The 90-kDa Heat Shock Protein, HSP90, Binds and Protects Casein Kinase II from Self-aggregation and Enhances Its Kinase Activity*

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We found that a preparation of the 90-kDa heat shock protein, HSP90, purified to apparent homogeneity, contains a serine/threonine kinase which phosphorylates HSP90. The protein kinase was identified as casein kinase II (CKII) according to its properties. The protein kinase was separable from HSP90 by adsorption to heparin-Sepharose or phosphocellulose. CKII was communoprecipitated with HSP90 by anti-HSP90 antibodies from cell extracts. Sucrose density gradient centrifugation analysis revealed that an addition of anti-HSP90 antibodies to cell extracts induces a shift of the sedimentation peak of CKII toward the bottom of a centrifuge tube. These results suggest that CKII is associated with HSP90 in cell lysates at low salt conditions. Furthermore, the CKII-HSP90 complex was reconstituted from purified HSP90-free CKII and CKII-free HSP90. In a buffer at low ionic strength, CKII forms large aggregates, but HSP90 dissociates the aggregates. Finally, we found that HSP90 activates CKII; an addition of HSP90 to CKII dramatically increased phosphorylation of exogenous substrates as well as the CKIIβ subunit. Taken altogether, these observations suggest that CKII is structurally and functionally active when it forms a complex with HSP90.

Various environmental stresses induce the expression of heat shock proteins, or stress proteins (for review, see Lindquist and Craig, 1988; Schlesinger, 1990). Recently, it has been widely accepted that heat shock proteins play important roles in living organisms. The 90-kDa major heat shock protein, HSP90, is synthesized abundantly in unstressed cells and is induced severalfold upon heat shock (Lindquist and Craig, 1988; Schlesinger, 1990). HSP90 is an essential protein for Saccharomyces cerevisiae, even under unstressful conditions (Borkovich et al., 1989), and is highly conserved during evolution (Hickey et al., 1989). Thus, HSP90 is likely to function as an essential protein in higher eukaryotic cells under normal conditions as well as stressful conditions. In fact, various functionally key proteins, such as those belonging to the steroid hormone receptor superfamily proteins (Joab et al., 1984; Catelli et al., 1985; Sanchez et al., 1985) and avian sarcoma virus transforming proteins (Opperman et al., 1981; Brugge et al., 1981; Schuh et al., 1985), have been shown to form complexes with HSP90. We have shown previously that HSP90 binds to filamentous actin in a Ca2+-calmodulin-regulated manner (Koyasu et al., 1986; Nishida et al., 1986) and, therefore, functions as an anchoring system of the above complexes to cytoskeletal structures (Miyata and Yahara, 1991). In addition, HSP90 may function in maintaining conformation of the counterpart protein in the complexes so that function(s) of the protein is expressed optimally. For instance, the affinity for glucocorticoids has been shown to be higher in the glucocorticoid receptor-HSP90 complex than in the free receptor (Bresnick et al., 1989). Furthermore, a positive role of HSP90 in signal transduction of glucocorticoid receptor has been also demonstrated (Picard et al., 1990).

In the course of study, we found that an incubation of highly purified HSP90 with [γ-32P]ATP resulted in its phosphorylation. This result seems to suggest that a protein kinase(s) might be copurified together with HSP90 and that the copurified kinase(s) phosphorylates HSP90. Among several protein kinases which have been reported to phosphorylate HSP90, casein kinase type TS (=type II) has been shown to be coeluted with HSP90 through a gel filtration column (Meggio et al., 1985; Dougherty et al., 1987). These facts led us to examine whether the kinase contained in the preparation of HSP90 is casein kinase II (CKII) or not.

