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The Carboxy-Terminal Region of Mammalian HSP90 Is Required for Its Dimerization and Function In Vivo

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The majority of mouse HSP90 exists as α-α and β-β homodimers. Truncation of the 15-kDa carboxy-terminal region of mouse HSP90 by digestion with the Ca^{2+}-dependent protease *m*-calpain caused dissociation of the dimer. When expressed in a reticulocyte lysate, the full-length human HSP90α formed a dimeric form. A plasmid harboring human HSP90α cDNA was constructed so that the carboxy-terminal 49 amino acid residues were removed when translated in vitro. This carboxy-terminally truncated human HSP90α was found to exist as a monomer. In contrast, loss of the 118 amino acid residues from the amino terminus of human HSP90α did not affect its in vitro dimerization. Introduction of an expression plasmid harboring the full-length human HSP90α complements the lethality caused by the double mutations of two HSP90-related genes, *hsp82* and *hsc82*, in a haploid strain of *Saccharomyces cerevisiae*. The carboxy-terminally truncated human HSP90α neither formed dimers in yeast cells nor rescued the lethal double mutant.

Like other stress proteins such as HSP70, the 90-kDa stress protein, HSP90, is conserved among all living organisms, from bacteria to humans (5, 12). HSP90 has been shown to be indispensable in the budding yeast *Saccharomyces cerevisiae* (1). HSP90 is abundantly expressed even in unstressed cells (38). A body of evidence has revealed that HSP90 interacts with various cellular and viral proteins, including steroid hormone receptors (4, 24, 31, 36), avian viral transforming tyrosine kinases such as $pp60^{v\text{-}src}$ (3, 6, 22, 32), serine/threonine protein kinases (11, 17, 28), and cytoskeletal proteins (9, 21). In addition, HSP90 forms a complex with another stress protein, HSP56 (30), which is known as an FK506-binding protein (10, 23, 34, 35). For some cases, functional significance of the complex formation of HSP90 and its target protein has been demonstrated. The affinity of glucocorticoid receptors to the ligands is a hundred times higher in the complex with HSP90 than in its HSP90-free form (20). Furthermore, activation of glucocorticoid receptors by the ligands in yeast cells requires the expression of a sufficient amount of HSP90 (24).

Mammalian HSP90 has two isoforms, HSP90α and HSP90β, which are encoded by separate genes (18, 19) and are present mainly as α-α and β-β homodimers (15). In addition, a minor population of HSP90β but not HSP90α exists as monomers (15). An electron microscopic study has revealed that the dimeric HSP90 has two heads linked with a flexible portion (9). As we have previously shown, HSP90 cross-links actin filaments probably by using its dimeric structure (9, 21). Steroid hormone receptor-HSP90 complexes have been reported to contain a dimeric HSP90 as their component (2, 14, 26). These results suggest that HSP90 exerts its function in a dimeric form.

This study consists of two parts. First, we have made an attempt to identify a domain(s) of HSP90 required for its dimerization. Second, we have examined whether the dimerization domain is necessary for the expression of the HSP90 functions.

MATERIALS AND METHODS

Materials. Polyacrylamide gradient gels were obtained from Daiichi Pure Chemical Co., Ltd. (Tokyo, Japan). Immobilon P membranes were from Millipore Corp. Restriction endonucleases, the Klenow fragment of DNA polymerase I, T4 DNA ligase, and T4 DNA polymerase were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan) and New England Biolabs, Inc. L-[^{35}S]methionine (370 MBq/ml) and Amplify were from Amersham. All other chemicals and reagents used were of the highest grade obtainable.

Proteolysis of HSP90 with *m*-calpain. HSP90 was purified from mouse lymphoma L5178Y cells as previously described (9). *m*-Calpain was purified from rabbit skeletal muscle (7). HSP90 (0.5 mg/ml) was treated with *m*-calpain (0.02 mg/ml) in F buffer [30 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 6.9, 2 mM $MgCl_2$, 100 mM KCl, 0.6 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)] in the presence of 1.5 mM Ca^{2+} at 30°C for various times indicated, and then leupeptin was added to stop the reaction. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and nondenaturing PAGE (native PAGE) were performed on a 10 to 20% polyacrylamide gradient and 7% polyacrylamide gel, respectively, as described before (16). HSP90 treated with *m*-calpain for 40 min was first resolved by 7% native PAGE and then subjected to SDS–10% PAGE.

