_2)/2} \quad \text{(Eq. 1)}$$

where I and I_0 are light intensities after and before the addition of the appropriate ligands, respectively; d is 0.5 cm; and E_1 and E_2 are the absorbances of the ligands at 295 and 330 nm, respectively. The necessary correction of fluorescence data was never higher than 0.1% of the total fluorescence.

Binding of hsp90 to Phenyl-Sepharose—Binding of hsp90 to phenyl-Sepharose was determined according to Yamamoto *et al.* (30). 25 μg of purified rat liver hsp90 in 60 μl of binding buffer containing 50 mM Hepes pH 7.4, 0.1 M NaCl, 0.2 mM dithiothreitol, 10 mM $CaCl_2$, ±2.5 mM ATP; and various concentrations of ethylene glycol were added to a 30-μl suspension of phenyl-Sepharose gel equilibrated with the same buffer. Samples were incubated at 10 °C for 12 min. The suspension was centrifuged at 6000 rpm for 1 min, and the supernatant was analyzed by SDS-PAGE (21). The amount of hsp90 was quantitated by densitometry of Coomassie Blue-stained gels on a Pharmacia Ultroscan XL laser densitometer using a standard curve of known amounts of hsp90.

Limited Proteolysis of hsp90—Tryptic digestion pattern of hsp90 was analyzed after Lees-Miller and Anderson (31). 15 μg of hsp90 was incubated with 0.1 μg of TPCK-treated trypsin in the presence of 20 mM Hepes pH 7.4, 0.15 M NaCl, 1 mM EDTA, 6 mM $MgCl_2$, 5% (v/v) glycerol, 1 mM dithiothreitol, ±2.5 mM ATP at 37 °C for the times indicated. Digestion was stopped by the addition of 10 μg of trypsin inhibitor, and the tryptic fragments were visualized by SDS-PAGE (21).

RESULTS

Effect of ATP on the Circular Dichroism of hsp90—CD spectrum of hsp90 showed an ellipticity maximum below 195 nm and two negative bands at 210 and 220 nm (Fig. 1). In the 200–270-nm range, the CD spectrum of hsp90 in 50 mM Hepes pH 7.4 was not significantly different from that measured in solutions with a strongly decreased buffer concentration (data not shown). Comparison of CD spectra at various buffer concentrations revealed that due to the intensive absorbance of the Hepes buffer there is a ±20% error in the magnitude and position of the positive band of the spectra below 195 nm.

While the addition of $MgCl_2$ and ATP at final concentrations of 10 and 0.5 mM, respectively, caused only slight changes in the shape of the spectrum, the effect of Ca-ATP was significant, giving rise to one single negative band at 219 nm (Fig. 1). Thus ATP induced a positive contribution to the overall ellipticity of hsp90 in the region of 195–220 nm. Control experiments showed no significant change in the CD spectrum of hsp90 if $MgCl_2$ or $CaCl_2$ was added alone. Ca-ATPγS induced changes similar to those of Ca-ATP in the CD spectrum of hsp90 (data not shown); thus the change in CD spectrum does not require the autophosphorylation of hsp90.

The analysis of CD spectra of hsp90 gave an average composition of 36% α-helix and 46% β-structure (Table I). The relative contribution of β-structure increased to 65% after ATP addition, whereas the α-helical content was essentially unchanged. Comparison of our experimental data with those

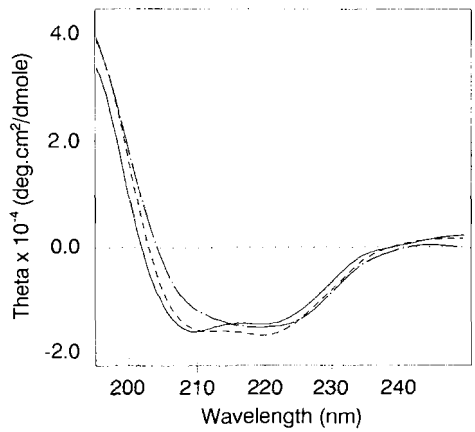

FIG. 1. **Effect of ATP on the circular dichroism of hsp90.** Circular dichroism spectra of hsp90 were recorded in the absence (*solid line*) or presence of 0.5 mM ATP and 10 mM $CaCl_2$ (*alternating dots* and *dashes*) or 10 mM $MgCl_2$ (*dashed line*) as described under "Materials and Methods." Spectra are representatives of three separate experiments.