CKII was identified as a second messenger-independent protein kinase and shown to be very ubiquitous and conserved during evolution (for review, see Pinn, 1990; Tuazon and Traugh, 1991). Although heparin inhibits while polylysine activates the kinase in vitro, little is known about the regulatory mechanism of the kinase in intact cells. The self-aggregation of purified CKII has been demonstrated at low ionic strength and also under physiological conditions (Glover, 1986), implicating the negative control mechanism of the protein kinase. A number of both cytosolic and nuclear proteins have been shown to be potential substrates of CKII (Pinna, 1990; Tuazon and Traugh, 1991). Among them, of interest are those concerning the regulation of cell growth that include the myc protein (Lüscher et al., 1989), p53 (Mek et al., 1990), SV40 large T antigen (Grasser et al., 1988), and serum response factor (Manuk et al., 1990). The CKII activity is stimulated significantly in response to several mitogens (Sommercorn et al., 1987; Klarlund and Czech, 1988; Ackerman and Osheroff, 1989), and microinjection of the kinase to Xenopus oocytes induces their maturation (Mulner-Lorillon et al., 1988). These results altogether suggest that CKII might be one of the key protein kinases involved in signal transduc-

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‡The abbreviations used are: HSP90, 90-kDa heat shock protein; CKII, casein kinase II; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol; EGTA, ethylenbis(oxyethylene)-N,N'-tetraacetic acid; Mops, 3-[N-morpholino]propanesulfonic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Pipes, piperazine-N,N'-bis[2-ethanesulfonic acid]; CBB, Coomassie Brilliant Blue R-250; BSA, bovine serum albumin.
tion of the cell cycle and cell growth regulation (Krebs et al., 1988; Carroll et al., 1988).

Here we report that CKII was copurified with HSP90 and that CKII was associated with HSP90 in cell lysates. Furthermore, we found that HSP90 disaggregates large polymeric forms of CKII and co-purifies them with CKII-HSP90 complexes. Since an addition of HSP90 to purified CKII prominently stimulated the kinase activity, the complex formation is necessary to keep CKII active. These results, together with the previous report that eIF2α kinase is activated by HSP90 (Rose et al., 1987; Matsa and Hurst, 1989), provide a new insight into functions of HSP90 and also into the regulation of CKII activity.

**EXPERIMENTAL PROCEDURES**

**Peptide**—A synthetic peptide Arg-Arg-Arg-Glu-Glu-Thr-Glu-Glu-Glu-Glu (RRREEETEEE), a specific substrate for CKII (Kuenzel et al., 1988), was synthesized on a Applied Biosysytem's solid-phase peptide synthesizer. The peptide was purified on Pepsilic column in a elution with acetonitrile (0-100%) in the presence of 0.1% trifluoroacetic acid.

**Buffers and Cells**—HEDG buffer: 25 mM Hepes, 1.5 mM EDTA, 1 mM DTT, and 10% (v/v) glycerol, pH 7.6. Assay buffer: 20 mM Tris-Cl, 20 mM KCl, 10 mM MgCl₂, 60 mM NaCl, 10 mM sodium metabol sulfate, 20 mM β-glycerophosphate, 6 mM EGTA, 0.5 mM p-nitrophenylphosphate (pNPP), 20 μM [γ-<sup>32</sup>P]ATP, 7.8 μM CKII buffer: 130 mM KHPo₄, 0.1 mM CaCl₂, 1 mM MgCl₂, 20 mM Hepes, 10 mM NaCl, pH 7.2. Mouse L cells were cultured in Eagle's medium supplemented with 5% fetal calf serum (GIBCO).

**Purification of Porcine Testis Casein Kinase II**—CKII was purified from porcine testis by the method described previously (Litchfield et al., 1990) with modifications and further purified using Mono Q fast protein liquid chromatography column (Pharmacia). Briefly, crude lysates of testis containing CKII activity were separated using sequential column chromatography on DE52 (Whatman), phosphocellulose P11 (Whatman), and hydroxylapatite (Calbiochem). Active fractions were collected, and proteins were precipitated with solid ammonium sulfate, solubilized, and applied sequentially to a HiLoad Superdex-200 gel filtration column (Pharmacia) and a heparin-Sepharose column (Pharmacia) as described (Litchfield et al., 1990). Eluted CKII was further purified with Mono Q fast protein liquid chromatography column using a 200-1,000 mM NaCl linear gradient in a buffer containing 15 mM Mops, 1.5 mM EDTA, 20% (v/v) glycerol, pH 7.0. The CKII activity from the column as a single peak coinciding with the protein peak, and SDS-PAGE analysis of the obtained CKII revealed three polypeptides of α (27 kDa), α* (40 kDa), and β (27 kDa) subunits.