Amino acid sequencing. HSP90 digested with *m*-calpain for

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40 min was separated on SDS–10 to 20% PAGE and then electroblotted onto an Immobilon P membrane as described by Matsudaira (13). After being stained with Coomassie brilliant blue R-250 (CBB), each stained band was cut out and subjected to amino acid sequence determination with a model 470A protein sequenator (Applied Biosystems, Inc.).

In vitro transcription and translation. A cDNA plasmid of human HSP90α constructed from pBluescriptII SK(−) (Stratagene), phsp90α/BS, was a gift from K. Yokoyama (39). A plasmid containing human HSP90β cDNA, pKN1-3, was a gift from N. F. Rebbe (25). The human HSP90β cDNA was transferred into pBluescriptII SK(−) to use for the in vitro transcription/translation. For the carboxy-terminal truncation of HSP90α, plasmid phsp90α/Nsp was constructed as follows. phsp90α/BS was digested with *Nsp*7524I, blunted with T4 DNA polymerase, and then digested with *Pst*I. The resulting fragment was inserted into pBluescriptII SK(−) digested with both *Pst*I and *Sma*I. The obtained plasmid was then cleaved with *Bam*HI and filled in with the Klenow fragment, and a synthetic termination linker, 5′-[CTAGCTAGCTAG]-3′, provided by M. Minami, was inserted. DNA sequencing of phsp90α/Nsp revealed that the carboxy terminus of its product was Thr-684–Gly–Asp–Pro–Ser, i.e., threonine of the residue 684 of human HSP90α followed by four additional residues derived from the termination linker. Next, for the amino-terminal truncation, phsp90α/BS was digested with *Nla*IV and *Bam*HI, and the obtained fragment was ligated to pBluescriptII SK(−) digested with *Eco*RI and *Bam*HI to yield phsp90α/Nla. The product was deduced to start from Met-119 of human HSP90α. The plasmids, phsp90α/BS, phsp90α/Nsp, and phsp90α/Nla, were separately linearized, and the respective transcripts were produced with T7 RNA polymerase by using the mCAP mRNA capping kit (Stratagene). In vitro translation was then performed in a rabbit reticulocyte lysate (Promega) in the presence of [^{35}S]methionine. The translation products were analyzed by SDS-PAGE (4 to 20%) and native PAGE (7%). In the cross-linking experiments, in vitro-synthesized products were treated with 0.002% glutaraldehyde at room temperature for 1 h and then subjected to SDS–4 to 20% PAGE. Gels were soaked in Amplify for 10 min after CBB staining and were then fluorographed.

Plasmid constructions and yeast transformations. Plasmid phsp90α/BS was digested with *Bsu*36I, filled in, and then digested with *Ssp*I. Plasmid phsp90α/Nsp was digested with *Bsu*I and *Not*I and then filled in. The resulting fragment from phsp90α/BS or phsp90α/Nsp, respectively, was inserted into a site intervening between the alcohol dehydrogenase 1 (ADH1) promoter and terminator of the expression vector pAD4 (a gift from J. Nikawa) (27), which was digested with *Sma*I and *Pst*I, and blunted. Each of the *Bam*HI fragments, cut out from the obtained plasmids and containing a DNA segment from the ADH1 promoter to terminator, was transferred into the *Bam*HI site of the 2μm-derived vector pTV3 (a gift from J. Nikawa) (29) to produce pTVh90α and pTVh 90Nsp, respectively. The plasmids, pTVh90α, pTVh90Nsp, and pTV3 (mock control), were transformed into haploid strain 5CG2, whose *HSC82* gene is disrupted and *HSP82* gene is controlled by the GAL1 promoter (8). The transformants were grown in minimal SGal medium (0.67% yeast nitrogen base without amino acids and with 2% galactose), and subsequently each colony was replicated onto either minimal SD (0.67% yeast nitrogen base without amino acids and 2% glucose) or SGal plates at 30°C for 4 to 5 days to monitor the viability.

Preparation of yeast cell extracts and immunoblotting. Yeast cells were grown in minimal SGal medium at 30°C. Cells pelleted by centrifugation were broken with glass beads in a buffer solution (50 mM Tris-HCl [pH 7.5], 1 mM EDTA, 1 mM sodium molybdate, 5% glycerol, 1 mg of α-casein per ml, 1% aprotinin, 1 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml). After being cleared by centrifugation at 100,000 × *g*, the yeast cell extracts were subjected to SDS–4 to 20% PAGE and 2–15% native PAGE, followed by electroblotting onto Immobilon P membranes. The blots were either stained with CBB or immunostained with rabbit anti-mouse HSP90 (anti-mHSP90) or anti-yeast HSP90 (anti-yHSP90) antibodies, which were prepared as previously described (17).