TABLE I

Secondary structure of hsp90

Calculation of secondary structure from CD spectra and its prediction from the primary structure of murine hsp90 (29) was done as described under "Materials and Methods." The numbers in parentheses denote the corresponding references.

	Contribution to secondary structure	
	α-Helix	β-Structure
	%	%
Experimental data		
hsp90	36	46
+ ATP	33	65
hsp70 (48)	40	40
Predicted values		
Chou-Fasman method (27)	37	26
Garnier method (28)	59	16
Chicken hsp90 (65)	58	11

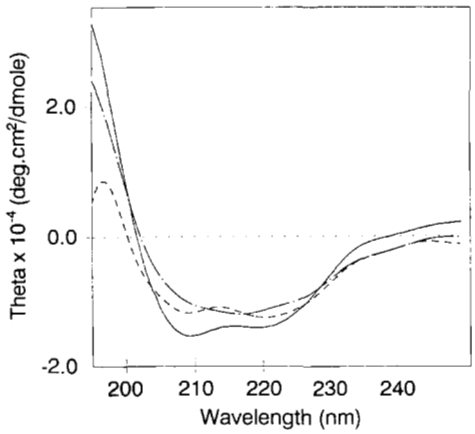

FIG. 2. **Effect of heat treatment on the circular dichroism of hsp90.** hsp90 was subjected to heat treatment by incubating in 50 mM Hepes buffer pH 7.4 at 56 °C for 15 min and cooling to room temperature within 3 min. Circular dichroism spectra of hsp90 were recorded before (*solid line*), immediately after (*alternating dots* and *dashes*), and 60 min after (*dashed line*) the heat treatment as described under "Materials and Methods." Spectra are representatives of three separate experiments.

of hsp70 and with the predicted secondary structure of hsp90 revealed a similar α-helical content and a slightly higher amount of β-structures. The difference in the amount of β-structures may arise both from the uncertainties of the determinations/predictions and from a possible thermodynamical unstability of hsp90, a molecular chaperone putatively involved in the unfolding/refolding of other proteins.

Effect of Temperature Change on the Circular Dichroism of hsp90—Since hsp90 is a heat shock protein and it displays temperature-induced changes in its hydrophobicity and self-aggregation around 40–45 °C (30, 32), we examined whether the elevation of the temperature resulted in any change in the CD spectrum of the protein. To avoid the distortion of the cells due to temperature differences, hsp90 was heated at 56 °C for 15 min in separate Eppendorf tubes, and the samples were rapidly cooled to room temperature before analysis. Heat treatment of hsp90 induced changes in its CD spectrum similar to those observed after the addition of Ca-ATP (*cf.* Figs. 1 and 2). The effect was at least partially reversible since leaving the samples at room temperature for 60 min partially restored the original, double-lobed negative peak at 210 and 220 nm (Fig. 2).

Effect of Vanadate and Molybdate on the Binding of Azido-ATP to hsp90 and on the CD Spectrum of the Protein—Molybdate and vanadate are known to stabilize the steroid receptor-hsp90 complex (33, 34). Since both ions are transition state analogs of phosphate (35, 36) and since hsp90 has an ATP-binding site (13), we wanted to examine whether molybdate and vanadate modulate the binding of ATP to hsp90. Indeed, both anions inhibited the affinity labeling of hsp90 by azido-ATP (Fig. 3). Molybdate had a half-maximal effect around 0.5 mM, whereas vanadate was more efficient, having an ED_{50} around 50 μM (Fig. 3).

After verifying that molybdate and vanadate are good inhibitors of ATP binding to hsp90, we analyzed whether these anions induced any change in the secondary structure of the protein by examining the CD spectra of hsp90 in the absence and presence of molybdate and vanadate. Vanadate induced a similar change in the CD spectrum of hsp90 similar to that induced by Ca-ATP or heat treatment (*cf.* Figs. 1, 2, and 4). The effect of molybdate at 10 mM final concentration was similar to that of 0.1 mM vanadate, whereas 0.5 mM molybdate did not induce any significant changes in the CD spectrum of

