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

(Received for publication, August 7, 1992)

Péter Csermely‡, Judit Kajtár‡, Miklós Hollósí‡, George Jalsovszky‡, Sándor Holly‡, C. Ronald Kahn‡, Péter Gergely, Jr.§, Csaba Sóf‡, Katalin Mihály‡, and János Somogyi‡

From the ‡Institute of Biochemistry I, Semmelweis University, School of Medicine, H-1444 Budapest, Hungary, the §Department of Organic Chemistry, Eotvos University, H-1518 Budapest, Hungary, the ¶Central Research Institute of Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, Hungary, and the ¶Research Division, Joslin Diabetes Center, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02215

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. (1951) 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 pp60c-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 hsp96 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). Spectrangel HA hydroxyapatite resin was from Spectrum Medical (Los Angeles, CA). Anti-hsp90 antibody (AC-89) was purchased from StressGen (Victoria, B. C., Canada). TPCK-trypsin was purchased from Worthington. ATP-S was from Boehringer Mannheim. 8-Azido-[32P]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 from 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; ATP-S, adenosine 5’-0(β,γ-impaphosphate).
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.

**Pheny!-Sepharose-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 CaCl₂, ±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 Ultrascan 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 MgCl₂, 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 intense 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 MgCl₂ 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 MgCl₂ or CaCl₂ was added alone. Ca-ATP-S 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.](image-url)
ATP-induced Conformational Change of hsp90

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             |
| hsp90 (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.

**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 primary structure of murine hsp90 (29) was done as described under "Materials and Methods." Spectra are representatives of three separate experiments.

**FIG. 3. Effect of vanadate and molybdate on azido-ATP binding to hsp90.** 5 μg of hsp90 was labeled with 4 mM azido-[α-32P]ATP in the presence of 10 mM CaCl2 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.

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 ED50 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 hsp90 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 instability of hsp90, a molecular chaperone putatively involved in the unfolding/refolding of other proteins.

Effect of Vanadate and Molybdate on the Binding of Azido-ATP to hsp90 and on the CD Spectrum of the Protein—
Most, if not all, prokaryotic and eukaryotic cells and may constitute 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


c, and Mg²⁺ + histone H1 in D₂O medium and in H₂O indicating that deuterium is unlikely to cause significant changes in the ATR-related functioning of hsp90 (data not shown). The interpretation of spectral changes in the amide II band is difficult in D₂O, 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 CaCl₂ alone did not induce any significant changes in the FT-IR spectrum of hsp90 (data not shown).

**Effect of ATP on the Trypsin 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 CaCl₂ or MgCl₂ (Table II). At lower ATP concentrations, however, the change in tryptophan fluorescence depended on the accompanying divalent cation with an apparent Kᵦ of 1.1 or 0.2 mM ATP in the presence of MgCl₂ or CaCl₂, 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.

**ATP-induced Changes in the Tryptic Digestion Pattern of hsp90—** 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).
ATP-induced Conformational Change of hsp90

**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 CaCl₂. 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 MgCl₂ (open circles) or CaCl₂ (filled circles). Data represent means of two experiments.

**TABLE II**

| 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 Kₐ 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 the nonhydrolyzable ATP analog, ATPTS.

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.

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.

Acknowledgments—We are thankful to Drs. Bonnie A. Wallace (Department of Crystallography, Birkbeck College, University of London), René Buchet (ETH, Zurich, Switzerland), and István Jóna (Central Research Laboratory, Medical University of Debrecen, Hungary) for help in evaluating the CD spectra, Fourier transform infrared spectra, and fluorometric data, respectively. We also thank Drs. Veronika Adam-Vizi and Zoltan Deri (Institute of Biochemistry II, Semmelweis University, School of Medicine, Budapest, Hungary) for the use of the Perkin-Elmer Cetus Instruments spectrofluorometer.

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-ssc 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.

