Characterization of the nucleotide binding properties of the 90 kDa heat shock protein (Hsp90)

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The recent crystallization and structural analysis of the ATP(ADP)-complex of the N-terminal domain of the 90 kDa heat shock protein (Hsp90) confirmed our earlier findings on the ATP-binding properties of Hsp90. Here we further characterize the nucleotide binding of Hsp90 by demonstrating that surface plasmon resonance measurements also indicate a low-affinity binding of ATP to Hsp90 and that [α-^{32}P]ATP seems to have an equal preference for monomers, dimers and oligomers of Hsp90 on native polyacrylamide gels. Finally we discuss some of our results which raise the possibility that Hsp90 has two nucleotide binding sites (one in its N-terminal and another in the C-terminal domain) and that the nucleotide binding to Hsp90 dimers may display a positive cooperativity under some special conditions. The submillimolar binding affinity of ATP to Hsp90 allows the regulation of some Hsp90-related functions just in the range of ATP-level fluctuations during stress or during the cell cycle.

1. Introduction

Molecular chaperones mediate the folding, assembly, transport and disposal of other proteins (Hartl 1996). Chaperones were highly conserved during the evolution and probably played a major role in the development of modern enzymes (Csermely 1997). The 90 kDa heat shock protein (Hsp90) is a ubiquitous molecular chaperone believed to play an organizational role in protein traffic (Pratt 1997; Csermely *et al* 1998).

In our earlier studies we have demonstrated that Hsp90 binds ATP, has an autokinase activity and that ATP induces a conformational change of the protein (Csermely and Kahn 1991; Csermely *et al* 1993). Hsp90 also possesses an associated ATP/GTPase activity (Nadeau *et al* 1992, 1993; Nardai *et al* 1996). Unlike in other chaperone families, such as those of Hsp60 and Hsp70 (Hartl 1996), ATP does not seem to play an important role in the chaperone function of Hsp90 (Miyata and Yahara 1992; Wiech *et al* 1992; Jakob and Buchner 1994). These findings and the low-affinity ATP-binding of Hsp90 led to the questioning of ATP-binding properties of Hsp90 (Jakob *et al* 1996). However, ATP induces the dissociation of Hsp90 from F-actin (Kellermayer and Csermely 1995) and was shown to be necessary for the interaction of Hsp90 and p23, another chaperone of the Hsp90-associated foldosome (Johnson *et al* 1996; Sullivan *et al* 1997). Recently the ATP- and ADP-complexes of the N-terminal domain of Hsp90 have been crystallized (Prodromou *et al* 1997) and methods with higher resolution also confirmed the binding of various nucleotides to Hsp90 (Scheibel *et al* 1997; Freitag *et al* 1997).

In our present studies we further characterize the nucleotide binding of Hsp90 using surface plasmon resonance and binding of radioactive ATP to Hsp90 on native polyacrylamide gels. Some of our data raise the possibility of a second nucleotide binding site presumably on the C-terminus of Hsp90 and indicate a cooperative interaction of the two or four nucleotide binding sites of the Hsp90-dimer.

2. Materials and methods

2.1 *Chemicals*

The chemicals used for polyacrylamide gel electrophoresis, Econo-Pac HTP and Q FPLC cartridges were

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from Bio-Rad (Richmond, CA, USA). Butyl-Sepharose 4B, DEAE-Sepharose Fast Flow and Sephacryl S-200 were purchased from Pharmacia LKB Biotechnology Inc. (Uppsala, Sweden). Chemicals for the coupling of Hsp90 to the surface plasmon resonance sensor chips were purchased from Pharmacia Biosensor AB (Uppsala, Sweden). [α-^{32}P]ATP (111 TBq/mmol) was from the Institute of Isotopes Co. Ltd. (Budapest, Hungary). All the other chemicals used were from Sigma Chemicals Co. (St. Louis, MO, USA).

2.2 *Isolation of Hsp90*

The 90 kDa heat shock protein was purified from rat liver using consecutive chromatographies on Butyl-Sepharose 4B, DEAE-Sepharose Fast Flow, Econo-Pac HTP and Q FPLC chromatography as described earlier (Csermely and Kahn 1991). The purity of these Hsp90 preparations was more than 95% as judged by silver staining of SDS polyacrylamide gels. Protein concentrations were determined according to Bradford (1976).

