Localization of calponin binding sites in the structure of 90 kDa heat shock protein (Hsp90)

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Received 21 July 1999

Abstract The structure of rabbit liver Hsp90 was reevaluated by limited trypsinolysis, N-terminal sequencing and determination of the site that is phosphorylated by casein kinase II. Limited proteolysis results in formation of four groups of large peptides with M_r in the range of 26–41 kDa. Peptides with M_r 39–41 kDa were represented by large N-terminal and central peptides starting at residue 283 of the α-isoform of Hsp90. All sites phosphorylated by casein kinase II were located in the large 39–41 kDa peptides. Peptides with M_r 26–27 kDa were represented by short N-terminal and central peptides starting at Glu-400 of the α-isoform of Hsp90. The data of affinity chromatography and light scattering indicate that smooth muscle calponin interacts with Hsp90. The calponin binding sites are located in the large (37–41 kDa) N-terminal and in a short (26–27 kDa) central peptide starting at Glu-400 of the α-isoform of Hsp90. Phosphorylation by casein kinase II up to 2 mol of phosphate per mol of Hsp90 does not affect interaction of Hsp90 with calponin.

Key words: Heat shock protein; Phosphorylation; Casein kinase; Calponin

1. Introduction

The cytosolic 90 kDa heat shock protein (Hsp90) is highly conserved, constitutively expressed abundant protein of eukaryotes [1,2]. Hsp90 interacts with many proteins and in this way prevents their aggregation, provides for proper folding or is involved in trafficking of certain proteins to their destination [1,2]. Localization of the sites involved in the interaction of Hsp90 with its substrate proteins is important for understanding of the molecular mechanism of action of this chaperone. Hsp90 interacts with steroid receptors and it is believed that at least two sites located in the N-terminal and central [3] or in the central and C-terminal [4] parts of Hsp90 participate in this interaction. Hsp90 interacts with a number of protein kinases [1,2], but the sites of its interaction with protein kinases are not precisely determined. Calmodulin binds to Hsp90 and the calmodulin binding sites of mouse Hsp90 are located in the central part of the molecule [5,6]. Intact Hsp90 prevents aggregation of denatured rhodanase. Two short peptides containing N-terminal (residues 1–236) or C-terminal (residues 629–732) fragments of Hsp90 are also effective in preventing aggregation of denatured rhodanase [7]. Although both the N-terminal and C-terminal fragments of Hsp90 prevent aggregation of denatured luciferase, only the C-terminal fragment of Hsp90 maintains the unfolded luciferase in a state competent for refolding [7]. Similar results were obtained on denatured insulin and citrate synthase [8]. These experiments indicate that for some protein substrates there are two independent chaperone sites located in the N- and C-terminal parts of Hsp90 [7,8].

It is well known that Hsp90 interacts with the cytoskeleton [2]. Hsp90 binds actin [9] and under certain conditions associates with microtubules, intermediate filaments and microfilaments [10]. Smooth muscle calponin is an actin binding protein which seems to be involved in the regulation of actomyosin ATPase, polymerization of G-actin and reorganization of the cytoskeleton [11]. In addition calponin is somehow involved in targeting of protein kinase C and mitogen-associated protein kinase to the cell membrane after chemical stimulation [12]. Taking into account the high concentration of Hsp90 in the cell [13] and its preferential interaction with basic substrates [14] we supposed that Hsp90 interacts with calponin and in this way may modulate calponin-induced reorganization of actin filaments [15,16]. We found that calponin can be crosslinked to Hsp90 [15]. Binding to Hsp90 affects calponin-induced bundling of actin filaments [16] and polymerization of G-actin (unpublished results). Thus, our in vitro results indicate that calponin may interact with Hsp90 and this interaction may be of physiological significance. In order to understand the mechanism of interaction of calponin with Hsp90 we tried to localize the calponin binding site of Hsp90 and to analyze the effect of phosphorylation of Hsp90 on its interaction with calponin.

