Selective Interaction of hsp90 with an Estrogen Receptor 
Ligand-binding Domain Containing a Point Mutation*

(Received for publication, February 13, 1997)

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The 90-kDa heat shock protein (hsp90) has been implicated in modulating steroid receptor function in vitro and in vivo. Previous studies have suggested that hsp90 interacts with large portions of the estrogen receptor (ER) ligand-binding domain and sequences of the receptor required for stable DNA binding. To characterize the interaction of the ER ligand-binding domain with hsp90, we have compared the properties of chimeras created by coupling the ligand-binding domain to the constitutive transactivator VP16-GAL. Two types of chimeras were created: VP16-GAL-ERG, containing the wild-type ligand-binding domain derived from the cDNA HEG0, and VP16-GAL-ERGV, containing the substitution G400V derived from the ligand-binding domain of the original ER cDNA isolate, HE0. The G400V mutation alters the physical properties of VP16-GAL-ERG by rendering it hormone-dependent for DNA binding and more strongly dependent on estradiol for transactivation compared with VP16-GAL-ERGV. Glycerol gradient analyses and chemical cross-linking/coimmunoprecipitation showed that, unlike VP16-GAL-ERGV, VP16-GAL-ERG formed stable complexes with hsp90 in vitro. These data show that hsp90 selectively recognizes the altered ER ligand-binding domain containing the G400V substitution and indicate that the wild-type ER ligand-binding domain of VP16-GAL-ERGV does not interact with hsp90 in vitro. Hormone binding studies showed that the ligand-binding domain of VP16-GAL-ERGV was stabilized by incubation in the presence of high concentrations of salt or in the absence of sodium molybdate, conditions that disrupt its interaction with hsp90. The ligand-binding domain of the Val-400 ER thus behaves similarly to that of the wild-type glucocorticoid receptor, which has previously been shown to interact with hsp90 in vitro. These results provide evidence for the action of hsp90 as a molecular chaperone by selectively recognizing destabilized proteins.

In the absence of hormone, the estrogen receptor (ER), similar to all steroid receptors, has been shown to form heteromerics complexes with accessory proteins both in vivo and in vitro (1–10). Among these accessory proteins are at least two members of the heat shock protein (hsp) family, hsp90 and hsp70, as well as members of the immunophilin family and several other receptor-associated proteins. It is thought that ligand-free steroid receptors interact directly with hsp90, stabilizing the receptor in an inactive state.

The ER is a member of a large family of nuclear receptors and, as such, is a hormone-dependent transcriptional regulator. The ER has been subdivided into six functionally distinct domains on the basis of sequence homology between receptors from different species (see Fig. 1 (11, 12). The highly conserved DNA-binding domain, encoded by region C, is composed of two zinc finger motifs, each with four conserved cysteine residues (13, 14). In addition to amino acids controlling site-specific DNA binding, region C contains a dimerization interface (15). Region D contains amino acids required for stable DNA binding (16) and has been shown to stabilize interactions with accessory proteins (1). Region E is also well conserved and contains domains required for ligand binding as well as the ligand-dependent transcriptional activation function known as AF-2 (17–20). Region E also contains sequences responsible for the interaction between the ER and accessory proteins (1, 2, 21, 22) as well as a strong dimerization interface (23, 24). An additional transactivation function (AF-1) is provided by the poorly conserved N-terminal A/B region of the receptor, which functions in a tissue- and promoter-specific context (18, 25).

It has been suggested that ligand binding induces a conformational change in the ER ligand-binding domain that releases hsp90, exposing those regions on steroid receptors required for homodimerization, nuclear localization, and DNA binding. Thus, hsp90 is thought to play a regulatory role by controlling several of the ligand-inducible functions of receptors. Studies in Saccharomyces cerevisiae showed that disruption of the interaction between hsp90 and the glucocorticoid receptor (GR) severely reduced the function of the GR in vivo (26, 27). Reduced expression of hsp90 strongly inhibits GR-dependent transactivation, suggesting that the GR can function properly only in the presence of hsp90. In parallel studies, however, ER-driven transactivation was less dependent on hsp90 expression, suggesting that hsp90 may be less important for controlling hormone-dependent function of the receptor in vivo.