RESULTS

Proteolysis of HSP90 with *m*-calpain. As we have previously shown, HSP90 is a calmodulin-binding protein (9, 16). Many of the calmodulin-binding proteins are known to be in vitro substrates of the Ca^{2+}-dependent protease calpain (37). Taken together, we decided to examine whether calpain does cleave purified mouse HSP90. The results showed that *m*-calpain restrictively cleaved HSP90 (Fig. 1). Another isotype of calpain, μ-calpain, also cleaved HSP90 in a somewhat different manner (data not shown).

Analysis by SDS-PAGE revealed that HSP90 underwent proteolysis by *m*-calpain in a time-dependent manner (Fig. 1A). After incubation for 10 min or longer, four polypeptides that migrated faster than the intact HSP90 on SDS-PAGE were detected. Bands I and II could be resolved by SDS-PAGE when the amount of HSP90 for loading on a gel was reduced (Fig. 1C). The apparent molecular masses of bands I/II, III/IV, and V were approximately 90, 82, and 75 kDa, respectively. Native PAGE patterns were also altered as the proteolysis proceeded (Fig. 1B). It should be noted that the monomeric band was reduced within 1 min and disappeared after digestion for 10 min (Fig. 1B, lanes 2 and 3). To determine which monomers or dimers the proteins corresponding to bands I to V formed in the native state, we performed two-dimensional PAGE as described previously (15) (Fig. 1C and D). The results revealed that band I and II proteins were mainly present as dimers, although minor parts of them were monomers. Approximately a half of the band III protein was present as a dimer, and the remaining half was a monomer. The majority of the band IV protein was present as monomers. As seen in Fig. 1B and C, band V was derived from three monomer bands (indicated by small arrows) on native PAGE.

Bands I/II, III, IV, and V were subjected to amino acid sequencing from their amino termini. The results showed that all of these proteolyzed products retained either the amino-terminal sequence of HSP90α or that of HSP90β (Table 1). This indicated that *m*-calpain trimmed the carboxy termini of HSP90α and HSP90β. All of the polypeptides corresponding to band V on SDS-PAGE were shown to exist as monomers under the nondenaturing conditions and to be derived from HSP90β. The results have also indicated that band III was derived from HSP90α, though the source(s) of the band IV was not clear (Table 1). Thus, the removal of the carboxy-terminal 10- to 15-kDa region from either constituent of the HSP90 dimer caused its dissociation into monomers.

Dimerization of carboxy-terminally truncated HSP90 synthesized in an in vitro translation system. We examined in vitro synthesis of HSP90α and HSP90β in a reticulocyte