2. Materials and methods

Rabbit liver Hsp90, duck gizzard calponin and rat liver casein kinase II were purified by earlier described methods [15,17,18]. Protein concentration was determined spectrophotometrically taking $E_{280}^{0.1\%}$ equal to 0.86 for Hsp90 [19] and 0.78 for calponin [20]. In some experiments the protein concentration was determined by the method of Spector [21].

Two methods were used for analyzing the interaction of Hsp90 with calponin. Binding of calponin to Hsp90 was followed by measuring light scattering at 340 nm a in Hitachi F-3000 spectrofluorometer. Aliquots of calponin were added to 1 ml samples containing 20 mM Tris-HCl, pH 7.4, in the presence or absence of Hsp90. The increase in light scattering (ΔI_s) was plotted against the total concentration of calponin added. Affinity chromatography was also used for analysis of the calponin-Hsp90 interaction. Calponin was immobilized on CNBr-activated Sepharose (Pharmacia) according to the procedure recommended by the supplier. Under the conditions used 2 mg of calponin were bound per 1 g of dry Sepharose. Phosphorylated or unphosphorylated Hsp90 (0.2–0.3 mg) was loaded on the column of calponin-Sepharose (3.0 ml) equilibrated by 20 mM Tris-HCl, pH 7.5, 75 mM NaCl. The column was washed by equilibrating buffer and

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Abbreviations: PVD, polyvinylidene difluoride; TFA, trifluoroacetic acid

0014-5793/99/$20.00
PII: S0014-5793(99)01056-X

Hsp90 bound to the column was eluted by the same buffer containing 300 mM NaCl. Since the samples of Hsp90 loaded on the column contained ATP, elution was followed by measuring the protein concentration by the dye binding method of Spector [21]. When the tryptic digest of Hsp90 was subjected to affinity chromatography the column of calponin-Sepharose was equilibrated by 20 mM Tris-HCl containing 25 mM NaCl and peptides bound to the matrix were eluted by the same buffer containing 300 mM NaCl. The composition of the fractions loaded on the affinity matrix and interacting with immobilized calponin was determined by SDS-gel electrophoresis and the peptides were detected on the gel using a silver staining kit (Sigma).

Phosphorylation of Hsp90 (0.4 mg/ml) by casein kinase II (0.01 mg/ml) was performed in 8 mM Tris-12 mM KH_2PO_4 (pH 7.0), 5 mM $MgCl_2$, 80–160 μM ATP containing [γ-^{32}P]ATP (0.1 mCi/ml). The samples were incubated at 37°C for 45–60 min and the reaction was stopped by addition of 1/10 volume of heparin-Sepharose. Casein kinase II bound to heparin-Sepharose was removed by low speed centrifugation. Phosphorylated Hsp90 was either directly loaded on the column of calponin-Sepharose or subjected to trypsinolysis. In order to analyze the effect of phosphorylation of Hsp90 on its interaction with calponin we loaded both phosphorylated and unphosphorylated Hsp90 on the column of calponin-Sepharose. The unphosphorylated Hsp90 was obtained as follows. Hsp90 was incubated in the buffer of phosphorylation without ATP and casein kinase II under conditions described earlier. ATP was added to the sample of unphosphorylated Hsp90 just before loading on the column of calponin-Sepharose or just before titration by calponin in the light scattering experiments.

Limited trypsinolysis was performed in 20 mM Tris-HCl, pH 7.5, 0.2 mM β-mercaptoethanol at a weight ratio of Hsp90/TPCK-treated trypsin equal to 150/1. The incubation lasted for 5–20 min at 35°C and the reaction was stopped by addition of phenylmethylsulfonyl fluoride (up to a final concentration of 1 mM). The peptide mixture was either directly subjected to SDS-gel electrophoresis [22] or separated by affinity chromatography on calponin-Sepharose which was followed by SDS-gel electrophoresis. After electrophoresis the fragments of Hsp90 were transferred to polyvinylidene difluoride (PVD) membranes (Sigma). The protein bands visualized with Coomassie R-250 were subjected to N-terminal sequencing with a model 491 cLC protein sequencer (Perkin-Elmer Applied Biosystems).