2.3 *Surface plasmon resonance measurements*

Surface plasmon resonance of carboxymethyl-dextrane coupled Hsp90 was measured using a BIA-Core apparatus as described earlier (Csermely *et al* 1997). Briefly, carboxymethyl-dextran coated sensor chip was activated with 200 mM of 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide and 50 mM N-hydroxy-succinimide. Rat Hsp90 (protein concentration: 30 µg/ml) was coupled to the chip in a buffer containing 10 mM Na-formate, pH 4·0, for 7 min at 25°C at a flow rate of 5 µl/min. The remaining active sites of the resin were inactivated by incubation with a 1 M ethanolamine. HCl solution (pH 8·5) for 7 min at 25°C (flow rate: 5 µl/min). After coupling the non-covalently bound Hsp90 was removed by 2 or 3 brief (2 min) fluxes of 20 mM HCl. The usual change in the angle of the SPR minimum of the reflected light after coupling of Hsp90 was approximately 1°, i.e. 10,000 resonance units corresponding to 10 ng Hsp90/mm^2 sensor chip (Johnsson *et al* 1991).

Binding of ATP to Hsp90 was analysed in a running buffer containing 10 mM Hepes, pH 7·4, 0·15 M KCl and 0·001% Tween-20. Binding experiments were repeated with "mock-coupled" sensor chips, where Hsp90 was omitted in the coupling procedure. ATP displayed a negligible binding in these control experiments. The portion of the sensorgrams which corresponds to the dissociation of ATP from Hsp90 was analysed by the BIA evaluation program 2·1 to obtain k_d. Experimental data fit well the single exponential kinetic model used. Experiments have been repeated at a flow rate of 10 µl/min to check the effect of possible rebinding. Rebinding and mass transfer limitation was also checked by repetition of the experiments at lower coupling ratios of Hsp90. Identical results under these conditions showed that these phenomena did not occur under the conditions we used.

2.4 *Binding of [α-^{32}P]ATP to Hsp90*

Eight µg of purified Hsp90 was incubated with 200 µM ATP (containing 5 to 10 µCi of [α-^{32}P]ATP) in 20 µl of 50 mM Hepes, pH 7·4, 5 mM $CaCl_2$ buffer for 30 min

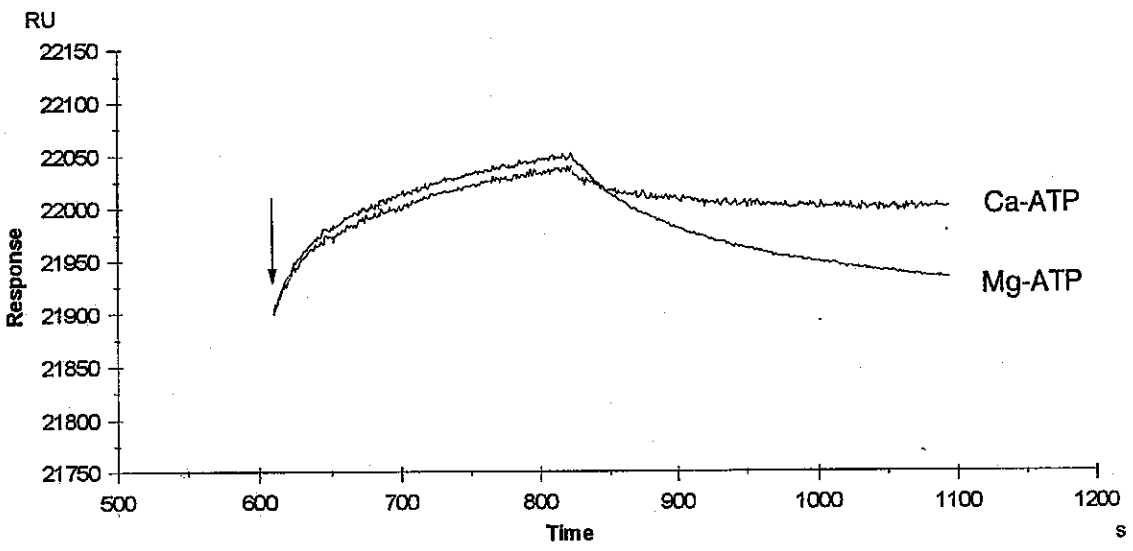

Figure 1. Surface plasmon resonance analysis of Hsp90-ATP interactions. Rat liver Hsp90 was immobilized and the surface plasmon resonance measurements were performed as described in § 2. A 4 min pulse of 4 mM ATP was initiated at the arrow in the presence of 5 mM $MgCl_2$ or $CaCl_2$ as indicated. The sensorgram is a representative of 3 independent experiments.