**Purification of Mouse HSP90**—Mouse HSP90 was purified to homogeneity as judged by CBB staining patterns on SDS-PAGE from mouse lymphoma L5178Y cells as described previously (Koyasu et al., 1986; Nishida et al., 1986; Yonezawa et al., 1988).

The expression plasmid of the cloned recombinant *S. cerevisiae* HSP90 in PET11d was expressed with the T7 RNA polymerase system in *Escherichia coli* (DE21 Lys E). HSP90 was induced by the addition of β-o-thiogalactopyranoside (37 °C 2 h) and was purified by the same method as in the case of the purification of mouse HSP90. Cloning, expression in *E. coli*, and purification of *S. cerevisiae* HSP90 were carried out in collaboration with Y. Kimura.

**Preparation of L Cell Lysates**—Confluent mouse L cells were washed with ice-cold phosphate-buffered saline and received HDG buffer containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 2 μg/ml leupeptin). The cell monolayers were immediately frozen with liquid nitrogen and then scraped, thawed, and solubilized on ice. The extracts were centrifuged at 22,000 × g for 20 min at 2 °C, and the supernatants were further centrifuged at 100,000 × g for 60 min at 2 °C, resulting in L cell lysates.

**Antibodies**—Rabbit antisera against mouse and yeast HSP90 were separately prepared by immunizing the corresponding purified proteins as antigens to rabbits. IgG fractions were prepared from the antisera using a protein A-Sepharose (Pharmacia) column.

**Immunoprecipitation of HSP90 and Associated Proteins**—L cell lysates were incubated with anti-HSP90 IgG (0.5 mg/ml) for 120 min on ice, further mixed with protein A-Sepharose suspension, and incubated at 4 °C for 60 min. The resin was washed five times with HEDG buffer, and adsorbed proteins including HSP90 and associated proteins were eluted by boiling in a SDS-PAGE sample buffer. The CKII activity in the immunocomplexes was analyzed using the active gel phosphorylation assay as described below.

**Assay for the CKII Activity**—CKII was assayed at 30 °C for 10 min in assay buffer containing 0.5 mg/ml CKII-specific synthetic peptide substrate (RRREEETEEE), and incorporation of radioactive ATP into the peptide was determined using P81 phosphocellulose papers as described elsewhere (Kuenzel and Krebs, 1985).

**Sucrose Density Gradient Centrifugation**—L cell lysates or purified CKII (diluted in HEDG buffer to 10 μg/ml) were incubated with or without anti-HSP90 IgG (0.5 mg/ml) for 120 min on ice. For the reconstitution experiments, purified CKII was preincubated for 60 min on ice with HSP90 (1.0 mg/ml) in HEDG buffer. Samples were layered onto a linear sucrose density gradient (5-20%) prepared in HEDG buffer and were centrifuged at 45,000 rpm for 16 h at 2 °C in a Beckman SW 50.1 rotor. Fractions were successively collected and diluted in assay buffer at start time of the CKII activity assay. Sedimentation coefficients were determined using ovalbumin, bovine serum albumin, aldolase, and catalase as marker proteins.

**Detection of CKII Activity after SDS-PAGE in Renatured Gels (Active Gel Phosphorylation Assay)**—The method described for calmodulin-dependent kinase II (Kameshita and Fujisawa, 1989) was used to determine the activity. Immunoprecipitation complexes were electrophoresed on a SDS-polyacrylamide gel containing 2 mg/ml of dephosphorylated casein (Sigma). After electrophoresis, SDS was removed and CKII in the gel was denatured with 6 x guanidine HCl in a buffer (50 mM Tris-Cl, 5 mM mercaptoethanol, pH 8.0) for 1 h and then renatured with the same buffer containing 0.04% Tween 40 at 4 °C for 16 h. Phosphorylation was performed by incubating the gel in a buffer (40 mM Tris-Cl, 50 mM NaCl, 20 mM KCl, 10 mM MgCl₂, 0.1 mM EGTA, 2 mM DTT, pH 8.0) containing 50 μM cold ATP and 3 MBq of [γ-<sup>32</sup>P]ATP for 60 min at room temperature. The gel was fixed and washed extensively with 5% (w/v) trichloroacetic acid containing 1% sodium pyrophosphate and dried for autoradiography.