Analysis of hsp90-ER interactions has been complicated by the overlap in domains D and E between regions of the receptor required for DNA binding, homodimerization, and nuclear localization and those required for interaction with hsp90 (1). To circumvent this problem, we recently constructed a series of chimeric ERs in which the DNA-binding domain has been replaced with that of the yeast transactivator GAL4. In addition, transactivation potential was increased by replacing the N-terminal AF-1 region with the strong acidic activator VP16, derived from herpes simplex virus, creating VP16-GAL-ER chimera. These chimeras do not interact stably with hsp90 in vitro. 

* This work was supported by Operating Grant MT-11704 from the Medical Research Council of Canada (to J. H. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: ER, estrogen receptor; hsp, heat shock protein; GR, glucocorticoid receptor; ERE, estrogen response element; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; SPDP, N-succinimidyl 3-(2-pyridyldithio)propionate.
vitro, bind DNA in a ligand-independent manner, and yet remain hormone-dependent for transactivation (28).

The original cDNA for the ER (HE0) contains the substitution G400V (29), which was subsequently found to destabilize the structure of the ligand-binding domain (29, 30). This suggests that the ligand-binding domain of HE0 may be like that of the hormone-free GR, which has been shown to be unstable in the absence of hsp90 (27). It was therefore of interest to determine the effect of such a destabilizing mutation on the interaction of the ER ligand-binding domain with hsp90 using VP16-GAL-ER chimeras as a model. To this end, VP16-GAL-ER

T-70 (Amersham Corp.) was constructed, containing the mutation equivalent to the G400V substitution of HE0. Unlike its wild-type counterpart, VP16-GAL-ER

interacts stably with hsp90 in vitro. The mutation destabilizes the ligand-binding domain, affecting its DNA binding and transactivation properties.

MATERIALS AND METHODS

Plasmid Recombinants—All chimeras were constructed in the pSG5 expression vector (31) by polymerase chain reaction amplification of the appropriate regions of VP16 and GAL4 and either wild-type ER HE0 or HE0. Duplication of each recombinant were tested for transactivation and verified by DNA sequencing.

Cell Culture—COS-7 cells were grown in 3.5-cm dishes in phenol red-free Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 5–10% charcoal-stripped fetal bovine serum. Lipofection was performed according to the manufacturer’s instructions (Life Technologies, Inc.). For luciferase assays, 100 ng of receptor expression vector was transfected along with 500 ng of 17M5-TATA-Luc or ERE2-TATA-Luc reporter vector and 1 µg of p610AZ-β-galactosidase expression vector for standardization. 16 h after lipofection, the medium supplemented with 10% charcoal-stripped fetal bovine serum was changed, and cells were incubated in the presence of 25 mM estradiol or ethanol vehicle for a further 20 h. Cells were lysed in 250 µl of lysis buffer (Promega). 50- and 45-µl aliquots of crude cell extracts were used for β-galactosidase and luciferase assays, respectively. For Western and gel retardation analyses, 1.0µg of ER expression vector was transfected by lipofection along with 1.0 µg of p610AZ-β-galactosidase expression vector as an internal control. For glycerol gradients, 10 µg of ER expression vector was transfected into COS-7 cells plated in 10-cm dishes.

Glycerol Gradient Analysis—Gradients were performed essentially as described (10). Briefly, cells from six individual 10-cm plates were scraped into 6 ml of ice-cold phosphate-buffered saline containing PMSF and protease inhibitors and resuspended in 1.5 volumes of TEGM buffer (20 mM Tris (pH 7.5), 1 mM EDTA, 10% (ν/v) glycerol, 30 mM sodium molybdate, and 5 mM DTT). Cells were lysed by drawing 20 times through a 25-gauge needle, and lysates were clarified by centrifugation at 10,000 rpm for 10 min at 4 °C, and supernatants were added to 15 min on ice in a 10-µl final volume of 25 mM Tris-Cl (pH 8.0), 1 mM EDTA, 50 mM KCl, and 20% glycerol containing 1 µg of poly(dI-dC) and then for a further 20 min at 23 °C after the addition of 50,000–100,000 cpm (5–10 fmol) of [3H]-labeled double-stranded oligonucleotide. Samples were run on 5% polyacrylamide gels (20:1), which were dried prior to autoradiography.