REFERENCES
1. Lindquist, S. (1986) Annu. Rev. Biochem. 55, 1151–1191
2. Burdon, R. H. (1986) Biochem. J. 240, 313–324
3. Subjeck, J. R., and Shoy, T. (1986) Am. J. Physiol. 250, C1–C17.
4. Lindquist, S., and Craig, E. A. (1988) Annu. Rev. Genet. 22, 631–677
5. Deshaies, R. J., Koch, R. D., Werner-Washburne, M., Craig, E. A., and Schekman, R. (1988) Nature 332, 800–805
6. Chirico, W. J., Waters, M. G., and Blobel, G. (1988) Nature 332, 800–810
7. Groyer, A., Schweizer-Groyer, G., Cadzow, F., Mariller, M. G., and Baulieu, E. E. (1987) Nature 328, 624–625
8. Denis, M., Poellinger, L., Wikström, A.-C., and Gustafsson, J.-A. (1988) Nature 332, 696–698
9. Oppermann, H., Levinson, W., and Bishop, J. M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 1067–1071
10. Rose, D. W., Wattenbarg, R. E., Dutton, W. E., Kramer, G., and Hardesty, B. (1987) Biochemistry 26, 6583–6587
11. Koyasu, S., Nishida, E., Kadowaki, T., Matsuzaki, F., Iida, K., Harada, F., Kasuga, M., Sakai, H., and Yabu, T. (1990) Proc. Natl. Acad. Sci. U. S. A. 83, 8045–8048
12. Schatz, G., Redmond, T., Scherrer, L. C., Brennich, E. W., Welsh, M. J., and Pratt, W. B. (1988) Mol. Endocrinol. 2, 756–760
13. Cassimery, P., and Kahn, C. R. (1991) J. Biol. Chem. 266, 4943–4950
ATP-induced Conformational Change of hsp90