**Effects of HSP90 on the Activity of Purified CKII**—Purified CKII (diluted in HEDG buffer at 4 μg/ml) was mixed with various concentrations of kinase-free HSP90 (0-0.4 mg/ml) in assay buffer and incubated for 2-30 min (normally 10 min) at 30 °C. The total assay volume (20 μl) and the ionic condition of the samples were kept constant. The mixtures were boiled in SDS sample buffer for 5 min and subjected to SDS-PAGE. When indicated, the synthetic peptide substrate was added to assay buffer as described above. To examine the effect of HSP90 on CKII activity in a physiological buffer, CKII was diluted in HEDG or KCM buffer containing increasing amounts of HSP90, and the phosphate incorporation into the substrate peptide was determined in KCM buffer at 37 °C for 10 min.

**RESULTS**

**Casein Kinase II Was Copurified with HSP90**—HSP90 was purified from L5178Y cells using sequential column chromatography on DE52, hydroxylapatite, gel filtration, and Mono Q (Welch and Feramisco, 1982; Koyasu et al., 1986; Yonezawa et al., 1988). The purified HSP90 appeared to be homogeneous as judged by SDS-PAGE followed by CBB staining (Fig. 1A). We found, however, that HSP90 in the above final preparation was phosphorylated upon incubation with [γ-<sup>32</sup>P]ATP without addition of other materials (Fig. 1B, lane 1). This implies that a protein kinase(s) might be copurified with HSP90 and phosphorylates HSP90. The copurified protein kinase(s) was adsorbed to phosphocellulose (Fig. 1B, lane 2) and heparin-Sepharose (Fig. 1B, lane 3) in high salt conditions (0.4 and 0.2 M NaCl, respectively). HSP90 itself did not bind to these resin as shown in Fig. 1A (lane 1–3).

Several characteristics of the copurified protein kinase in the HSP90 preparation were investigated. The protein kinase was identified as CKII according to its properties; inhibition by liruparin, stimulation by polyvinyl pyrrolidone, and its sensitivity as well as ATP as phosphate donors, substrate specificity, and molecular mass (43/40 kDa) of its catalytic subunit (data not shown).

2 Y. Kimura, unpublished data.
A highly purified HSP90 fractions contained a HSP90-kinase(s) which were adsorbed to phosphocellulose and heparin-Sepharose. 360 μg of highly purified HSP90 fractions were mixed with 30 μl of control Sepharose 4B (lanes 1), phosphocellulose (lanes 2), or heparin-Sepharose (lanes 3) in a buffer (40 mM Pipes, 1.2 mM MgCl2, 0.8 mM EGTA, 0.4 mM DTT, 3.2 mM β-glycerophosphate, 60 mM sucrose, pH 6.8) containing 0.2 M (for NaCl at 4°C for 120 min, and adsorbed proteins to resins were removed by a centrifugation. Proteins in the supernatants (5 μg) were incubated with [γ-32P]ATP in the assay buffer for 10 min at 30°C and analyzed by SDS-PAGE. CBB staining of the gel with marker proteins (A) and the autoradiogram (B) are shown.

We pursued the CKII activity during the purification steps of HSP90. The results shown in Fig. 2 revealed that CKII coeluted with HSP90 from DE52 (A), hydroxylapatite (B), and Mono Q (D) columns. The CKII activity was eluted slightly faster than HSP90 from a gel filtration column (Fig. 2C). We interpreted these results as an indication that CKII possibly forms a complex with HSP90 and that the HSP90-CKII complex is larger than HSP90 dimer. We then critically examined this possibility.