at 37°C after a 5 min preincubation in the absence or presence of competitors. After addition of 5 µl Laemmli buffer without sodium dodecyl sulphate and dithiothreitol samples were placed on ice and loaded immediately to a 6% native polyacrylamide gel with a discontinuous Laemmli buffer system without sodium dodecyl sulphate (Laemmli 1970). Gels were run at 4°C at a constant current of 15 mA/gel. Bands of Hsp90 oligomers were visualized by Coomassie blue staining. Destaining by 7·5% acetic acid was continued until the radioactivity of the washing solution decreased to the background level. After drying, gels were subjected to autoradiography (1 to 3 days exposure).

3. Results and discussion

3.1 *Binding of ATP to Hsp90*

Binding of ATP to Hsp90 has been characterized by a novel method, viz., surface plasmon resonance. This method, which measures the changes in the refractive index of carboxymethyl-dextrane immobilized Hsp90 and of the compounds associated with this chaperone has already been successfully applied to characterize Hsp90-protein or Hsp90-vanadate interactions (Miyata and Yahara 1995; Csermely *et al* 1997; Sőti *et al* 1998). Figure 1 shows the changes of Hsp90 surface plasmon resonance after a 4 min pulse of 4 mM ATP in the presence of 5 mM $CaCl_2$ or $MgCl_2$. The maximal change in the deflection of the reflected light has been calculated as 180 ± 30 resonance units (0·018° ± 0·003°). This would correspond to 180 pg ATP/mm^2 sensor chip, or to 2·7 ± 0·5 mol ATP/mol Hsp90. Though changes in surface plasmon resonance may arise by changes in the refractive index of Hsp90 itself after ATP addition (a phenomenon likely to occur due to the ATP-induced conformational change of the protein; Csermely *et al* 1993), or from the increased binding of ions, buffer components or Tween-20 to Hsp90 after ATP-addition, the magnitude of surface plasmon resonance changes suggests a stoichiometry of the ATP/Hsp90 complex higher than 1 : 1. The association of Mg-ATP and Ca-ATP is rather similar to Hsp90. However, Mg-ATP tends to dissociate at a much faster speed from the protein than Ca-ATP. The looser binding of Mg-ATP than Ca-ATP to Hsp90 is in agreement with our earlier findings showing a minimum two-fold higher dissociation constant for the Mg-ATP/Hsp90 complex than for Ca-ATP/Hsp90 (Csermely and Kahn 1991; Csermely *et al* 1993). Due to the tighter binding of Ca-ATP to Hsp90, ATP binding has been usually analysed in the presence of calcium, in the subsequent experiments.

Binding of ATP to various oligomeric forms of Hsp90 was assessed by analysing the interaction of Hsp90 complexes with [α-^{32}P]ATP. Figure 2 shows the Coomassie blue-stained gel pattern and the corresponding autoradiogram of rat liver Hsp90 incubated with [α-^{32}P]ATP. In agreement with earlier findings (Minami *et al* 1991) the majority of Hsp90 formed dimers under physiological conditions. A minor amount of monomers, tetramers, octamers and higher oligomers could also be detected. Quantitative analysis of the protein and protein-bound ATP revealed that there was no major preference of radioactive ATP binding to the monomeric, dimeric and oligomeric structures of Hsp90 (table 1). Excess of cold ATP and ADP efficiently competed with the binding of radioactive ATP (figure 2).