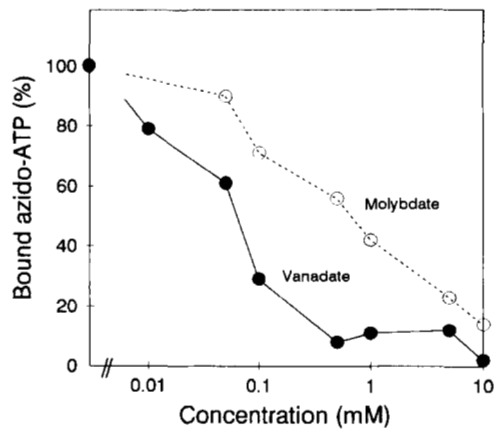

FIG. 3. **Effect of vanadate and molybdate on azido-ATP binding to hsp90.** 5 μg of hsp90 was labeled with 4 μM azido-[α-^{32}P]ATP in the presence of 10 mM $CaCl_2$ and various concentrations of sodium molybdate (*open circles*) and sodium vanadate (*filled circles*) by illuminating with UV light for 5 min at 4 °C as described under "Materials and Methods." Samples were subjected to SDS-PAGE and autoradiography. The amount of bound azido-ATP was calculated by densitometric analysis of the hsp90 band in autoradiograms with or without vanadate or molybdate. Data are means of two separate experiments.

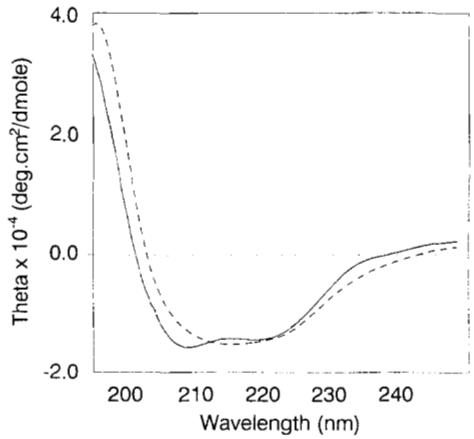

FIG. 4. **Effect of vanadate on the circular dichroism of hsp90.** Circular dichroism spectra of hsp90 were recorded in the absence (*solid line*) or presence of 0.1 mM sodium vanadate (*dashed line*) as described under "Materials and Methods." Spectra are representatives of three separate experiments.

hsp90 (data not shown). In the experiments with vanadate, attention was paid to avoid even a transient acidification of the samples that would cause the accumulation of the kinetically stable decavanadate species distorting the CD measurements due to its high absorbance (bright yellow color; Ref. 37).

Effect of ATP on the Fourier Transform Infrared Spectrum of hsp90—The high absorbance of water in the amide I region hides the subtle differences of FT-IR spectra induced by conformational changes in the protein backbone. Therefore, we analyzed the Fourier transform infrared spectrum of hsp90 in D_2O. hsp90 displayed a similar autophosphorylation pattern in the presence of Ca^{2+}, Mg^{2+}, and Mg^{2+} + histone H1 in D_2O medium and in H_2O indicating that deuteration is unlikely to cause significant changes in the ATP-related functioning of hsp90 (data not shown). The interpretation of spectral changes in the amide II band is difficult in D_2O, since the extent of hydrogen to deuterium exchange varies with the residual amount of water in different samples inducing larger absorbance differences in this section of the spectrum than in the amide I region. Therefore, the analysis was restricted to the amide I band in these samples. In Fig. 5, the amide I band of the FT-IR spectrum of hsp90 is shown before and after the addition of Ca-ATP (*dashed lines* in *panels A* and *B*, respectively). The *solid lines* represent the same FT-IR spectra after Fourier deconvolution using a bandwidth of 15 cm^{-1} and a resolution enhancement factor of 2.0. Addition of ATP induces a significant increase at 1625–1627 cm^{-1} and several less pronounced changes at 1660, 1670, 1680, and 1695 cm^{-1}. In control experiments, the addition of $CaCl_2$ alone did not induce any significant changes in the FT-IR spectrum of hsp90 (data not shown).