Association of CKII with HSP90 in Cell Lysates—Total lysates of L cells were prepared in HEDG buffer, a low salt buffer. Aliquots were incubated with or without anti-HSP90 IgG and centrifuged in 5–20% sucrose density gradient in HEDG buffer. First, the specificity of the antibody was examined by western blotting (Fig. 3A). The antibody specifically recognized HSP90 in L cell lysate (lane 1), reacted with purified HSP90 (lane 2), but did not cross-react with purified CKIIα/α'β (lane 3). Fig. 3B (●) revealed that the CKII activity exists as a single peak with a sedimentation coefficient of ~8 S. It has been reported that purified CKII under high salt conditions exists as a ~6 S αβ2 heterotrimer (Glover, 1986; Pinna, 1990; Tuazon and Traugh, 1991), suggesting that CKII contained in L cell lysates exists as a larger oligomeric structure than the αβ2 form. To examine the possibility that the 8 S CKII complex contains HSP90 as a component, we determined an effect of anti-HSP90 antibody on the sedimentation profile of 8 S CKII. The result shown in Fig. 3B (●) clearly indicated that a sedimentation coefficient of CKII was increased to ~10 S by anti-HSP90 antibodies. The shift of sedimentation peak of CKII by anti-HSP90 antibodies is similar to that of steroid hormone receptor-HSP90 complexes (Joab et al., 1984). This result strongly suggests that CKII exists in cell lysates as an ~8 S complex with HSP90. Nonimmune bovine IgG at a similar concentra-

![Fig. 1](image1.png)

![Fig. 2](image2.png)

![Fig. 3](image3.png)
tion did not result in any shift of the sedimentation peak of CKII (data not shown). Both purified CKII (α,β) and CKII in cell extracts in a high salt buffer existed as a ~6 S form (Fig. 3B, arrow), and that anti-HSP90 antibody did not affect the sedimentation profile of 6 S-CKII (data not shown). This indicates that an interaction of HSP90 with CKII is sensitive to high salt conditions as is the case for the glucocorticoid receptor-HSP90 complex (Joab et al., 1984; Pratt et al., 1989; Denis and Gustafsson, 1989).

Next, we have examined whether CKII is coimmunoprecipitated with HSP90. L cell lysates prepared in HEDG buffer were separately incubated with control IgG, anti-mouse HSP90 IgG, and anti-S. cerevisiae HSP90 IgG. The resulting immunocomplexes were subjected to active gel phosphorylation method using dephosphorylated casein as a casein followed by autoradiography (Fig. 4). The result revealed that radioactive phosphate was incorporated into casein, forming two bands of ~43 and ~40 kDa. These values of molecular mass are comparable with those reported for α/α′ catalytic subunits of CKII (Tuazon and Traugh, 1991). These results indicated that the casein kinase activity was coimmunoprecipitated with HSP90 (Fig. 4).

HSP90 Associates and Disaggregates CKII in Vitro—It is of interest to examine whether or not CKII-HSP90 complexes can be re-constructed in vitro from separately purified components. HSP90-free CKII was purified from porcine testis. The CKII-free HSP90 was prepared from conventionally purified HSP90 by adsorbing the kinase with heparin-Sepharose. Purified CKII was incubated with or without HSP90 at the same ionic condition, and the mixtures were analyzed by sucrose density gradient centrifugation. CKII formed large polymeric structures at low ionic strength in the absence of HSP90. An incubation of aggregated CKII with an excess amount of HSP90 clearly resulted in 8 S form of CKII (Fig. 5, □). Free HSP90 existed in the mixture as a dimeric form with a sedimentation coefficient of ~6 S (indicated by an arrow). When the CKII-specific substrate peptide was omitted from the assay mixture, an incorporation of radioactive phosphate was negligible (Fig. 5, ○, △), indicating that the 8 S peak did not result from phosphate incorporation into added HSP90.

An addition of anti-HSP90 antibodies to the mixture of CKII and HSP90 caused a decrease of the 8 S peak and a shift of the 8 S peak toward the bottom (data not shown).