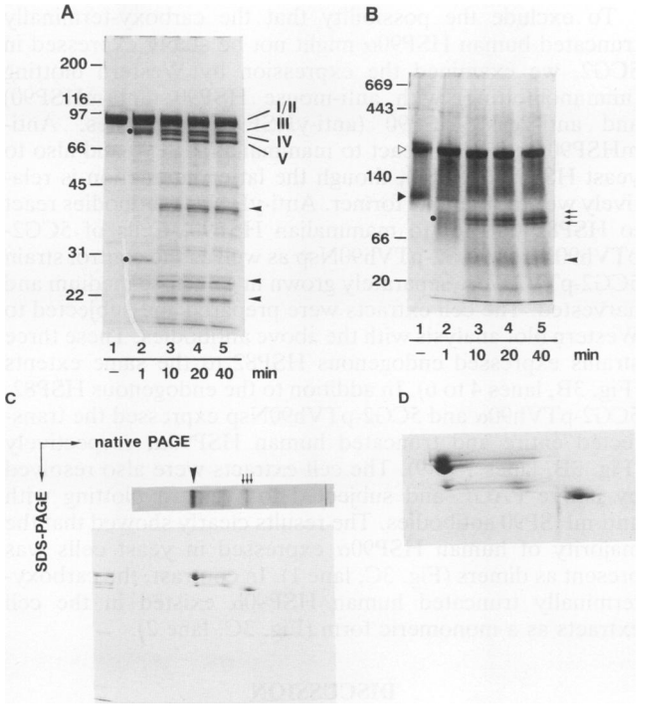

FIG. 1. Proteolysis of mouse HSP90 with *m*-calpain. (A) Proteolytic products of mouse HSP90 with *m*-calpain were analyzed by SDS-PAGE (10 to 20%). (B) The same samples were analyzed by native PAGE (7%). (A and B) Lane 1, results with intact HSP90; lanes 2 to 5, results with samples proteolyzed for indicated times. The 80- and 30-kDa subunits of *m*-calpain are indicated by dots in lane 2 of panel A. *m*-Calpain was obscurely stained on native PAGE as indicated by a dot in lane 2 of panel B. The polypeptides derived from *m*-calpain by autolysis are also indicated by arrowheads in both panels A and B. The major proteolyzed products of HSP90 are numbered (I to V) by SDS-PAGE (A). The native HSP90 dimer and monomer and the proteolyzed products are indicated by native PAGE by an open and closed triangle and small arrows, respectively (B). The positions of molecular mass markers in kilodaltons are indicated to the left of panels. (C) HSP90 digested with *m*-calpain for 40 min was first resolved by native PAGE (7%) and subsequently subjected to SDS-PAGE (10%). The patterns of native PAGE and SDS-PAGE are also presented on the top and left of the two-dimensional gel, respectively. An arrowhead indicates the dimer slightly reduced in molecular weight by proteolysis. Arrows indicate the proteolyzed products, which are shown also in panel B. (D) A magnified part of the two-dimensional gel (C) is shown.

TABLE 1. The amino-terminal amino acid sequences of mouse HSP90 proteolyzed with *m*-calpain

Protein	Amino acid sequence
HSP90α[a]	PEETQTQDQPM
HSP90β[a]	PEEVHHGEEEV
I/II[b]	PEETQTQDQPM V G
III[c]	PEXTQXQ
IV	P
V	PEEVH

[a] The amino-terminal 11 residues of the native mouse HSP90α and HSP90β, which were previously determined (15), are shown along with the determined amino acid sequences of the proteolyzed products (I to V) of mouse HSP90.
[b] Multiple residues were detected for two of the cycles, and all of them are shown.
[c] X indicates the cycle where there was no significant peak.

lysate. We found that human HSP90α efficiently dimerized when synthesized in vitro (Fig. 2B, lane 1), whereas human HSP90β dimerized only in a minor population (data not shown). For this reason, the system using HSP90α mRNA was employed to confirm that the carboxy-terminal 10- to 15-kDa domain of HSP90 is necessary for its dimerization. A couple of modified human HSP90α cDNAs to be expressed were constructed as described (see Materials and Methods). When the plasmid pHSP90α/Nsp was expressed, the carboxy-terminal 49 amino acid residues of the intact human HSP90α were expected to be truncated, and 4 extra amino acid residues were added instead. When another plasmid, pHSP90α/Nla, was expressed, the translation was expected to start from Met-119 of HSP90α.

Three plasmids, phsp90α/BS, phsp90α/Nsp, and phsp90α/Nla, were separately transcribed in vitro by T7 RNA polymerase. The transcripts were translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. The translation products were shown to have the expected molecular sizes when resolved by SDS-PAGE (Fig. 2A). Analysis by native PAGE revealed that the carboxy-terminally truncated HSP90α existed as a monomer while the intact and amino-terminally truncated HSP90α dimerized, respectively (Fig. 2B).

The dimerization of the translated products could be examined also by a chemical cross-linking experiment. The above translation products were treated with glutaraldehyde and resolved by SDS-PAGE (Fig. 2C). Cross-linked products were expected to be yielded only when the translation product was present as dimeric forms. In accord with this expectation, the cross-linked products were detected for both the intact and amino-terminally truncated HSP90α but not for the carboxy-terminally truncated HSP90α.

These results strongly suggested that the carboxy-terminal

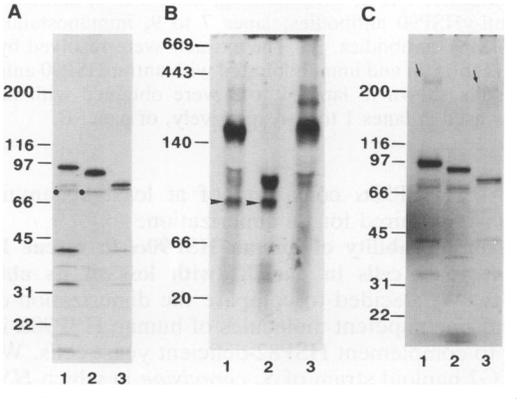

FIG. 2. In vitro-translated products from human HSP90α cDNAs. The full-length, carboxy-, and amino-terminally truncated HSP90α were synthesized in vitro in a reticulocyte lysate from phsp90α/BS (lane 1), phsp90α/Nsp (lane 2), and phsp90α/Nla (lane 3), respectively. The [^{35}S]methionine-labeled products were analyzed by SDS-PAGE (4 to 20%) (A) or native PAGE (7%) (B) and visualized by fluorography. The products indicated by arrowheads in lanes 1 and 2 of panel B were premature polypeptides from human HSP90α mRNA and correspond to bands indicated by dots in lanes 1 and 2 of panel A. (C) The products were treated with 0.002% glutaraldehyde and resolved by SDS-PAGE (4 to 20%). The cross-linked products are indicated by arrows.