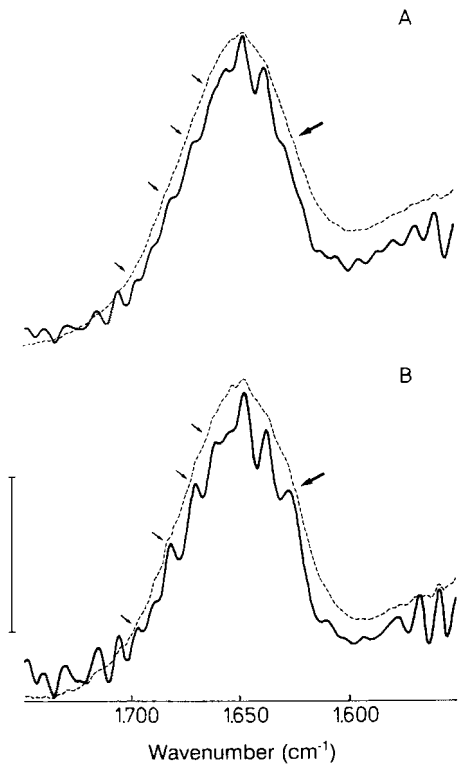

FIG. 5. **Effect of ATP on the Fourier transform infrared spectrum of hsp90.** Rat liver hsp90 was equilibrated with a Hepes-buffered solution of D_2O by repeated cycles of dilution/concentration using a Centricon 30 concentrator. FT-IR spectra of hsp90 were recorded in the absence (*panel A*) and presence (*panel B*) of 0.5 mM ATP and 5 mM $CaCl_2$ as described under "Materials and Methods." *Solid lines* represent the same FT-IR spectra after Fourier deconvolution using 15 cm^{-1} bandwidth and a resolution-enhancing factor of 2.0. *Arrows* indicate changes in the spectrum after addition of ATP. *Vertical bar* denotes an absorbance of 0.1. Spectra are representatives of three separate experiments.

Effect of ATP on the Tryptophan Fluorescence of hsp90—Murine hsp90 contains 4 tryptophan and 23 tyrosine residues (29). At the 295/330 excitation/emission wavelength pair, however, the only significant contribution to the intrinsic fluorescence comes from the tryptophan residues (38). Since tryptophan fluorescence is a sensitive marker of the conformational changes of proteins (38), we examined whether ATP induces any change in this property of hsp90. Indeed, addition of ATP resulted in a large decrease in the intrinsic fluorescence of hsp90 similarly to that of the DnaK protein (Fig. 6, *panel A, trace a*; Ref. 18). The ATP-induced decrease in tryptophan fluorescence could be repeatedly observed after several cycles of dialysis and ATP readdition (data not shown). The change of tryptophan fluorescence was almost identical if we used the nonhydrolyzable ATP analog, ATPγS (Fig. 6, *panel A, trace b*). ATP induces a similar decrease in tryptophan fluorescence at a final ATP concentration of 2.5 mM in the absence or presence of $CaCl_2$ or $MgCl_2$ (Table II). At lower ATP concentrations, however, the change in tryptophan fluorescence depended on the accompanying divalent cation with an apparent K_d of 1.1 or 0.2 mM ATP in the presence of $MgCl_2$ or $CaCl_2$, respectively (Fig. 6, *panel B*).

Effect of ATP on the Hydrophobicity of hsp90—ATP induced a small but significant ($p < 0.1$) increase in the binding of hsp90 to phenyl-Sepharose (Fig. 7). The ATP-induced differences increased with increasing concentration of ethylene glycol, a known modulator of interactions between hsp90 and phenyl-Sepharose (30). In control experiments, ATP did not cause any significant change in binding of bovine serum albumin to phenyl-Sepharose (data not shown). In contrast to these results, ATP diminished the binding of hsp90 to DNA-cellulose and cellulose.[2]

ATP-induced Changes in the Tryptic Digestion Pattern of hsp90—Since ATP induces significant changes in the tryptic digestion pattern of grp78 (16) and DnaK (17), we examined if there was any change in the limited proteolysis of hsp90 after ATP addition. In the absence of ATP, trypsin (4 μg/ml) produced a rapid proteolysis of hsp90 to fragments of 68–20 kDa. In the presence of ATP, hsp90 was less susceptible to tryptic digestion than in its absence (Fig. 8). Besides an ATP-induced increase in the amount of uncleaved hsp90, at later time points of tryptic digestion there was also an increase in the amount of peptide fragments *c* and *d* in the presence of ATP compared with control samples (Fig. 8). These latter changes may reflect a local protection of ATP around its binding site, which is located in tryptic fragments *c* and *d* (*cf.* Refs. 13, 29, and 31).