**Fig. 4. Coimmunoprecipitation of casein kinase II with HSP90.** L cell lysates were prepared in HEDG buffer and separately incubated with nonimmune IgG, anti-mouse HSP90 IgG, and anti-S. cerevisiae HSP90 IgG (0.5 mg/ml). Resulting immunocomplexes were adsorbed to protein A-Sepharose, washed extensively, and analyzed by active gel phosphorylation assay for CKII activity, followed by autoradiography.

**Fig. 5. Reconstitution of CKII-HSP90 complexes from purified CKII and HSP90.** Purified CKII (10 μg/ml) was incubated with (□) or without (○) HSP90 (1 mg/ml) in HEDG buffer and analyzed by sucrose density gradient centrifugation. CKII activity in each fraction was measured with (●, ■) a substrate peptide (0.7 mg/ml). As a control experiment, the peptide was omitted (○, △) in the assay mixture. The position of free HSP90 dimer is indicated by an arrow.

This result suggests that the reconstituted 8 S-CKII contains HSP90 as has been shown for endogenous 8 S-CKII (Fig. 3B) and 8 S-glucocorticoid receptor (Joab et al., 1984). Taken together, we conclude that HSP90 disaggregated the CKII large polymer observed in a low salt buffer by forming an 8 S CKII-HSP90 complex that is probably identical to the CKII complex in cell lysates.

**HSP90 Activates Casein Kinase II—** As is observed in Fig. 5, the total kinase activities of CKII in the absence of HSP90 (large aggregates) were always lower than those of CKII determined in the presence of HSP90 (8 S complex). This implied that HSP90 enhances CKII activity, thus, we precisely determined an effect of HSP90 on the CKII activity. A fixed amount of purified CKII (diluted in HEDG buffer at 4 μg/ml) was mixed in assay buffer containing different amounts of kinase-free yeast recombinant HSP90. The total assay volume and the ionic condition were kept constant. The mixture was incubated with [γ-32P]ATP. CKII induced very little autophosphorylation of the α and β subunits in the absence of HSP90 in the condition used (Fig. 6A). An addition of HSP90 to the phosphorylation mixtures dramatically increased the autophosphorylation of CKIIβ (Fig. 6A). At the same time, HSP90 was also phosphorylated by CKII. CKIIα was slightly autophosphorylated by the "hyperactive" CKII in the presence of HSP90. Phosphorylation by CKII of exogenous substrates such as a synthetic peptide substrate was also dramatically enhanced by yeast HSP90 in an HSP90 dose-dependent manner (Fig. 6B), and the kinase activity of CKII toward exogenous substrate was very well correlated with the amount of autophosphorylation of CKIIβ (compare Figs. 6, A and B). Essentially the same results were obtained with kinase-free purified mouse HSP90. Taken together, we conclude that HSP90 enhances CKII activity toward all of exogenous substrates, HSP90 moiety of CKII-HSP90 complex, and CKII itself.

We next addressed a question of whether the enhancing effect of HSP90 on CKII activity was seen in buffers containing physiological concentrations of salt. Purified CKII was diluted in KCM buffer, a buffer with the physiological salt composition, or HEDG buffer, and CKII activity toward the synthetic substrate peptide was determined in KCM buffer. The data shown in Fig. 7 clearly indicated that both purified mouse HSP90 (●) and recombinant yeast HSP90 (■) enhanced the CKII activity in KCM buffer in a dose-depend-
Association and Activation of Casein Kinase II by HSP90

A. Association and Activation with \([\gamma\-^{32}P]ATP\) at analysis and an autoradiogram was shown. The CKII activities toward a synthetic substrate peptide were determined and shown. The CKII activities toward a synthetic substrate peptide (RRREEETEEE) were determined and shown. The CKII activities toward a synthetic substrate peptide/min/mg CKII at 37 °C for 10 min in the presence of 0-0.40 mg/ml of protein kinase-free recombinant yeast HSP90 produced in E. coli in assay buffer. The mixtures were subjected to SDS-PAGE analysis and an autoradiogram was shown. B. Phosphorylation by CKII was performed as described in A with increasing concentrations of HSP90. The CKII activities toward a synthetic substrate peptide (RRREEETEEE) were determined and shown. The radioactivity of 10,000 cpm corresponds to 660 nmol of phosphate from ATP into the synthetic peptide substrate/min/mg CKII at 30 °C.