Exhaustive trypsinolysis of Hsp90 phosphorylated by casein kinase II was performed in 100 mM NH_4HCO_3, 5 mM $MgCl_2$ at a weight ratio of Hsp90/TPCK-treated trypsin equal to 50/1. After overnight incubation at 37°C the reaction was stopped by addition of a mixture of trifluoroacetid acid (TFA) and EDTA up to final concentrations of 1% and 1 mM respectively. The mixture of peptides was freeze-dried, dissolved in 0.05% TFA and loaded on an Ultrasphere ODS 18 column (4.6×250 mm). Elution by a linear gradient (0–50%) of acetonitrile in 0.05% TFA results in two major radioactive peaks. The first peak was rechromatographed on the same column using a shallow (0–21%) gradient of acetonitrile. The purified radioactive peptide was transferred to the PVD membrane and sequenced with 477 A protein sequencer (Applied Biosystems). The second peak was lost during further purification due to its poor solubility.

Since the complete primary structure of rabbit liver Hsp90 is unknown we used numbering corresponding to the primary structure of human α- or β-isoform of Hsp90 (P07900 or P08238 respectively) from the Swiss-Prot data bank. Computer analysis of the primary structure and physico-chemical properties of Hsp90 peptides was performed by the PC GENE program package (subprogram PHYSCHEM).

3. Results

3.1. Identification of tryptic peptides of Hsp90

Limited trypsinolysis of Hsp90 results in the formation of a number of peptides with M_r in the range of 14–41 kDa (Fig. 1A). These peptides can be separated into six groups. Four groups of high molecular weight peptides form doublets with M_r 39–41 kDa (group 1), 37–38 kDa (group 2), 31–32 kDa (group 3) and 26–27 kDa (group 4). Two groups of low molecular weight peptides migrate with M_r 17–18 kDa (group 5) and 14–15 kDa (group 6). The overall pattern of trypsinolysis is similar to that described in the literature [23–26]. However, there are still problems in attributing different peptides to certain domains in the Hsp90 structure. For example, peptides with M_r 39–41 kDa are supposed to be presented either by the N-terminal peptide [24,25] or by the peptides starting at Tyr-283 of the α-isoform of human Hsp90 [23]. Lees-Miller and Anderson [23] and Nemoto et al. [25] supposed that peptides with M_r 30–33 kDa are represented by the N-terminal peptides of Hsp90, whereas the data of Itoh and Tashima [24] indicate that this band contains peptides starting at Tyr-283. In order to localize the position of tryptic peptides in the structure of Hsp90 we determined the N-terminal sequence of some of these peptides.

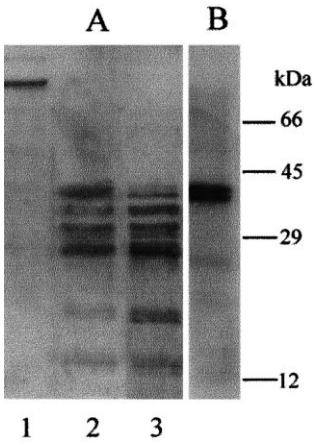

Fig. 1. Tryptic peptide mapping of rabbit liver Hsp90 phosphorylated by casein kinase II (about 1 mol of phosphate per mol of Hsp90). A: SDS-gel electrophoresis of intact Hsp90 (1) and its tryptic peptides obtained after 5 (2) and 20 (3) min incubation with trypsin. B: Autoradiogram of lane 3.