14. Welch, W. J., and Feramisco, J. R. (1985) Mol. Cell. Biol. 5, 1229–1236
15. Zilnic, M., LeBoitwiz, J. H., McMacken, R., and Georgopoulos, C. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 6451–6455
16. Kassensbrock, C. K., and Kelly, R. B. (1989) EMBO J. 8, 1461–1467
17. Liberet, K., Showy, D., Zilnic, M., Johnson, C., and Georgopoulos, C. (1991) J. Biol. Chem. 266, 14491–14496
18. Pallares, D. R., Reid, K. L., McCarty, J. S., Walker, G. C., and Fink, A. L. (1992) J. Biol. Chem. 267, 5279–5285
19. Pallares, D. R., Welch, W. J., and Fink, A. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5719–5723
20. Yonezawa, N., Nishida, E., Sakai, H., Koyashu, S., Matsuzaki, F., Iida, K., and Yahara, I. (1988) Eur. J. Biochem. 177, 1–7
21. Lassini, U. K. (1970) Nature 227, 680–685
22. Lowey, O. H., Rosebroch, N. J., Fazz, A. L., and Randall, R. J. (1991) J. Biol. Chem. 266, 265–270
23. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
24. Udenfriend, S. Stein, S. Bohlen, P., Dairman, W., Leimburger, W., and Weigle, M. (1972) Science 178, 871–872
25. Provencher, S. W., and Glocner, J. (1981) Biochemistry 20, 33–37
26. Provencher, S. W. (1982) Comput. Physica Commun. 27, 229–242
27. Chou, P. Y., and Fassman, G. D. (1974) Biochemistry 13, 222–245
28. Garnier, J., Osguthorpe, D. J., and Robinson, B. (1978) J. Mol. Biol. 120, 97–120
29. Moore, S. K., Kozak, C., Robinson, E. A., Ullrich, S. J., and Appella, E. (1989) J. Biol. Chem. 264, 5343–5351
30. Yamamoto, M., Takahashi, Y., Inano, K., Horigome, T., and Sugano, H. (1991) J. Biochem. (Tokyo) 110, 141–145
31. Lees-Miller, S. P., and Anderson, C. W. (1989) J. Biol. Chem. 264, 2431–2437
32. Lansky, K. W. (1988) J. Cell. Physiol. 140, 601–607
33. Nishigori, H., and Toft, D. (1989) Biochemistry 28, 77–83
34. Nielsen, C. J., Sande, J. J., Vogel, W. M., and Pratt, W. B. (1977) J. Biol. Chem. 252, 753–758
35. VanRonen, R. I., Waymack, P. P., and Rehkop, D. M. (1974) J. Am. Chem. Soc. 96, 6782–6785
36. Nechay, H. R., Nisang, L. B., Nechay, P. S., Post, R. L., Grantham, J. J., Macara, I. G., Kubena, L. F., Phillips, T. D., and Nielsen, F. H. (1986) Fed. Proc. 45, 123–132
37. Dreyfuss, R., Maniachi, A., Levy, G. C., and Ejchart, A. J. (1985) Biochem. J. 230, 807–815
38. Lakowitz, J. R. (1983) Principles of Fluorescence Spectroscopy, Plenum Press, New York
39. Renoir, J.-M., Radanyi, C., Faber, L. E., and Baulieu, E.-E. (1990) J. Biol. Chem. 265, 10746–10745
40. Sanchez, E. R., Faber, L. E., Henzel, W. J., and Pratt, W. B. (1990) Biochemistry 29, 5145–5152
41. Sanchez, E. R. (1990) J. Biol. Chem. 265, 22067–22070
42. Perdue, G. H., and Whitehead, M. L. (1991) J. Biol. Chem. 266, 6706–6713
43. Dougherty, J. J., Rabideau, D. A., Ianetti, A. M., Sullivan, W. P., and Toth, D. O. (1987) Biochem. Biophys. Acta 927, 74–80
44. Walker, A. L., Hunt, T., Jackson, R. J., and Anderson, C. W. (1985) EMBO J. 4, 139–145
45. Lipich, L. A., Cut, J. R., and Brugge, J. S. (1982) Mol. Cell. Biol. 2, 875–880
46. Adams, B. Hunter, T., and Sefton, B. M. (1982) J. Biol. Chem. 257, 4485–4459
47. Ziemiecki, A., Catelli, M., Joab, I., and Monacharm, B. (1986) Biochem. Biophys. Res. Commun. 138, 1299–1307
48. Sadar, S., Raghadvendra, K., and Hightower, L. E. (1990) Biochemistry 29, 8199–8206
49. Woody, R. W. (1985) The Peptides, Vol. 7, Academic Press, Orlando, FL
50. Sureswiz, W. K., and Mantsch, H. H. (1988) Biochem. Biophys. Acta 952, 115–120
51. Schwab, D. A., and Wilson, J. E. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2563–2567
52. Banks, R. D., Blake, C. C. F., Evans, P. R., Hase, R., Rice, D. W., Hardy, G. W., Merrett, M., and Phillips, W. (1979) Nature 279, 773–777
53. Flaherty, K. M., DeLuca-Flaherty, C., and McKay, D. (1990) Nature 346, 625–635
54. Kabsch, W., Mannherz, H. G., Suck, D., Pai, E. F., and Holmes, K. C. (1990) Nature 347, 37–44
55. Mossak, V. K., and John, J. K. (1990) Biochem. J. 190, 799–806
56. Meshinchi, S., Matic, G., Hutchinson, K. A., and Pratt, W. B. (1991) J. Biol. Chem. 266, 11643–11649
57. Sando, J. J., Le Forest, A. C., and Pratt, W. B. (1979) J. Biol. Chem. 254, 4772–4778
58. Dorn, J. A., Wasily, L. C., and Kaufman, R. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7429–7432
59. Alfaro, C., and McMacken, R. (1989) J. Biol. Chem. 264, 10699–10708
60. Alfaro, C., and McMacken, R. (1989) J. Biol. Chem. 264, 10708–10718
61. Hwang, D. S., Cooke, E., and Kornberg, A. (1990) J. Biol. Chem. 265, 19244–19249
62. Beckmann, R. P., Mizzen, L. A., and Welch, W. J. (1990) Science 248, 850–854
63. Lee, M. J., and Pelham, H. R. B. (1985) EMBO J. 4, 3137–3145
64. Leustek, T., Toled, H., Brot, N., and Weissbach, H. (1991) Arch. Biochem. Biophys. 286, 256–261
65. Buxart, N., Chambray, B., Dumas, B., Rowlands, D. A., Bigogone, C., Levin, J. M., Garnier, J., Baulieu, E.-E., and Catelli, M. G. (1989) Biochem. Biophys. Res. Commun. 159, 140–147
66. Bork, P., Sander, C., and Valencia, A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7290–7294
67. Hutchinson, K. A., Stancato, L. F., Jove, R., and Pratt, W. B. (1992) J. Biol. Chem. 267, 13952–13957