3.2 *Possible properties of Hsp90 nucleotide binding*

Prodromou *et al* (1997) reported the crystallization of the ATP- and ADP-complex of the N-terminal domain of Hsp90 establishing a non-canonical ATP-binding site in this segment of the protein. Our earlier assumption predicted a nucleotide binding site in the C-terminus of Hsp90 (Csermely and Kahn 1991). Alhough the significance of the homology between this site and the usual Walker-type ATP-binding sites of other molecular chaperones has been questioned in later studies (Jakob *et al* 1996), a low affinity binding of ATP and other

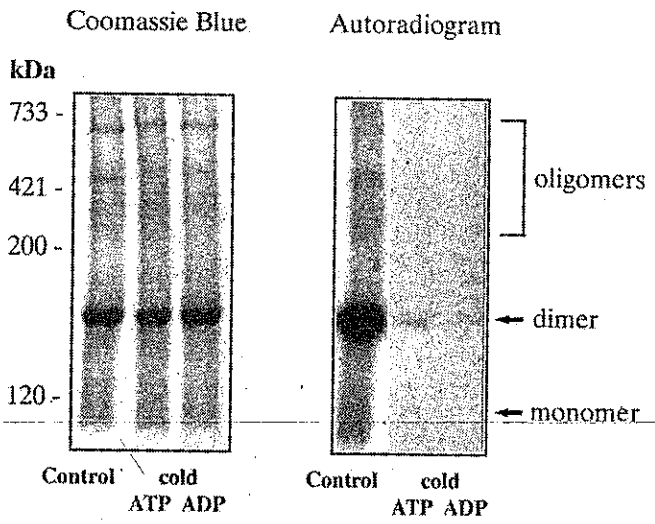

Figure 2. Binding of [α-^{32}P]-ATP to Hsp90. Eight µg purified rat liver Hsp90 was incubated with [α-^{32}P]ATP and analysed by native polyacrylamide gel electrophoresis as described in § 2. Molecular weight markers indicate the position of Pharmacia high molecular weight Gel-Filtration Calibration Kit markers. Positions of Hsp90 monomers, dimers, tetramers, hexamers, octamers and higher oligomers are marked. Cold ATP and ADP were added at final concentrations of 10 mM each. The Coomassie blue stained gel picture and the corresponding autoradiogram are representatives of 4 experiments.

Table 1. Quantitative evaluation of [α-^{32}P]ATP binding to various oligomeric forms of Hsp90.

	Monomer	Dimer	Tetramer	Octamer and hexamer
	(Expressed as % of total)			
Protein	11·8 ± 0·3	73·9 ± 2·8	10·6 ± 2·2	3·6 ± 0·5
Bound [α-^{32}P]ATP	8·3 ± 1·6	83·5 ± 1·2	5·8 ± 0·7	2·4 ± 0·7

Binding of [α-^{32}P]ATP to Hsp90 was analysed as described in § 2. Bands corresponding to Hsp90 oligomers on Coomassie blue-stained gels and to bound ATP on autoradiograms of four experiments were quantified using a Pharmacia LKB laser-densitometer.

Table 2. Comparison of apparent Hill coefficients of Hsp90/ATP interactions.

Interaction	Hill coefficient	Correlation
Conformational change—Ca-ATP (Csermely *et al* 1993)	0·93	0·98
Conformational change—Mg-ATP (Csermely *et al* 1993)	1·65	0·98
Autophosphorylation—Ca-ATP (Csermely and Kahn 1991)	0·93	0·97
Hydrolysis of methylfluorescein phosphate—Ca-ATP inhibition (Nardai *et al* 1996)	0·78	0·93

Apparent Hill coefficients were determined using the equation $\lg(y/1-y) = n_H \times \lg c$ where "y" is the measure of the interaction, "n_H" is the Hill coefficient and "c" is the concentration of ATP (Endrenyi *et al* 1975; Weiss 1997). Apparent Hill and correlation coefficients were calculated from a linear fit to the Hill plot of 3 to 10 measurements originally published in references in parentheses.