**Fig. 6.** HSP90 activates CKII in a dose-dependent manner. A, purified CKII (diluted in HEDG buffer at 4 µg/ml) was incubated with \([\gamma\-^{32}P]ATP\) at 30 °C for 10 min in the presence of 0-0.40 mg/ml of protein kinase-free recombinant yeast HSP90 produced in E. coli in assay buffer. The mixtures were subjected to SDS-PAGE analysis and an autoradiogram was shown.  B, phosphorylation by CKII was performed as described in A with increasing concentrations of HSP90. The CKII activities toward a synthetic substrate peptide (RRREEETEEE) were determined and shown. The radioactivity of 10,000 cpm corresponds to 660 nmol of phosphate from ATP into the synthetic peptide substrate/min/mg CKII at 30 °C.

**Fig. 7.** HSP90 enhanced CKII activity in a physiological buffer. Purified CKII (pre-diluted in HEDG buffer (○, ■) or in KCM buffer (□)) was incubated with \([\gamma\-^{32}P]ATP\) at 37 °C for 10 min in the presence of 0-0.40 mg/ml of CKII-free mouse HSP90 (○, □) or recombinant yeast HSP90 produced in E. coli (■) in KCM buffer. As controls, the effect of purified BSA and actin on the CKII activity were shown (×, +), respectively. The CKII activities toward a synthetic substrate peptide (RRREEETEEE) were determined and shown.

**DISCUSSION**

Purified CKII exists in high salt buffers as a heterotetrameric structure, α2β2, which has a sedimentation coefficient of 6 S (Glover, 1986; Pinna, 1990; Tuazon and Traugh, 1991). We have shown, in this study, that CKII prepared from cultured L cells in HEDG buffer exists as an 8 S complex with HSP90. The HEDG buffer is a buffer generally used for preparation of cytoplasmic 8 S steroid hormone receptors (Joab et al., 1984; Sanchez et al., 1985). Various steroid hormone receptors and arylhydrocarbon receptors have been shown to interact with HSP90 in HEDG buffer. It has been suggested recently that steroid hormone receptors form complexes with HSP90 under physiological ionic strength and, therefore, also in intact cells (Howard and Distelhorst, 1988). Analogously, it is likely that HSP90 interacts with CKII under physiological conditions. In fact, we could observe the activation of CKII with HSP90 in a physiological buffer (see Fig. 7). Copurification of HSP90 with CKII would be ascribed to the complex formation of CKII with HSP90. These results are consistent with the previous report by others which showed that CKII is coeluted from a gel filtration column with HSP90 in rat liver cytosol (Meggio et al., 1985; Dougherty et al., 1987).

Mixing separately prepared CKII-free HSP90 and HSP90-free CKII, the complex of CKII and HSP90 was re-constructed. When purified CKII was exposed to relatively low salt conditions, it formed large aggregates as has been previously reported (Glover, 1986). An addition of HSP90 to aggregated CKII induced dissociation of the aggregates, forming soluble CKII-HSP90 complexes. This dissociation of CKII aggregates is associated with augmentation of the CKII activity. HSP90 thus appears to function to allow CKII to become soluble and active. It should be noted that a relatively high concentration of HSP90 (0.4 mg/ml) is required for the optimum activation of CKII (4 µg/ml). We have preliminary results that HSP90 as low as 50 µg/ml significantly activated CKII in HEDG buffer containing 10 mM MgCl2 and 150 mM NaCl at 37 °C. This activation could not be attributed to a protective effect of high concentrations of proteins from denaturation; high concentrations of BSA or actin did not disaggregate nor activate CKII at all (see Fig. 7). On the other hand, we should consider intracellular concentration of HSP90 in unstressed cells. The content of HSP90 in unstressed cells was approximately estimated to be ~0.3 mg/ml for rat 3Y1 cells and ~1.0 mg/ml for mouse L5178Y cells. Local concentrations of HSP90 in particular regions such as membrane ruffles may be higher than these values. Thus, the physiological concentrations of HSP90 are sufficient to form complex with and activate CKII. It would be possible, therefore, that CKII really interacts with HSP90 in intact cells.