DISCUSSION

The 90-kDa heat shock protein (hsp90) is present in most, if not all, prokaryotic and eukaryotic cells and may constitute up to 1–2% of the total cytosolic protein (4). hsp90 is associated with steroid receptors, hsp70, hsp56/59, actin, tubulin, and other yet unidentified proteins with molecular masses of 188, 63, and 50 kDa (7, 8, 11, 12, 39–42) and forms complexes with a number of protein kinases such as casein kinase II, double-stranded DNA-activated protein kinase, heme-regulated initiation factor-2 kinase, protein kinase C, and various tyrosine kinases (4, 9, 10, 43–47). Conformational changes of the 90-kDa heat shock protein may significantly influence its

[2] T. Schnaider and P. Csermely, unpublished observations.

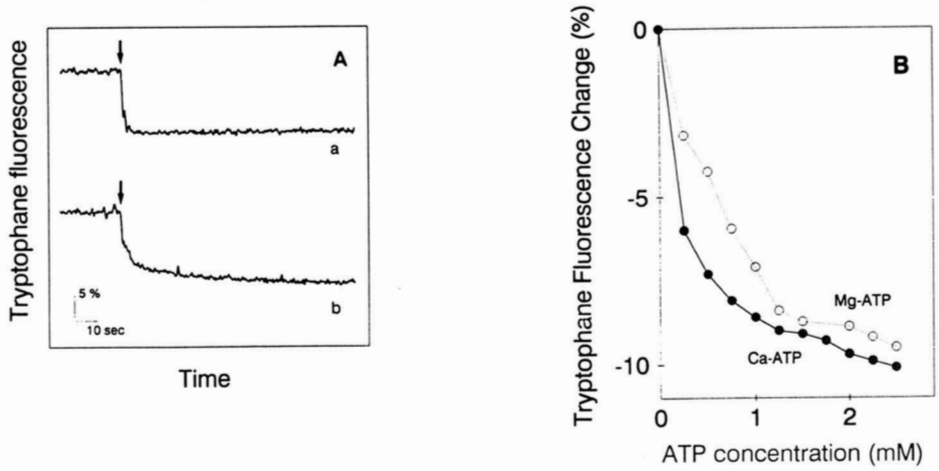

FIG. 6. **Effect of ATP and ATPγS on the tryptophan fluorescence of hsp90.** The intrinsic fluorescence of hsp90 was measured as described under "Materials and Methods." *Panel A,* fluorescence traces of hsp90 recorded in the presence of 5 mM $CaCl_2$. At the *arrow,* ATP or ATPγS was added at a final concentration of 2.5 mM in *curve a* and *b,* respectively. *Traces* are representatives of five separate experiments. *Panel B,* hsp90 tryptophan fluorescence as a function of ATP concentration in the presence of 5 mM $MgCl_2$ (*open circles*) or $CaCl_2$ (*filled circles*). Data represent means of two experiments.

TABLE II

Changes of the tryptopha fluorescence of hsp90

Tryptophan fluorescence of hsp90 was measured as described under "Materials and Methods." The final concentrations of ATP or $MgCl_2$ and $CaCl_2$ were 2.5 or 5 mM, respectively. Data are means ± S.D. of five separate experiments.

Added compound	Fluorescence decrease at 295/330 nm
	%
ATP	11.6 ± 1.9
Mg-ATP	12.1 ± 1.3
Ca-ATP	12.8 ± 0.7

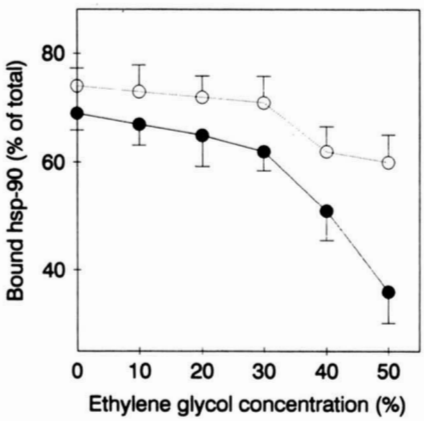

FIG. 7. **Effect of ATP on binding of hsp90 to phenyl-Sepharose.** Binding of hsp90 to phenyl-Sepharose was studied in the presence of ethylene glycol at concentrations indicated as described under "Materials and Methods." *Open* and *filled circles* represent data points in the presence or absence, respectively, of ATP at a final concentration of 2.5 mM. Data are means ± S.D. of three separate experiments. Control values (0% ethylene glycol) represent means ± S.D. of seven experiments.