The first group of peptides with M_r 39–41 kDa revealed two sequences, namely PEETQ and YIDQX. These sequences correspond to the N-terminal peptide of the α-isoform of Hsp90 and to the peptide starting at Tyr-283 of the α- or Tyr-275 of the β-form of Hsp90 (Table 1). The peptides of the second group (M_r 37–38 kDa) form a rather diffuse zone on the SDS-gel electrophoresis and contained bands differently stained by silver. Since these peptides were not homogeneous and were poorly separated on the SDS-gel electrophoresis we did not try to determine their N-terminal sequence. (Affinity chromatography on calponin-Sepharose showed that peptides migrating in this zone were indeed heterogeneous. One component of this mixture is represented by the N-terminal peptide with the N-terminal sequence PEEVH corresponding to residues 1–5 of the β-isoform of Hsp90.) The third group of peptides with M_r 31–32 kDa was also heterogeneous. One of the components of this mixture revealed the sequence PEETQ once again corresponding to the N-terminus of the α-isoform of Hsp90. The N-terminal sequences of the fourth group of peptides migrating with M_r 26–27 kDa were EMLQQ and PXEVX. The first sequence corresponds to peptides starting at Glu-400 of the α- or Glu-392 of the β-isoform of human Hsp90. The second sequence (PXEVX) corresponds to the N-terminus of the β-isoform of Hsp90 (Table 1).

