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THE 90 kDa HEAT SHOCK PROTEIN (hsp90) INDUCES THE CONDENSATION OF THE CHROMATIN STRUCTURE

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Summary: The 90 kDa heat shock protein (hsp90) is a member of the "chaperone-complex" of steroid receptors believed to be partially or transiently localized in the cell nucleus. Demonstrating that hsp90 has an ATP binding site and autophosphorylating activity we have observed that histones, especially histone H1, are able to modulate the autophosphorylation of hsp90 [*Csermely, P. and Kahn, C.R. (1991) J. Biol. Chem. 266, 4943-4950*]. Our present data suggest a direct interaction of hsp90 with histones, showing that hsp90 is able to bind histone-agarose and enhances the binding of histones to DNA. Circular dichroism spectra of rat liver chromatin indicate that hsp90 induces a tighter, condensed state of the chromatin structure which is resistant against disruption by high salt treatment. Interactions of hsp90 with the chromatin may be important in regulating the transcriptional activity of steroid receptors and other transcription factors.

Sequential action of chaperones is required for many proteins to fold or re-fold into native structures and for their oligomeric assembly (1-3). In *Escherichia Coli* three out of the nine proteins forming the origin-recognizing complex of DNA replication, DnaJ, DnaK and GrpE are heat shock proteins showing high homology with their eukaryotic counterparts (4-6). Recently a similar, ATP-dependent protein complex was described for the recognition of DNA replication origins in eukaryotes (7).

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Abbreviations: CD, circular dichroism; DnaJ, 42 kDa heat shock protein of *E. Coli*; DnaK, the hsp70 homolog of *E. Coli*; GrpE, heat shock protein of *E. Coli*; hsp70 and hsp90, 70 and 90 kDa heat shock proteins, respectively.

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Besides a putative role of heat shock proteins in prokaryotic and eukaryotic replicative processes there is increasing evidence for their role in modifying gene transcription. In *E. Coli* the "vegetative" regulating subunit of RNA polymerase, σ70 is a heat shock protein and after heat shock it is replaced by σ32 whose synthesis and stability is regulated by the DnaJ/DnaK/GrpE heat shock protein complex (8,9). Conformational changes are likely to play an important role in the binding of several eukaryotic transcription factors, such as fos, jun and GCN4 to DNA (10,11). Nuclear heat shock proteins may participate in these processes.

The 90 kDa heat shock protein (hsp90) is a highly conserved, abundant protein, which is mostly cytosolic, but has been also localized in the cell nucleus in normal (12-14) and in stressed cells (15-18). hsp90 has an ATP-binding site, undergoes autophosphorylation and profound conformational changes after ATP addition (19,20). hsp90 participates in the "8S" nuclear form of the progesterone receptor (21), binds to the heat-shock factor (22), chaperones E12/myoD (23), associates with heat shock puffs (24), is a good substrate of the double-stranded DNA-dependent protein kinase (25) and a regulator of casein kinase II (26), a predominantly nuclear enzyme (27). These observations suggest that hsp90 may also function as a nuclear chaperone.

Transcriptionally active chromatin contains histone H1 and probably H2A/B in a partially dissociated form (28-31). Association of histones with DNA is regulated by several posttranscriptional modifications, such as acetylation and phosphorylation (28-30,32). Molecular chaperones, such as nucleoplasmin or other heat-shock proteins, may assist in this process (33).

Our earlier findings showing that histones are able to modulate the autophosphorylation of hsp90 (19) prompted us to investigate the direct interactions of hsp90 with histones. Here we demonstrate that hsp90 is able to bind histone-agarose and enhances the binding of histones to DNA. Circular dichroism spectra of rat liver chromatin indicate that hsp90 induces a condensed state of the chromatin which has a higher resistance against salt extraction. Interactions of hsp90 with the chromatin may thus be of importance in regulating the transcriptional activity of steroid receptors and other transcription factors.

MATERIALS AND METHODS

Chemicals -- The chemicals used for polyacrylamide gel electrophoresis were from Bio-Rad (Richmond, CA). Butyl-Sepharose 4B, DEAE Sepharose Fast Flow and Sepharose S-200 were purchased from Pharmacia LKB Biotechnology Inc. (Uppsala, Sweden). Spectragel HA hydroxyapatite resin was from Spectrum Med. Ind. (Los Angeles, CA). All the other chemicals used were from Sigma Chemicals Co. (St. Louis, MO).

Isolation of hsp90 -- The 90 kDa heat shock protein was isolated from livers of 2-3 months old, male Sprague-Dawley rats using the method of Yonezawa et al (34) as described earlier (19). The purity of this preparation was higher than 95% (usually higher than 98%) as judged by densitometry of Coomassie Blue stained SDS slab gels (35). Protein concentrations were determined using the method of Bradford (36) and bovine serum albumin as standard.