Western Blot Analysis—Immunoprecipitates were run on 7.5% polyacrylamide gels containing 0.1% SDS. Resolved proteins were transferred to nitrocellulose membrane (Hybond-C, Amersham Corp.) and probed with an anti-hsp90 monoclonal antibody (SPA 835) diluted 1:1000 in Tris-buffered saline/ Tween and 1% milk powder. Aliquots of extracts expressing ER chimeras were taken before or after precipitation, resolved on 10% polyacrylamide gels containing 0.1% SDS, transferred to Hybond-C membranes, and probed with anti-GAL4 DNA-binding domain monoclonal antibodies 2GV3 and 3GV2 diluted 1:5000 in Tris-buffered saline/Tween and 1% milk powder. Blots were developed using the ECL detection system (DuPont NEN).

RESULTS

Construction of ER Chimeras—We have recently reported the construction of a series of VP16-GAL-ER fusion receptors to study the role of hsp90 interaction in modulating ER function (28). Addition of the ER ligand-binding domain rendered constitutively active VP16-GAL receptorially dependent on hormone for transactivation. The VP16-GAL-ER chimeras bound to DNA equally well in the presence or absence of estradiol and are at least partially nuclear in the absence of ligand (28). More important, glycerol gradient analysis and communoprecipitation studies suggested that the chimeras do not interact with hsp90 in vitro (28). Assays have thus been established to measure transactivation, DNA binding, and hsp90 association by

were treated with 0.5 mM N-ethylmaleimide (10 min at 23 °C) to block all free SH groups that could prematurely cleave cross-links and divided into two equal aliquots. Intermolecular disulfide bonds formed during cross-linking were either reduced by incubation with 50 mM DTT for 30 min on ice or left intact. Protein complexes were immunoprecipitated by incubation for 12 h at 4 °C in radioimmunoprecipitation assay buffer (50 mM Tris (pH 7.4), 4 mM EDTA, 150 mM NaCl, 1% Triton X-100, and 0.1% SDS). Samples were sedimented by centrifugation at 10,000 rpm for 30 s, washed three times in radioimmunoprecipitation assay buffer, and then resuspended in 20 µl of SDS-polyacrylamide gel electrophoresis sample buffer supplemented with 50 mM DTT. Western analysis using the anti-hsp90 monoclonal antibody SPA 835 (Stressgen Biotech Corp.) was carried out as described below.

Hormone Binding Assays—Hormone binding assays were performed essentially as described (11). Briefly, COS-7 cells were transfected as described for glycerol gradient experiments. Cells (10-cm dishes) were harvested in 1 ml of phosphate-buffered saline containing PMSF and centrifuged, and the cell pellet was resuspended and lysed as described for glycerol gradient analysis in 100 µl of hormone binding buffer (50 mM Tris (pH 7.5), 1.5 mM EDTA, 50 mM NaCl, 10% (ν/v) glycerol, 1 µM β-mercaptoethanol, and the protease inhibitors PMSF, leupeptin, aprotonin, and 10 µg/mL soybean Bowman-Birk protease inhibitor) containing 10 µM molybdate where indicated. Lysates were diluted to 200 µl in hormone binding buffer and clarified by centrifugation. For each binding reaction, 20-µl aliquots were diluted to 50 µl with hormone binding buffer. For preincubations with high salt, KCl was added to a final concentration of 400 mM. Aliquots were preincubated at room temperature as indicated, prior to incubation on ice for 5 h in the presence of 20 nM 17β-[3H]estradiol (Amersham Corp.). To determine nonspecific binding, 4 µM estradiol was also included. Unbound estradiol was removed by incubation with 0.5% Nor-A-Charcoal and 0.05% dextran T-70 for 10 min and then centrifuged. Radioactive estradiol in the supernatant was determined by liquid scintillation counting.