If Hsp90 phosphorylated by casein kinase II was subjected

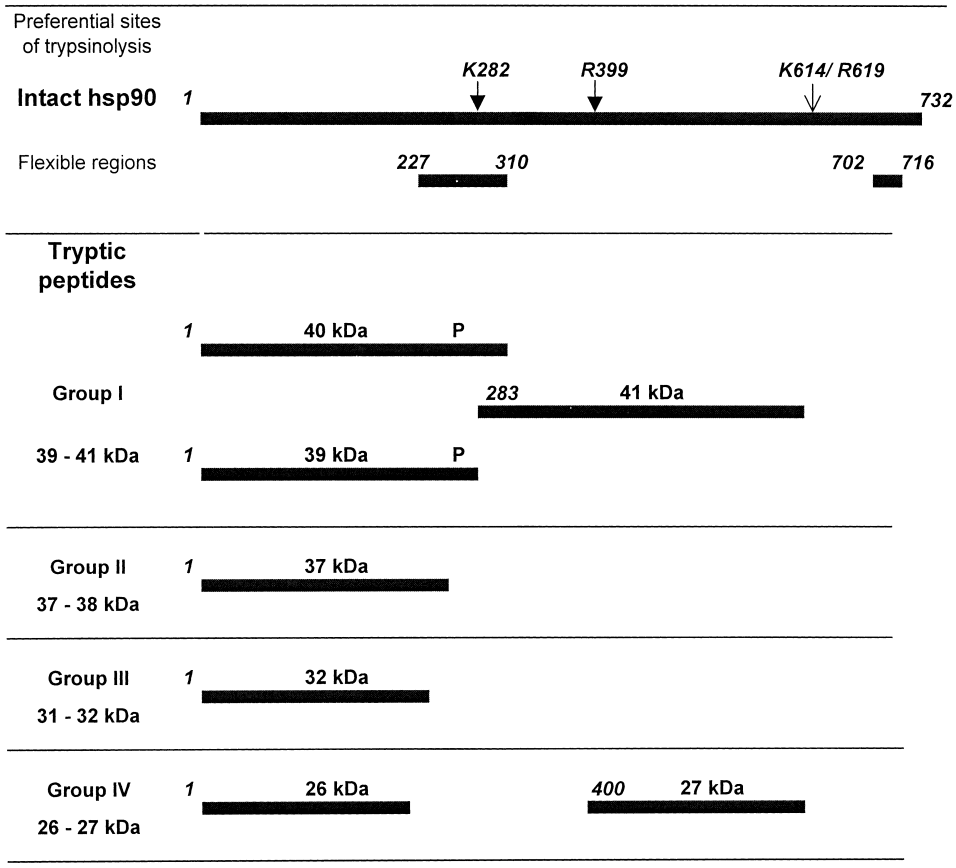

Fig. 2. Hypothetical scheme of trypsinolysis of the α-isoform of Hsp90. Full-length Hsp90 and its fragments are represented as proportional rectangles with their M_r values in kDa. Two heavy arrows (K282 and R399) indicate preferential sites of trypsinolysis detected in this paper. The light arrow (K614/K619) indicates the preferential site of trypsinolysis detected by Nemoto et al. [25]. Highly flexible immunogenic regions (227–310 and 702–716) were described by Nemoto et al. [25]. Black rectangles with labeling of N-terminal amino acids show peptides characterized in this paper. One of the sites phosphorylated by casein kinase II (Ser-262) is labeled with a P. Peptides with M_r 31–38 kDa (groups II and III) are heterogeneous, and only peptides with determined N-terminal sequence are represented in the scheme.

to limited trypsinolysis and subsequent SDS-gel electrophoresis the whole radioactivity was detected in the doublet of largest peptides with M_r 39–41 kDa (Fig. 1B). When Hsp90 phosphorylated by casein kinase II was exhaustively cleaved by trypsin and phosphorylated peptides were separated by reverse phase HPLC we detected two main radioactive peptides. About 15% of radioactivity incorporated by casein kinase II was detected in the peptide having the N-terminal sequence IEDVX. This sequence corresponds to the tryptic peptide starting at Lys-248 of the β-isoform of human Hsp90. This peptide contains Ser-254 in the sequence SDEED that ideally corresponds to the motif phosphorylated by casein kinase II [27]. The α-isoform of Hsp90 has an identical primary structure at Ser-262, which is homologous to Ser-254 of the β-isoform. The second radioactive peptide was poorly soluble and we failed to determine its primary structure. In any case, our data mean that at least one site phosphorylated by casein kinase II is located in the N-terminal part of Hsp90.

Based on the data of SDS-gel electrophoresis, N-terminal sequencing and location of the sites of phosphorylation we tried to reevaluate the localization of tryptic peptides in the primary structure of Hsp90. The data in the literature [23–25] indicate that there are three main sites of trypsinolysis located approximately at residues 282, 399 and 614/616 of the α-isoform of Hsp90 (Fig. 2). The cleavage at these sites produces N-terminal (residues 1–282, 1–399), central (residues 283–614/616, 400–614/616) and C-terminal (residues 615/620–732) peptides. The C-terminal end of the large N-terminal peptides is susceptible to further trypsinolysis and contains highly flexible and surface-exposed region (residues 227–310) [25]. This leads to accumulation of a number of N-terminal peptides with M_r in the range of 26–41 kDa (Fig. 2). One of the sites phosphorylated by casein kinase II is Ser-254/Ser-262 of Hsp90. This means that all N-terminal peptides containing the first 260–270 residues with an estimated molecular weight of 30–32 kDa should contain radioactive phosphate. In contrast, we found that only peptides with M_r 39–41 kDa contained sites of phosphorylation whereas the second and the third groups of peptides with M_r 31–38 kDa (containing among others the N-terminal fragments) were not radioactive (Fig. 1B). This discrepancy can be explained by the fact that the N-terminal part of Hsp90 is highly acidic (the calculated pI value of the peptide containing residues 1–300 of the α-isoform of Hsp90 is equal to 4.44). Acidic proteins anomalously interact with SDS and have a lower mobility on SDS-gel electrophoresis [28]. Therefore we might expect that the N-terminal peptides would migrate with an anomalously high molecular weight on SDS-gel electrophoresis. Indeed we found that the N-terminal peptide containing 282 residues and the central peptide (residues 283–614/619) containing more than 330 residues (Fig. 2)

Table 1
N-terminal sequencing of proteolytic fragments of rabbit liver Hsp90

Peptide M_r (kDa)	Detected amino acids	Corresponding sequence in Hsp90
39–41	PEETQ YIDQE	^{1}PEETQ^{5} (α-isoform) ^{283}YIDQE^{287} (α-isoform) ^{275}YIDQE^{279} (β-isoform)
37–38	Not determined	
31–32	PEETQ	^{1}PEETQ^{5} (α-isoform)
26–27	EMLQQ PXEVX	^{400}EMLQQ^{405} (α-isoform) ^{392}EMLQQ^{396} (β-isoform) ^{1}PEEVH^{5} (β-isoform)

migrated on SDS-gel electrophoresis with similar M_r of 39–41 kDa (Table 1, Fig. 2).