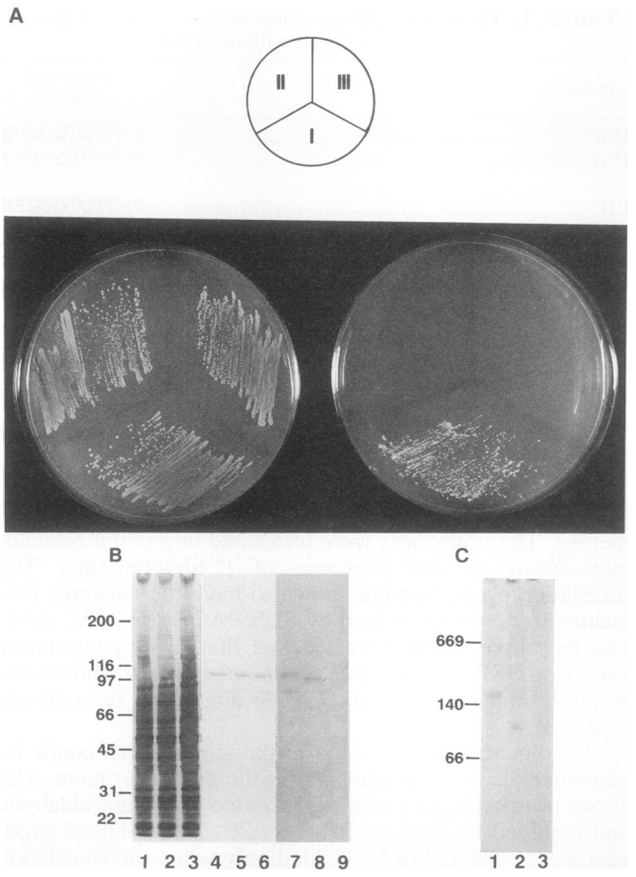

FIG. 3. Rescue of yeast HSP82-deficient cells by human HSP90α but not by the carboxy-terminally truncated HSP90α. (A) 5CG2 strains transformed with pTVh90α (I), pTVh90Nsp (II), or pTV3 (III) were plated on a galactose (left) or glucose (right) plate. (B) 5CG2 transformants with pTVh90α (lanes 1, 4, and 7), pTVh90Nsp (lanes 2, 5, and 8), or pTV3 (lanes 3, 6, and 9) were grown in galactose medium. The cell extracts were resolved by SDS-PAGE (4 to 20%). Lanes 1 to 3, CBB staining; lanes 4 to 6, immunostaining with anti-yHSP90 antibodies; lanes 7 to 9, immunostaining with anti-mHSP90 antibodies. (C) The extracts were resolved by native PAGE (2 to 15%) and immunoblotted with anti-mHSP90 antibodies. The results shown in lanes 1 to 3 were obtained with the same samples used in lanes 1 to 3, respectively, of panel B.

domain of HSP90α consisting of at least 50 amino acid residues is required for its dimerization.

Loss of the ability of human HSP90α to rescue HSP82-deficient yeast cells in parallel with loss of its ability to dimerize. We decided to compare the dimerization-competent and -incompetent molecules of human HSP90α in their ability to complement HSP82-deficient yeast cells. We used the 5CG2 haploid strain of *S. cerevisiae* in which *HSC82* is disrupted and the expression of *HSP82* is controlled by the GAL1 promoter (8). 5CG2 cells were separately transformed by multicopy plasmids harboring cDNAs encoding the entire (pTVh90α) and carboxy-terminally truncated (pTVh90Nsp) human HSP90α, respectively. The vector plasmid pTV3 was also used for transformation as a control. The transformants grown on galactose plates were replicated onto glucose and galactose plates. In accord with the previous report (24), transformants with pTVh90α grew on both galactose and glucose plates (Fig. 3A). Transformants with pTVh90Nsp or pTV3 did not grow on a glucose plate, however.