Binding of hsp90 to histone agarose -- 25 μg rat liver hsp90 was mixed with 15 μl histone-agarose containing 45 μg of calf thymus histones. Histone-agarose was equilibrated with 50 mM Hepes, pH 7.4 in a final volume of 30 μl and incubated with hsp90 for 10 minutes at

room temperature. Agarose beads were sedimented by spinning the samples in a microfuge and 20 µl of the supernatant was analysed by SDS-PAGE. The amount of free and bound hsp90 was determined by densitometric analysis.

Binding of histone H1 to DNA -- Binding of histone H1 to DNA was studied by electrophoretic mobility shift assay using a double stranded oligonucleotide with the H1 binding site of the SL3-3 virus enhancer as described earlier (37). To minimize nonspecific H1-DNA interactions samples contained a 2,000-fold excess of double stranded poly(dI-dC), poly(dA-dT) and 0.1 pmol of single-stranded DNA.

Preparation of rat liver chromatin -- Long fragments of native soluble chromatin were prepared as described by Noll (38). Rat liver nuclei (OD_{260} = 40 in 0.3 n NaOH) were prepared as described earlier (39), digested with DNase I (250 units Sigma enzyme/ml nuclei suspension) for 30 sec at 37 °C and lysed with ten volumes of 1 mM EDTA, 1 mM Tris.HCl, pH 8.0. (More extensive digestion significantly decreased the observed changes in CD spectra.) Chromatin fragments were dialysed overnight against 1000 volumes of 5 mM Tris.HCl, pH 7.4, 1 mM DTT buffer at 4 °C and their circular dichroism was recorded.

Circular dichroism spectra of rat liver chromatin -- Circular dichroism (CD) spectra were recorded on a Jobin Yvon VI dichrograph. Measurements were made at room temperature in a 1.0 cm pathlength cylindrical quartz cell. (Repeated recording of some spectra at 10 °C gave identical results.) Rat liver chromatin (OD_{260} = 4) was suspended in a 5 mM Tris.HCl, pH 7.4, 1 mM DTT buffer. Baselines were obtained using protein-free buffer solution with the appropriate additions. The ligands were introduced from 200 times concentrated stock solution and their equilibration was facilitated with gentle agitation for approximately 1 minute. Longer incubation did not cause any further difference in the CD spectra.

RESULTS

Binding of hsp90 to histone agarose -- As a first step we have demonstrated that hsp90 directly interacts with histones. hsp90 strongly associates with histone-agarose, while it does not bind to the agarose matrix (Figure 1A). Histone-bound hsp90 can be dissociated by high-salt treatment or by ionic detergents.

Effect of hsp90 on the binding of histone H1 to DNA -- We have analysed the effect of hsp90 on the binding of histones to DNA. Our experiments were focussed on histone H1,

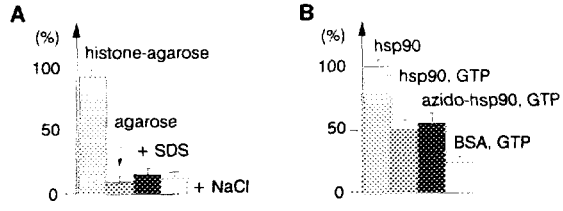

Figure 1. ***Binding of hsp90 to histone agarose (panel A) and effect of hsp90 on the binding of histone H1 to DNA (panel B)*** -- hsp90 was isolated and its binding to histone agarose was studied as described in Materials and Methods. Binding of histone H1 to DNA was analysed as described earlier (37). Additions: NaCl: 2 M NaCl; SDS: 1 % SDS; hsp90: 10 µg hsp90; GTP: 2 mM GTP-γ-S; azido-hsp90: 10 µg azido-ATP-labelled hsp90 (19); BSA: 20 µg bovine serum albumin. Data represent means±SDs of three individual experiments.

since it has the most profound effect of hsp90 autophosphorylation among all the histones (19). In the absence of nucleotides histone H1 has a very high affinity for DNA (100 % binding in Figure 1B). This strong binding was not influenced by hsp90. On the other hand, hsp90 significantly enhanced the binding of histone H1 in the presence of 2 mM GTP-γ-S, which alone induces the dissociation of histone H1 from DNA (37). Equal amount of bovine serum albumine was ineffective and hsp90 did not work via sequestering the GTP-γ-S to its nucleotide binding site (19) since azido-ATP-labelled hsp90 induced a similar increase in histone H1-DNA binding.