As mentioned earlier, two groups of peptides migrating with M_r 37–38 and 31–32 kDa contained a complex mixture of different peptides. N-terminal sequencing revealed the presence of the N-terminal peptides in both 37–38 and 31–32 kDa bands, but we cannot exclude the presence of other peptides in these mixtures. The fourth group of peptides migrating with M_r 26–27 kDa on SDS-gel electrophoresis may also consist of a mixture of short N-terminal and central peptides containing residues 400–614/616 (Table 1, Fig. 2).

3.2. Localization of calponin binding sites of Hsp90

The method of affinity chromatography was used for localization of calponin binding sites of Hsp90. To avoid weak electrostatic interaction between acidic Hsp90 (p*I* 5.4) and basic calponin (p*I* 9.0) we equilibrated the column of calponin-Sepharose with buffer containing 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. If the protein mixture containing Hsp90 and a five-fold weight excess of bovine serum albumin was loaded on the calponin-Sepharose, Hsp90 was quantitatively retarded on the column whereas the vast majority of albumin was detected in the flow-through. Thus, among two proteins

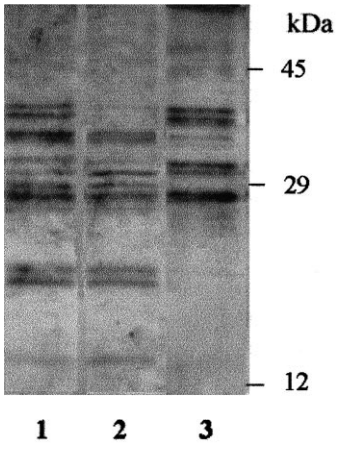

Fig. 3. Identification of tryptic peptides of Hsp90 interacting with calponin. SDS-gel electrophoresis of the initial mixture of tryptic peptides of Hsp90 loaded on the calponin-Sepharose column (1), peptides which were not retarded on the affinity matrix (2) and peptides bound on calponin-Sepharose and eluted by 300 mM NaCl (3).

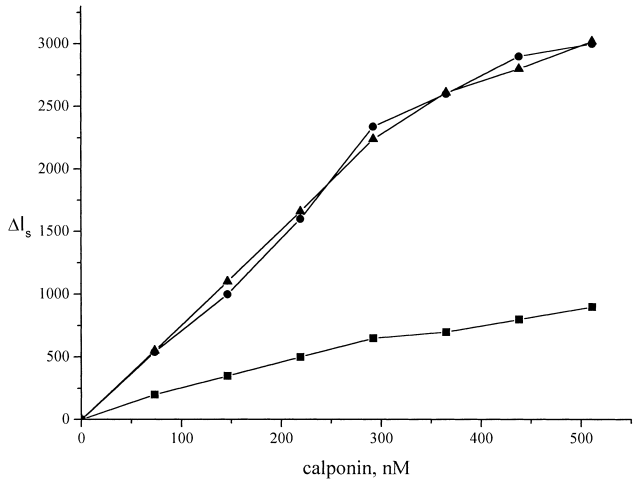

Fig. 4. Titration of 400 nM unphosphorylated (circles) and phosphorylated (2 mol phosphate/mol protein) (triangles) Hsp90 by calponin. Light scattering induced by addition of calponin to the control sample without Hsp90 is shown by the curve labeled by squares. The increase in light scattering at 340 nm (ΔI_s) is plotted against the total concentration of calponin added.

having similar p*I* only Hsp90 was specifically bound to calponin-Sepharose. Therefore affinity chromatography seems to be the method of choice for determination of calponin binding sites of Hsp90.