To exclude the possibility that the carboxy-terminally truncated human HSP90α might not be stably expressed in 5CG2, we examined the expression by Western blotting (immunoblotting) with anti-mouse HSP90 (anti-mHSP90) and anti-yeast HSP90 (anti-yHSP90) antibodies. Anti-mHSP90 antibodies react to mammalian HSP90 and also to yeast HSP90 (HSP82), though the latter interaction is relatively weaker than the former. Anti-yHSP90 antibodies react to HSP82 but not to mammalian HSP90. Cells of 5CG2-pTVh90α and 5CG2-pTVh90Nsp as well as the control strain 5CG2-pTV3 were separately grown in galactose medium and harvested. The cell extracts were prepared and subjected to Western blot analysis with the above antibodies. These three strains expressed endogenous HSP82 to the same extents (Fig. 3B, lanes 4 to 6). In addition to the endogenous HSP82, 5CG2-pTVh90α and 5CG2-pTVh90Nsp expressed the transfected entire and truncated human HSP90α, respectively (Fig. 3B, lanes 7 to 9). The cell extracts were also resolved by native PAGE and subjected to Western blotting with anti-mHSP90 antibodies. The results clearly showed that the majority of human HSP90α expressed in yeast cells was present as dimers (Fig. 3C, lane 1). In contrast, the carboxy-terminally truncated human HSP90α existed in the cell extracts as a monomeric form (Fig. 3C, lane 2).

DISCUSSION

The 90-kDa stress protein, HSP90, has been shown to exist mainly in a dimeric form (9, 16). In mammals, there are two HSP90 isoforms, HSP90α and HSP90β, which are encoded by separate genes (18, 19). HSP90α is present as a homodimer in a purified preparation and also in crude cell lysates, whereas HSP90β exists as both a homodimer and a monomer (15). In this study, we addressed the question of which domain(s) of HSP90 is responsible for its dimerization. We interpreted the result shown in Fig. 1C as indicating that trimming of the carboxy-terminal 10- to 15-kDa region caused dissociation of the HSP90α and HSP90β homodimers into monomers. Band V on SDS-PAGE is carboxy-terminally truncated HSP90β (Table 1) and could be derived from the HSP90β homodimer, because monomeric HSP90β was significantly reduced (Fig. 1B, lane 2), but band V did not appear at this time (Fig. 1A, lane 2). Band III was identified as products from HSP90α. Although only a half of the molecules existed as monomers, the residual might have formed dimers in combination with the band I/II proteins.

Dimeric HSP90α was synthesized in vitro in a reticulocyte lysate which contains endogenous rabbit HSP90. Thus, it is likely that homodimers of each of the full-length and amino-terminally truncated HSP90α were constructed in a reticulocyte lysate. We have shown that the truncation of the carboxy-terminal 50 amino acid residues from HSP90α causes loss of its ability to dimerize. Consistent with the results of digestion of purified mouse HSP90 by *m*-calpain, this also suggests that the carboxy-terminal region is necessary for dimerization of HSP90.

A region from Ala-558 to Pro-588 of mouse HSP90β and corresponding regions of HSP90 of other species have been suggested to contain a hydrophobic heptad repeat (33). This region may be involved in transient interactions between HSP90 and MyoD1, a helix-loop-helix protein (33). This region was contained in the carboxy-terminally truncated HSP90 examined in this study and is, therefore, not involved in its self-dimerization.

Like other stress proteins, HSP90 is also well conserved

among species. Double disruption of the HSP90-related genes of *S. cerevisiae*, *HSP82* and *HSC82*, in a haploid strain caused cell lethality (1). Taking advantage of the ability of mammalian HSP90 to rescue the yeast double mutant (24), we examined the carboxy-terminally truncated HSP90 in its ability to complement the double mutant. We found that the truncated HSP90 failed in the complementation, although it was stably expressed in yeast cells. The truncated human HSP90 was found to exist as monomers in yeast cells, whereas the full-length HSP90 formed dimers. These results are consistent with the proposal that the dimerization of HSP90 is required for its function in intact cells.