Effect of hsp90 on the circular dichroism spectrum of rat liver chromatin -- Far UV (240-300 nm) circular dichroism (CD) spectra are sensitive markers of changes in the chromatin structure (40). Addition of 0.6 M NaCl markedly increased the $\Delta\varepsilon$ values (Cotton-effect) of the CD spectrum which corresponds to the partial dissociation of histones from the DNA backbone, *i.e.* to the decondensation of the chromatin structure (40, Figure 2A). On the contrary, addition of hsp90 diminished the Cotton-effect (Figure 2A) which, by analogy, would correspond to a condensation of the chromatin structure (the negligible contribution of hsp90 addition to buffer alone were taken into correction). Vanadate, which effectively binds to the ATP binding sites of hsp90 (20) did not cause further significant changes in the CD spectrum after hsp90 addition. hsp90 induced a similar decrease in the CD spectrum of rat liver chromatin in the presence of 2 mM $MgCl_2$ and also promoted the association of a

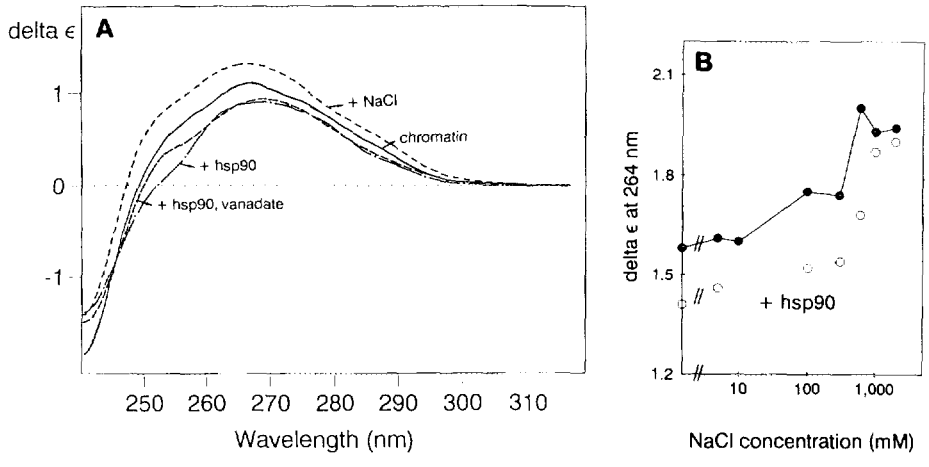

Figure 2. ***Effect of hsp90 on the circular dichroism spectrum (panel A) and on the stability (panel B) of rat liver chromatin*** -- Rat liver chromatin was isolated and circular dichroism (CD) spectra were recorded as described in Materials and Methods. *Panel A*: CD spectrum of rat liver chromatin (solid line) representing approximately 0.2 mg/ml DNA. CD spectrum of the chromatin sample was also recorded in the presence of 0.1 mg/ml hsp90 (alternating dots and dashes), 0.6 M NaCl (dashed line) or 0.1 mg/ml hsp90 plus 0.1 mM sodium-vanadate (long dashes). *Panel B*: $\Delta\varepsilon$ of rat liver chromatin at 264 nm. The Cotton-effect was monitored after sequential addition of NaCl in the absence (open circles) and presence of 0.1 mg/ml hsp90 (filled circles). Data are representative of three independent experiments.

mixture of purified calf thymus DNA and histones as judged from a decrease in the Cotton-effect (data not shown).

Effect of hsp90 to the stability of rat liver chromatin -- To get a better insight of hsp90/chromatin interactions we sequentially dissociated the histone components from the DNA by adding increasing amounts of NaCl. As it is expected the Cotton-effect of the chromatin showed a gradual increase (Figure 2B). hsp90 delayed the dissociation of histones from the chromatin which also suggests that this heat shock protein induces a condensation of the chromatin structure. Dissociation of histones H2A/H2B at a NaCl concentration of 0.95 M was similar in the absence and presence of hsp90 (41). However, another -- presumably intranucleosomal-- rearrangement of the chromatin structure at smaller NaCl concentrations was significantly hindered by hsp90 (Figure 2B).

DISCUSSION

The present report extends our previous observations that histones induce profound changes in the autophosphorylation of hsp90 (19) by demonstrating a direct interaction of histones and hsp90 and an hsp90-induced condensation of the chromatin structure.

hsp90 has numerous negatively charged residues in the central domain of the protein and chicken hsp90 has been shown to possess a region with homologous charge distribution with that of the DNA (42). This may provide a structural element for the direct interactions of hsp90 and histones.

Heat shock induces an increased protein content of the cell nucleus which may be the consequence of an increased association of various proteins with the nuclear scaffold (43,44). Warters and Roti Roti (38) observed an increased resistance of the chromatin against nuclease treatment after heat shock. Since hsp90 is known to be translocated to the nucleus after heat shock (15-18), its ability to promote the condensation of the chromatin may contribute to the tighter nuclear structure of heat shocked cells revealed by these studies.

The overall transcriptional activity --with the notable exception of that of the heat shock proteins-- is diminished after heat shock (46). The hsp90-induced tighter binding of histones to DNA may hinder the binding of various transcription factors and the protease activity of hsp90 (47) may also participate in the termination of various transcriptional activities, such as those of the heat shock factor and steroid receptors.

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