When the mixture of tryptic peptides of Hsp90 was loaded on the column, only high molecular weight peptides were bound to the affinity matrix whereas all short tryptic peptides with M_r of 17–19 and 14–15 kDa were detected in the flow-through (Fig. 3). The peptides retarded on the column were subjected to SDS-gel electrophoresis, transferred to the PVD membrane and sequenced on the protein sequencer. Analysis of the peptide doublet with M_r 39–41 kDa retarded on calponin-Sepharose revealed the sequence PEEVH corresponding to residues 1–5 of the β-isoform of Hsp90. Part of the peptides having M_r in the range of 37–38 kDa was not bound to immobilized calponin (Fig. 3). The portion of these peptides retarded on the affinity matrix has the N-terminal sequence PEEVH. This means that these peptides also derive from the very N-terminal part of Hsp90. We detected multiple peptides with M_r in the range of 31–32 kDa (Fig. 3). Part of these peptides were not bound to immobilized calponin, whereas other peptides having a similar molecular weight were tightly bound to calponin. Unfortunately, this fraction was heterogeneous and we failed to determine its N-terminal sequence. Only one N-terminal sequence was detected for peptides having M_r 27 kDa and retarded on calponin-Sepharose. The sequence EMLQQ corresponds to peptides starting at residue 392 of the β- or 400 of the α-isoform of Hsp90.

The data presented mean that the large N-terminal peptides having M_r 37–41 kDa and a rather short (M_r 27–29 kDa) peptide derived from the central portion of the molecule and starting at residues 392/400 of Hsp90 were able to interact with calponin.

3.3. Effect of phosphorylation of Hsp90 on its interaction with calponin

Since the N-terminal part of Hsp90 containing the sites phosphorylated by casein kinase II seems to be involved in

the interaction with calponin, we analyzed the effect of phosphorylation on the binding of Hsp90 to immobilized calponin. Under the conditions used casein kinase II transferred more than 2 mol of phosphate per mol of Hsp90. All phosphate incorporated by casein kinase II was detected in the largest 39–41 kDa tryptic peptides of Hsp90 (Fig. 1).

When an excess of unphosphorylated Hsp90 in the presence of 0.1 mM ATP was loaded on the column of immobilized calponin (equilibrated by buffer containing 75 mM NaCl), about 80% was detected in the flow-through and 20% was bound to the affinity matrix (data not shown). Similar results were obtained with Hsp90 phosphorylated by casein kinase II. Thus, under the conditions used phosphorylation of Hsp90 does not affect its binding to immobilized calponin. Similar results were obtained by the method of light scattering. Titration of Hsp90 by calponin is accompanied by an increase in light scattering which is significantly larger than the scattering induced by addition of calponin to the control sample without Hsp90 (Fig. 4). Therefore we may suppose that the increase in light scattering reflects the interaction of calponin with Hsp90. Since the titration curves obtained with phosphorylated (2 mol phosphate per mol of protein) and non-phosphorylated Hsp90 were very similar, we suppose that phosphorylation by casein kinase II does affect the interaction of Hsp90 with calponin.

4. Discussion

Proteolysis was successfully used for analysis of the structure of Hsp90 [23–26]. It is supposed that there are two main sites of proteolytic cleavage of Hsp90 located around residues 360–400 and 600–620 [25,26]. Cleavage at these sites results in accumulation of large (40–50 kDa) N-terminal, intermediate (22–27 kDa) central and short (about 15 kDa) C-terminal fragments. One additional preferential site of cleavage detected in this investigation and in a number of other papers [23,24] is Lys-282 of the α-isoform of Hsp90 (see Fig. 2). The presence of at least three main sites of trypsinolysis together with the susceptibility of large tryptic fragments to further degradation from their C-terminal ends [25] leads to accumulation of a set of tryptic peptides with similar molecular weight derived from completely different regions of Hsp90. For instance, the largest 39–41 kDa peptides may be presented by both the N-terminal and central peptides (Fig. 2) and the same is true for practically all groups of peptides. This partly explains the discrepancy in the literature when peptides having similar molecular weights were attributed to different parts of Hsp90. For example the largest peptides with M_r 39–41 kDa were attributed to the N-terminal [25] or to the central part [23] of Hsp90.