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REFERENCES

1. **Borkovich, K. A., F. W. Farrelly, D. B. Finkelstein, J. Taulien, and S. Lindquist.** 1989. Hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Mol. Cell. Biol. **9**:3919–3930.
2. **Bresnick, E. H., F. C. Dalman, and W. B. Pratt.** 1990. Direct stoichiometric evidence that the untransformed Mr 300 000, 9S, glucocorticoid receptor is a core unit derived from a larger heteromeric complex. Biochemistry **29**:520–527.
3. **Brugge, J. S., E. Erikson, and R. L. Erikson.** 1981. The specific interaction of the Rous sarcoma virus transforming protein, $pp60^{src}$, with two cellular proteins. Cell **25**:363–372.
4. **Dalman, F. C., E. H. Bresnick, P. D. Patel, G. H. Perdew, S. J. Watson, Jr., and W. B. Pratt.** 1989. Direct evidence that the glucocorticoid receptors binds to hsp90 at or near the termination of receptor translation *in vitro*. J. Biol. Chem. **264**:19815–19821.
5. **Gething, M.-J., and J. Sambrook.** 1992. Protein folding in the cell. Nature (London) **355**:33–45.
6. **Hutchison, K. A., L. F. Stancato, R. Jove, and W. B. Pratt.** 1992. The protein-protein complex between $pp60^{v\text{-}src}$ and hsp90 is stabilized by molybdate, vanadate, tungstate, and an endogenous cytosolic metal. J. Biol. Chem. **267**:13952–13957.
7. **Inomata, M., M. Nomoto, M. Hayashi, M. Nakamura, K. Imahori, and S. Kawashima.** 1984. Comparison of low and high calcium requiring forms of the calcium-activated neutral protease (CANP) from rabbit skeletal muscle. J. Biochem. **95**:1661–1670.
8. **Kimura, Y., S. Matsumoto, and I. Yahara.** Temperature-sensitive mutants of *hsp82* of the budding yeast *Saccharomyces cerevisiae*. Mol. Gen. Genet., in press.
9. **Koyasu, S., E. Nishida, T. Kadowaki, F. Matsuzaki, K. Iida, F. Harada, M. Kasuga, H. Sakai, and I. Yahara.** 1986. Two mammalian heat shock proteins, HSP90 and HSP100, are actin-binding proteins. Proc. Natl. Acad. Sci. USA **83**:8054–8058.
10. **Lebeau, M.-C., N. Massol, J. Herrick, L. E. Faber, J.-M. Renoir, C. Radanyi, and E.-E. Baulieu.** 1992. P59, an hsp90-binding protein. J. Biol. Chem. **267**:4281–4284.
11. **Lees-Miller, S. P., and C. W. Anderson.** 1989. The human double-stranded DNA-activated protein kinase phosphorylates the 90-kDa heat-shock protein, hsp90α at two NH_2-terminal threonine residues. J. Biol. Chem. **264**:17275–17280.
12. **Lindquist, S., and E. A. Craig.** 1988. The heat-shock proteins. Annu. Rev. Genet. **22**:631–677.
13. **Matsudaira, P.** 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. **262**:10035–10038.
14. **Mendel, D. B., and E. Orti.** 1988. Isoform composition and stoichiometry of the 90-kDa heat shock protein associated with glucocorticoid receptors. J. Biol. Chem. **263**:6695–6702.
15. **Minami, Y., H. Kawasaki, Y. Miyata, K. Suzuki, and I. Yahara.** 1991. Analysis of native forms and isoform compositions of the mouse 90-kDa heat shock protein, HSP90. J. Biol. Chem. **266**:10099–10103.
16. **Minami, Y., H. Kawasaki, K. Suzuki, and I. Yahara.** 1993. The calmodulin-binding domain of the mouse 90-kDa heat shock protein. J. Biol. Chem. **268**:9604–9610.
17. **Miyata, Y., and I. Yahara.** 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.
18. **Moore, S. K., C. Kozak, E. A. Robinson, S. J. Ullrich, and E. Appella.** 1987. Cloning and nucleotide sequence of the murine *hsp84* cDNA and chromosome assignment of related sequences. Gene **56**:29–40.
19. **Moore, S. K., C. Kozak, E. A. Robinson, S. J. Ullrich, and E. Appella.** 1989. Murine 86- and 84-kDa heat shock proteins, cDNA sequences, chromosome assignments, and evolutionary origins. J. Biol. Chem. **264**:5343–5351.
20. **Nemoto, T., Y. Ohara-Nemoto, M. Denis, and J.-A. Gustafsson.** 1990. The transformed glucocorticoid receptor has a lower steroid-binding affinity than the nontransformed receptor. Biochemistry **29**:1880–1886.
21. **Nishida, E., S. Koyasu, H. Sakai, and I. Yahara.** 1986. Calmodulin-regulated binding of the 90-kDa heat shock protein to actin filaments. J. Biol. Chem. **261**:16033–16036.
22. **Oppermann, H., W. Levinson, and J. M. Bishop.** 1981. A cellular protein that associates with the transforming protein of Rous sarcoma virus is also a heat-shock protein. Proc. Natl. Acad. Sci. USA **78**:1067–1071.
23. **Perdew, G. H., and M. L. Whitelaw.** 1991. Evidence that the 90-kDa heat shock protein (HSP90) exists in cytosol in heteromeric complexes containing HSP70 and three other proteins with Mr of 63,000, 56,000, and 50,000. J. Biol. Chem. **266**:6708–6713.
24. **Picard, D., B. Khursheed, M. J. Garabedian, M. G. Fortin, S. Lindquist, and K. R. Yamamoto.** 1990. Reduced levels of hsp90 comprise steroid receptor action *in vivo*. Nature (London) **348**:166–168.
25. **Rebbe, N. F., J. Ware, R. M. Bertina, P. Modrich, and D. W. Stafford.** 1987. Nucleotide sequence of a cDNA for a member of the human 90-kDa heat-shock protein family. Gene **53**:235–245.
26. **Rexin, M., W. Busch, B. Segnitz, and U. Gehring.** 1988. Tetrameric structure of the nonactivated glucocorticoid receptor in cell extracts and intact cells. FEBS Lett. **241**:234–238.
27. **Rose, A. B., and J. R. Broach.** 1990. Propagation and expression of cloned genes in yeast: 2-μm circle-based vectors. Methods Enzymol. **185**:234–279.
28. **Rose, D. W., R. E. H. Wettenhall, W. Kudlicki, G. Kramer, and B. Hardesty.** 1987. The 90-kilodalton peptide of the heme-regulated eIF-2α kinase has sequence similarity with the 90-kilodalton heat shock protein. Biochemistry **26**:6583–6587.
29. **Rose, M. D., and J. R. Broach.** 1991. Cloning genes by complementation in yeast. Methods Enzymol. **194**:195–230.
30. **Sanchez, E. R.** 1990. Hsp56: a novel heat shock protein associated with untransformed steroid receptor complexes. J. Biol. Chem. **265**:22067–22070.
31. **Schowalter, D. B., W. P. Sullivan, N. J. Maihle, A. D. W. Dobson, O. M. Conneely, B. W. O'Malley, and D. O. Toft.** 1991. Characterization of progesterone receptor binding to the 90- and 70-kDa heat shock proteins. J. Biol. Chem. **266**:21165–21173.
32. **Schuh, S., W. Yonemoto, J. Brugge, V. J. Bauer, R. M. Riehl, W. P. Sullivan, and D. O. Toft.** 1985. A 90,000-dalton binding protein common to both steroid receptors and the Rous sarcoma virus transforming protein, $pp60^{v\text{-}src}$. J. Biol. Chem. **260**:14292–14296.