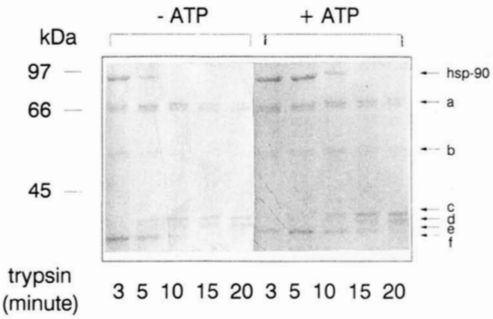

FIG. 8. **Effect of ATP on the tryptic digestion pattern of hsp90.** Limited proteolysis of rat liver hsp90 was performed in the absence or presence of ATP at a final concentration of 2.5 mM as described under "Materials and Methods." The tryptic digestion was stopped after the times indicated, and the samples were subjected to SDS-PAGE. The *letters a–f* denote tryptic fragments of hsp90 identified by Lees-Miller and Anderson (31). The Coomassie Blue-stained gel is a representative of five separate experiments.

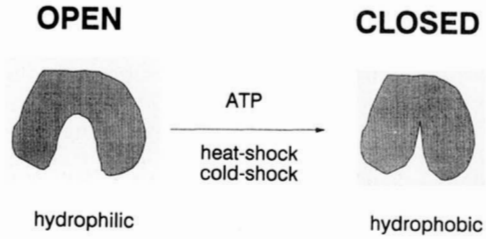

FIG. 9. **ATP- and temperature-induced conformational changes of hsp90.** The figure summarizes our present view about the shift of hsp90 from an *open* to a *closed* conformation after ATP addition or change in temperature. See details in text.

interaction with the proteins mentioned above. In our earlier studies we demonstrated that hsp90 has an ATP-binding site and is able to phosphorylate itself (13). In the present paper we report that ATP is inducing significant changes in the secondary/tertiary structure of hsp90.

The CD spectrum of hsp90 shows a great similarity with the CD spectrum of the closely related hsp70 (19, 48). The ATP-induced overall spectral change reflects an enrichment in β-structures (49) similar to the changes in the 1630–1620 and 1695–1660 cm^{-1} regions of the FT-IR spectrum of hsp90 (50). Mg-ATP induces smaller changes in the CD spectrum of hsp90 than Ca-ATP. This difference may simply reflect that the 0.5 mM Mg-ATP used in these experiments was not enough to saturate hsp90, since tryptophan fluorescence data revealed that Mg-ATP has an approximately 5-fold higher apparent K_d than Ca-ATP. Unfortunately, the high UV absorbance of ATP did not permit the direct analysis of this assumption.

Heat treatment induces a conformational change of hsp90 similar to the effect of ATP. The CD spectrum of hsp90 displays almost identical changes with the CD spectrum of hsp70 after heat treatment (19). Palleros *et al.* (19) reported that the heat-induced conformational change of hsp70 parallels with its oligomerization. hsp90 is also known to form oligomers at higher temperatures (32). However, the oligomerization of hsp90 is strongly detergent-dependent (32), so it is not likely that oligomerization occurred in our detergent-free samples. This may explain why the temperature-induced changes in hsp90 conformation proved to be reversible in contrast to those reported for hsp70 (19). Interestingly, the temperature-induced conformational change of DnaK, the *Escherichia coli* hsp70 homologue, is also reversible and does not result in an oligomerization of the protein similar to hsp90 (18). Our results that the ATP-induced decrease in tryptophan fluorescence of hsp90 could be repeatedly observed after several cycles of dialysis and ATP readdition suggest that ATP also induces a reversible change in the conformation of hsp90. The reversal of the ATP-induced conformational change of hsp90 may also require the presence of other proteins.