In order to determine the sites of Hsp90 involved in the binding of calponin we used the method of affinity chromatography (Fig. 3). The peptides retarded on immobilized calponin derived from the N-terminal (peptides with M_r 37–41 kDa) or from the central (peptides with M_r 26–27 kDa) part of Hsp90 (Figs. 2 and 3). As mentioned earlier, the N-terminal part of Hsp90 is highly acidic and the p*I* value of the peptide containing the first 300 residues is equal to 4.44. Therefore binding of acidic N-terminal fragments of Hsp90 to basic calponin having p*I* 9.6 is at least partially based on electrostatic interactions. The calculated p*I* value of the central peptide of Hsp90 restricted by residues 400–614 is equal to 8.5. Therefore interaction of this peptide with calponin cannot be primarily dependent on electrostatic interactions.

At present it is more or less accepted that Hsp90 contains two chaperone sites in the N- and the C-terminal parts of the molecule [7,8] and some of the potential substrates have at least two binding sites on Hsp90 [3,4,7,8]. Our results with the extremely basic calponin corroborate this point of view and indicate that either Hsp90 has only one extended calponin binding site (covering approximately residues 1–620 of Hsp90) or two different sites located in the N-terminal and the central parts of molecule. We also cannot exclude the possibility that the calponin binding site of Hsp90 is formed by short stretches of amino acids belonging to the N-terminal and central peptides which approach each other in the tertiary structure of Hsp90.

Hsp90 is copurified with casein kinase II and this protein kinase seems to be involved in Hsp90 phosphorylation [29]. Lees-Miller and Anderson [23] found that Ser-231 and Ser-263 were the primary sites phosphorylated by casein kinase in the α-isoform of Hsp90, whereas Rose et al. [30] detected the sites of phosphorylation in the peptide corresponding to residues 55–68 of the β-isoform of Hsp90. In agreement with the data of Lees-Miller and Anderson [23], we found that one of the sites phosphorylated in Hps90 indeed corresponded to Ser-254 of the β- or to Ser-263 of the α-isoform of Hsp90. In our case radioactive phosphate was detected only in a rather large N-terminal peptides with M_r 39–41 kDa. The mixture of tryptic peptides of Hsp90 contains a number of short N-terminal fragments (with M_r 26–32 kDa) but neither of them contained sites of phosphorylation (Figs. 1 and 2). These results contradict the data of Rose et al. [30] and indicate that the main sites of phosphorylation are located in the surface flexible region in the C-terminal part of the large N-terminal peptides of Hsp90.

Since the sites of phosphorylation were located in the N-terminal part of Hsp90 close to the potential sites of calponin binding we analyzed the effect of Hsp90 phosphorylation on its interaction with calponin. The data of affinity chromatography and light scattering indicate that phosphorylation of Hsp90 by casein kinase II (up to 2 mol of phosphate per mol of protein) has no significant effect on the binding of calponin (Fig. 4). Although the role of phosphorylation of Hsp90 remains enigmatic some data in the literature indicate that phosphorylation of Hsp90 may affect its interaction with $pp60^{src}$ [31]. We may suppose that either interaction of Hsp90 with calponin is independent of Hsp90 phosphorylation or protein kinases other than casein kinase II participate in the regulation of this interaction.

Acknowledgements: This work was supported by Grant 98-04-48116 from the Russian Foundation for Basic Research. The authors are grateful to Prof. J.H. Collins (Department of Biological Chemistry, School of Medicine, University of Maryland, Baltimore, MD, USA) and Dr. M.A. Krymsky (Institute of Experimental Cardiology, Russian Cardiological Research Center, Moscow, Russia) for their help in purification of peptides and their sequencing. The authors thank K. Gromadsky for his help in performing some of the experiments.

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