33. **Shaknovich, R., G. Shue, and D. S. Kohtz.** 1992. Conformational activation of a basic helix-loop-helix protein (MyoD1) by the C-terminal region of murine HSP90 (HSP84). Mol. Cell. Biol. **12:**5059–5068.
34. **Smith, D. F., W. P. Sullivan, T. N. Marion, K. Zaitsu, B. Madden, D. J. McCormick, and D. O. Toft.** 1993. Identification of a 60-kilodalton stress-related protein, p60, which interacts with hsp90 and hsp70. Mol. Cell. Biol. **13:**869–876.
35. **Tai, P.-K. K., M. W. Albers, H. Chang, L. E. Faber, and S. L. Schreiber.** 1992. Association of a 59-kilodalton immunophilin with the glucocorticoid receptor complex. Science **256:**1315–1318.
36. **Tai, P.-K. K., Y. Maeda, K. Nakao, N. G. Wakim, J. L. Duhring, and L. E. Faber.** 1986. A 59-kilodalton protein associated with progestin, estrogen, androgen, and glucocorticoid receptors. Biochemistry **25:**5269–5275.
37. **Wang, K. K. W., A. Villalobo, and B. D. Roufogalis.** 1989. Calmodulin-binding proteins as calpain substrates. Biochem. J. **262:**693–706.
38. **Welch, W. J., and J. R. Feramisco.** 1982. Purification of the major mammalian heat shock proteins. J. Biol. Chem. **257:** 14949–14959.
39. **Yamazaki, M., K. Akaogi, T. Miwa, T. Imai, E. Soeda, and K. Yokoyama.** 1989. Nucleotide sequence of a full-length cDNA for 90 kDa heat-shock protein from human peripheral blood lymphocytes. Nucleic Acids Res. **17:**7108.