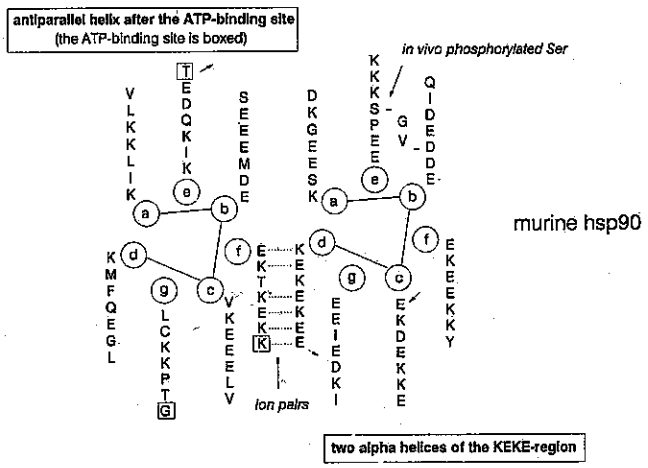

Figure 3. Putative ion-paired alpha-helical structures of Hsp90 around the consensus C-terminal nucleotide-binding sequence (Csermely and Kahn 1991) of the protein. Alpha-helical prediction has been made by the method of Chou and Fasman (1974) using the murine Hsp90 sequence described by Moore *et al* (1989).

nucleotides might occur at this putative second nucleotide binding site of the protein.

In the present studies our surface plasmon resonance measurements indicating a higher stoichiometry of ATP binding to Hsp90 monomers than 1 : 1, together with the scattered recovery of radioactivity of [α-^{32}P]-azido-ATP label in Hsp90 tryptic fragments (data not shown) might suggest that Hsp90 has more than one nucleotide binding sites.

Interestingly, a positive synergism could be observed by monitoring the tryptophane fluorescence of Hsp90, where sequential addition of Mg-ATP and Mg-ADP induced a significant increase in the fluorescent signal (data not shown). Analysis of the Hill plots of various Hsp90-ATP interactions also suggest a positive cooperativity in the effects of Mg-ATP (table 2). Binding of nucleotides to Hsp90 may reflect a cooperation between the two N-terminal nucleotide binding sites of the Hsp90-dimer. However, since the Hsp90-dimers are connected by their C-termini (Minami *et al* 1994) and the recently observed dimerization of the N-terminal domains (Prodromou *et al* 1997) might occur because of the N-terminal truncation of Hsp90, cooperation of N-terminal ATP-binding sites would imply a cooperative behaviour of sites several nm-s apart from each other. In case a C-terminal nucleotide binding site of Hsp90 were established, a cooperation of the N- and C-terminal nucleotide binding sites would be a much more likely phenomenon to occur. Interestingly, the N-terminal nucleotide binding site identified by Prodromou *et al* (1997) resembles more to the "regulatory" ATP-binding sequences of e.g., aspartate carbamoyl transferase than to the catalytic sites of usual kinases (Traut 1994).

Noteworthyly, ATP-binding to Hsp90 behaves several times as a non-reversible phenomenon. As an example for this the ATP-dependent binding of p23 to Hsp90 requires a prolonged incubation with ATP (Johnson *et al* 1996; Sullivan *et al* 1997). These observations might imply that a conformational change must preceed a higher affinity ATP-binding to Hsp90. This change may be

promoted by the occupancy of an ATP-binding site of the Hsp90-dimer. Interestingly, a predicted alpha-helix of the highly charged, middle "KEKE"-region of Hsp90 (Nardai *et al* 1996; Csermely *et al* 1998) has an almost complete complementary charge distribution with an alpha-helix containing the putative C-terminal ATP-binding site (figure 3). This raises the possibility of the formation of several ion-pairs hindering the binding of nucleotides to this putative second nucleotide binding site of Hsp90. The predicted ion-paired region is only 14 amino acids apart from the so-called "alpha-peptide" of Hsp90, a C-terminal peptidic segment forming the calmodulin binding site of the protein and inducing an "opening" of the Hsp90 structure which results in an increased ability to form higher oligomers (Minami *et al* 1993). In agreement with the proposed interaction between calmodulin, alpha-peptide and nucleotide binding, CTP has also been reported to induce the oligomerization of Hsp90 (Freitag *et al* 1997).

Nucleotide binding to Hsp90 occurs with a rather low affinity (Csermely and Kahn 1991; Csermely *et al* 1993; Nardai *et al* 1996; Scheibel *et al* 1997). The loose binding of ATP and GTP probably allows the use of the same nucleotide binding sites for the binding of other nucleotides such as CTP, UTP or NAD (Freitag *et al* 1997) and for the binding of nucleic acids such as DNA or RNA (Csermely *et al* 1994, 1998; Morcillo *et al* 1993; Szántó *et al* 1996; T Schnaider, E Nagy and P Csermely, unpublished observations). The functional interaction of Hsp90 with the non-protein coding heat shock RNA, hsr-omega has also been supported by genetic studies (Lakhotia and Ray 1996).