The FT-IR spectrum of hsp90 shows a significant increase of the band at 1625–1627 cm^{-1} after the addition of ATP. This region is characteristic of interchain interaction of β-pleated or extended peptide chains (50), which seem to increase in hsp90 after binding of ATP. Ethylene glycol affects the binding of hsp90 to phenyl-Sepharose much less in the presence than in the absence of ATP. Finally, ATP renders hsp90 less susceptible to tryptic digestion. These results may all reflect the fact that ATP induces a tighter folding, a change from open to closed conformation of hsp90. This conformational change is analogous with the "closure" of grp78 (BiP, the immunoglobulin heavy chain binding protein), hexokinase, and phosphoglycerokinase after addition of ATP (16, 51, 52). Interestingly, these proteins, together with the nucleotide-binding domain of hsp70 and actin, all have a bilobular, hinge-type three-dimensional structure, which is similar to the structure of hsp90 (11, 51–54). Hydrolysis of ATP induces an opposite, closed → open conformational change of the *E. coli* hsp70 homolog, DnaK (18), which may be the reversal of the closure of hsp90 inducible by both ATP and the nonhydrolyzable ATP analog, ATPγS.

Fig. 9 summarizes the properties of the putative open and closed conformations of hsp90. Tryptic digestion and FT-IR data support the induction of a closed conformation by ATP. hsp90 is more hydrophobic in this conformation as revealed by its increased association with phenyl-Sepharose and its decreased binding to cellulose. CD data show that the closed conformation of hsp90 can be induced by heating the protein to 56 °C. The results of Yamamoto *et al.* (30) showing that hsp90 is indeed more hydrophobic at this extreme temperature fit well to the properties of the two hsp90 conformations detailed above.

Vanadate and molybdate also induce an enrichment of the secondary structure of hsp90 in β-strands. The effect is similar to the ATP-induced conformational change. Both anions inhibit the binding of azido-ATP to hsp90, which suggests a common mechanism of the observed changes in secondary structure. Molybdate and vanadate are potent stabilizers of the steroid receptor-hsp90 complex (33, 34). Their efficiency in stabilizing the untransformed steroid receptors parallels their half-maximal concentration in inhibiting the binding of azido-ATP to hsp90 and in inducing a conformational change of the 90-kDa heat shock protein, and in both cases vanadate is more potent than molybdate. Though the effects of molybdate on steroid receptors are attributed to its inhibitory action on various phosphatases, to a possible competition of molybdate for a nucleotide binding site on steroid receptors, and/or to complex formation between molybdate and SH groups of steroid receptors (33, 34, 55, 56), our findings raise the possibility that an interaction of molybdate and hsp90 may also play a role in the molybdate-induced stabilization of the nontransformed steroid receptor complex.

The effects of ATP on steroid receptors are more complex. On the one hand, ATP is known to increase the steroid binding capacity of the steroid receptor ("activation") (39, 40, 55, 57); on the other hand, ATP also induces the dissociation of the steroid receptor-hsp90-hsp70-hsp56/59 complex "transforming" the steroid receptor, thus enabling it to bind to DNA (39, 40, 55). The ATP-induced conformational change and autophosphorylation of hsp90 (13) may contribute to these effects of ATP on steroid receptors. A simple mechanism hypothesizing a role of hsp90 in the molybdate- and ATP-induced changes of steroid receptors, however, cannot be proposed yet, since molybdate and ATP have rather opposite effects on steroid receptors while they induce similar conformational changes of hsp90.

Proteins related to hsp70, such as grp78 (BiP), the immunoglobulin heavy chain binding protein, and DnaK, the hsp70 homolog of *E. coli,* and hsp70 itself require ATP for their function (15, 58–63). In our previous and present studies we demonstrated that hsp90 is similar to these proteins regarding its nucleotide-binding site, autophosphorylation, and ATP-induced conformational change (13–19, 64). This raises the possibility that interactions of hsp90 with ATP are important/necessary elements of the heretofore elusive function of hsp90.

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Addendum—After the submission of our manuscript, Bork *et al.* (66) gave further evidence for the bilobular, hinge-type structure of both sugar kinases and heat shock proteins, and Pratt and co-workers (67) demonstrated that molybdate and vanadate also stabilize the complex of hsp90 and $pp60^{v\text{-}src}$ similar to the stabilization of the hsp90-steroid receptor complex. This latter finding raises the possibility that hsp90 contains a binding site for molybdate and vanadate, which is in agreement with our findings, demonstrating a competition of these ions with ATP and their ability to induce a similar conformational change of hsp90 like ATP.

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