HSP90 interacts with a variety of functionally key proteins, including steroid hormone receptors (Joab et al., 1984; Catelli et al., 1985; Sanchez et al., 1985), dioxin receptors (Perdew, 1988; Wilhelmssohn et al., 1990), avian sarcoma virus transforming proteins such as pp60csrc (Brugge et al., 1981; Opperman et al., 1981; Schuh et al., 1985), and eIF-2α kinase (Rose et al., 1987; Mats and Hurst, 1989). Positive regulatory roles of HSP90 have been suggested for the glucocorticoid receptor complex containing HSP90. The association of glucocorticoid receptor polypeptide with HSP90 increases the affinity of the receptor to the ligand (Bresnick et al., 1989). In addition, HSP90 is necessary for gene expression in response to glucocorticoid (Picard et al., 1990). More recently, we have shown that the glucocorticoid receptor complex binds to actin filaments through the HSP90 moiety and suggested that HSP90 might function as an anchoring system of the receptor to the cytoskeleton (Miyata and Yahara, 1991). Negative regulatory roles of HSP90 have been also suggested for the steroid hormone receptor complexes; steroid hormone receptors lose the DNA-binding ability in the complexes with HSP90 (for review, see Denis and Gustafsson, 1989; Pratt et al., 1989). The tyrosine-specific protein kinase activity of pp60csrc has been shown to be suppressed in the complex with HSP90 and

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3 Y. Miyata and I. Yahara, unpublished results.
pp50, another cellular protein (Courtneidge and Bishop, 1982). In all of these cases, HSP90 appears to regulate functions of the target proteins by forming complexes with the proteins. It should be pointed out that both steroid hormone receptors and pp60c-src form complexes with HSP90 soon after synthesis (Dalmann et al., 1985; Brugge et al., 1983) and in vitro reconstitution of the complexes from separately isolated molecules have not been succeeded without denaturation or additional factors (Inano et al., 1990; Scherrer et al., 1990).

By contrast, as has been shown above, an addition of HSP90 to CKII prevents aggregation of CKII by readily forming soluble CKII-HSP90 complexes.

The activation of CKII by HSP90 appears to be similar to that of eIF2a kinase by HSP90 (Rose et al., 1989; Mats and Hurst, 1989). However, recombinant yeast HSP90 produced in E. coli as well as purified mouse HSP90 were found to similarly activate CKII, whereas phosphorylated HSP90 does but dephosphorylated HSP90 does not activate eIF2a kinase (Szymska et al., 1989). In addition, it has not been shown whether or not the complex formation of CKII with HSP90 correlate with the activation of eIF2a kinase. Present, it is not clear whether similar mechanisms operate in the activation of CKII and eIF2a kinase. As these two protein kinases can phosphorylate the initiation factor complexes (for review, see Hershey, 1989), HSP90 may be involved in the regulation of protein synthesis.

Finally, we should consider whether or not the activation of CKII by HSP90 is biologically significant. Since the activation requires a relatively high concentration of HSP90 as discussed above, an increase in the concentration of HSP90 in stressed cells may trigger the further activation of CKII. Alternatively, HSP90 is thought to function as scaffold for CKII and perhaps for other protein kinases including eIF2a kinase. This is rather likely because HSP90 is very abundant in most eukaryotic cells. As was shown in this study, HSP90 is present as a complex with HSP90 in cell lysates and, therefore, possibly in intact cells. The complex may interact with cytoskeleton and nuclear skeleton through binding to actin filaments as we have recently shown for the glucocorticoid receptor-HSP90 complex (Miyata and Yahara, 1991). If this is the case, HSP90 would be involved not only in activation of CKII but also in intracellular positioning of CKII, as CKII is known to exist in nucleus as well as in cytoplasm (Filhol et al., 1990). As both HSP90 and CKII are well conserved and highly expressed in most eukaryotic cells.