The submillimolar half-saturation values for ATP render Hsp90 an excellent subject for ATP-dependent regulation since this concentration range is involved in the stress- or cell cycle-induced changes of ATP-level of our cells (Kabakov and Gabai 1997; Marcussen and Larsen 1996). In the light of this possibility further studies exploring ATP-dependent interactions between Hsp90 and other proteins besides p23 and F-actin would be of high interest for a better understanding of the physiological function of this chaperone.

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References

Bradford M M 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding; *Anal. Biochem.* **72** 248–254

Chou P Y and Fasman G D 1974 Conformational parameters for amino acids in helical, beta-sheet and random coil regions calculated from proteins; *Biochemistry* **13** 222–245

Csermely P 1997 Proteins, RNA-s and chaperones in enzyme evolution: a folding perspective; *Trends Biochem. Sci.* **22** 147–149

Csermely P and Kahn C R 1991 The 90 kDa heat shock protein (Hsp90) possesses an ATP-binding site and autophosphorylating activity; *J. Biol. Chem.* **266** 4943–4950

Csermely P, Kajtár J, Hollósi M, Jalsovszky G, Holly S, Kahn C R, Gergely P Jr, Sőti Cs, Mihály K and Somogyi J 1993 ATP induces a conformational change of the 90 kDa heat shock protein (Hsp90); *J. Biol. Chem.* **268** 1901–1907

Csermely P, Kajtár J, Hollósi M, Oikarinen J and Somogyi J 1994 The 90 kDa heat shock protein (Hsp90) induces the condensation of the chromatin structure; *Biochem. Biophys. Res. Commun.* **202** 1657–1663

Csermely P, Miyata Y, Sőti Cs and Yahara I 1997 Binding affinity of proteins to Hsp90 correlates with both hydrophobicity and positive charges. A surface plasmon resonance study; *Life Sci.* **61** 411–418

Csermely P, Schnaider T, Sőti Cs, Prohászka Z and Nardai G 1998 The 90 kDa molecular chaperones: structure, function and clinical applications. A comprehensive review; *Pharmacol. Therapeut.* **79** 129–168

Endrenyi L, Fajszi Cs and Kwong F H F 1975 Evaluation of Hill slopes and Hill coefficients when the saturation binding or velocity is not known; *Eur. J. Biochem.* **51** 317–328

Freitag D G, Ouimet P M, Girvitz T L and Kapoor M 1997 Heat shock protein 80 of *Neurospora crassa*, a cytosolic molecular chaperone of the eukaryotic stress 90 family, interacts directly with heat shock protein 70; *Biochemistry* **36** 10221–10229

Hartl F-U 1996 Molecular chaperones in cellular protein folding; *Nature (London)* **381** 571–580

Jakob U and Buchner J 1994 Assisting spontaneity: the role of Hsp90 and small Hsps as molecular chaperones; *Trends Biochem. Sci.* **19** 205–211

Jakob U, Scheibel T, Bose S, Reinstein J and Buchner J 1996 Assessment of the ATP binding properties of Hsp90; *J. Biol. Chem.* **271** 10035–10041

Johnson J, Corbisier R, Stengard B and Toft D 1996 The involvement of p23, Hsp90, immunophilins in the assembly of progesterone receptor complexes; *J. Steroid Biochem. Mol. Biol.* **56** 31–37

Johnsson B, Löfas S and Lindquist G 1991 Immobilization of proteins to a carboxymethyldextran-modified gold surface for biospecific interaction analysis in surface plasmon resonance sensors; *Anal. Biochem.* **198** 268–277

Kabakov A E and Gabai V L 1997 *Heat shock proteins and cytoprotection: ATP-deprived mammalian cells* (Austin: Springer—RG Landes Co)

Kellermayer M S Z and Csermely P 1995 ATP induces dissociation of the 90 kDa heat shock protein (Hsp90) from F-actin: interference with the binding of heavy meromyosin; *Biochem. Biophys. Res. Commun.* **211** 166–174

Laemmli U K 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4; *Nature (London)* **227** 680–685

Lakhotia S C and Ray P 1996 hsp83 mutation is a dominant enhancer of lethality associated with absence of the non-protein coding hsr-omega locus in *Drosophila melanogaster*; *J. Biosci.* **21** 207–219

Marcussen M and Larsen P J 1996 Cell cycle-dependent regulation of cellular ATP concentration, and depolymerization of the interphase microtubular network induced by elevated cellular ATP concentration in whole fibroblasts; *Cell Motil. Cytoskel.* **35** 94–99

Minami Y, Kawasaki H, Miyata Y, Suzuki K and Yahara I 1991 Analysis of native forms and isoform compositions of the mouse 90-kDa heat shock protein; *J. Biol. Chem.* **266** 10099–10103

Minami Y, Kawasaki H, Suzuki K and Yahara I 1993 The calmodulin-binding domain of the mouse 90-kDa heat shock protein; *J. Biol. Chem.* **268** 9604–9610

Minami Y, Kimura Y, Kawasaki H, Suzuki K and Yahara I 1994 The carboxy-terminal region of mammalian Hsp90 is required for its dimerization and function; *Mol. Cell. Biol.* **14** 1459–1464

Miyata Y and Yahara I 1992 The 90 kDa heat shock protein, Hsp90, binds and protects casein kinase II from self-aggregation and enhances its kinase activity; *J. Biol. Chem.* **267** 7042–7047

Miyata Y and Yahara I 1995 Interaction between casein kinase II and the 90-kDa stress protein, Hsp90; *Biochemistry* **34** 8123–8129

Moore S K, Kozak C, Robinson E-A, Ullrich S J and Appella E 1989 Murine 86- and 84-kDa heat shock proteins, cDNA sequences, chromosome assignments, and evolutionary origin; *J. Biol. Chem.* **264** 5343–5351

Morcillo G, Diez J L, Carbajal M E and Tanguay R M 1993 Hsp90 associates with specific heat shock puffs (hsr omega) in polytene chromosomes of *Drosophila* and *Chironomus*; *Chromosoma* **102** 648–659

Nadeau K, Sullivan M A, Bradley M, Engman D M and Walsh C T 1992 83-kilodalton heat shock proteins of trypanosomes are potent peptide-stimulated ATPases; *J. Prot. Sci.* **1** 970–976

Nadeau K, Das A and Walsh C T 1993 Hsp90 chaperonins possess ATPase activity and bind heat shock transcription factors and peptidyl-prolyl isomerases; *J. Biol. Chem.* **268** 1479–1487

Nardai G, Schnaider T, Sőti Cs, Ryan M T, Hoj P B, Somogyi J and Csermely P 1996 Characterization of the 90 kDa heat shock protein-(Hsp90)-associated ATP/GTP-ase; *J. Biosci.* **21** 171–190

Pratt W B 1997 The role of Hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling *via* MAP kinase; *Annu. Rev. Pharmacol. Toxicol.* **37** 297–326

Prodromou C, Roe S M, O'Brien R, Ladbury J E, Piper P W and Pearl L H 1997 Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone; *Cell* **90** 65–75

Scheibel T, Neuhofen S, Weikl T, Mayr C, Reinstein J, Vogel P D and Buchner J 1997 ATP-binding properties of human Hsp90; *J. Biol. Chem.* **272** 18606–18613

Sőti Cs, Radics L, Yahara I and Csermely P 1998 Interaction of vanadate oligomers and permolybdate with the 90 kDa heat shock protein, Hsp90; *Eur. J. Biochem.* (in press)

Sullivan W P, Stensgard B, Caucutt G, Bartha B, McMahon N, Alnemri E S, Litwack G and Toft D O 1997 Nucleotides and two functional states of Hsp90; *J. Biol. Chem.* **272** 8007–8012

Szántó I, Schnaider T and Csermely P 1996 Topoisomerase activity of the 90 kDa heat shock protein (Hsp90); *Cell Biol. Int.* **20** 246–247

Traut T W 1994 The functions and consensus motifs of nine types of peptide segments that form different types of nucleotide-binding sites; *Eur. J. Biochem.* **222** 9–19

Weiss J N 1997 The Hill equation revisited: uses and misuses; *FASEB J.* **11** 835–841

Wiech H, Buchner J, Zimmermann R and Jakob U 1992 Hsp90 chaperones protein folding in vitro; *Nature (London)* **358** 169–170