Gel Retardation Assays—Cells were harvested by combining those scraped from two or three 3-cm plates in 500 µl of ice-cold phosphate-buffered saline. Cells were centrifuged at 2500 rpm for 10 min at 4 °C, the supernatant was carefully removed; and the pellet was resuspended in 100 µl of high salt extraction buffer (25 mM Tris (pH 7.9), 0.3 mM DTT, 0.1 EDTA, 400 mM NaCl, and 10% (ν/v) glycerol). Cells were lysed by three cycles of freezing at −70 °C and thawing at room temperature and then centrifuged at 10,000 rpm for 10 min at 4 °C, and supernatants were centrifuged at 70,000 g for 30 min at 4 °C, and supernatants were added to 15 min on ice in a 10-µl final volume of 25 mM Tris-Cl (pH 8.0), 1 mM EDTA, 50 mM KCl, and 20% glycerol containing 1 µg of poly(dI-dC) and then for a further 20 min at 23 °C after the addition of 50,000–100,000 cpm (5–10 fmol) of [3H]-labeled double-stranded oligonucleotide. Samples were run on 5% polyacrylamide gels (20:1), which were dried prior to autoradiography.


VP16-GAL-ER chimeras. We were interested next in determining the effect on the interaction of chimeras with hsp90 of the destabilizing mutation G400V, found in the original ER cDNA, HE0 (29). A well characterized chimera was chosen to study the functional differences between the Gly-400 and Val-400 homologues, containing ER ligand-binding domain sequences from amino acid 258 (Fig. 1). VP16-GAL-ERG and VP16-GAL-ERV are used to denote chimeras derived from HEG0 and HE0, respectively (Fig. 1).

**Glycerol Gradient Analysis of Extracts Expressing VP16-GAL-ERV or VP16-GAL-ERG Chimeras—**hsp90-ER chimera interactions were first probed by glycerol gradient analysis. Sedimentation profiles of full-length receptors were used as positive controls. In the presence of molybdate ions, HEG0 formed a stable complex, characteristic of its interaction with hsp90, that migrated in a glycerol gradient with a sedimentation coefficient of 8–9 S. Under high ionic strength conditions, the molybdate-stabilized complex dissociated, resulting in an "activated" ER that migrated at 4–5 S (Fig. 2A). Conversely, VP16-GAL-ERG did not exhibit a salt-dependent shift in its migration and sedimented at 4–5 S under both low and high salt conditions (Fig. 2B). To test the characteristics of Val-400 mutants, we performed identical analyses on HE0 and VP16-GAL-ERV. As expected, HE0 was found to form molybdate-stabilized, salt-sensitive 8 S complexes in vitro (Fig. 2C). Significantly, VP16-GAL-ERG also formed a salt-sensitive 8 S complex (Fig. 2D), suggesting that hsp90 may recognize the altered ligand-binding domain of the chimera.

**Stable Interaction of hsp90 with VP16-GAL-ERV, but Not VP16-GAL-ERG, Determined by Chemical Cross-linking/Immunoprecipitation—**To verify that hsp90 is indeed a component of the 8–9 S complex containing VP16-GAL-ERV, a combined chemical cross-linking/coimmunoprecipitation protocol was developed using extracts of transiently transfected COS-7 cells expressing either VP16-GAL-ERG or VP16-GAL-ERV. Cells were lysed in the presence of molybdate ions under conditions in which an 8–9 S complex formed in the absence of salt. Extracts were divided into two equal aliquots, treated with 400 mM KCl (high salt; closed circles) or water (low salt; open circles), and loaded into 10–35% gradients. Gradients were centrifuged at 49,000 rpm for 16 h, and reactions were collected in 150-μl aliquots from the top of the gradient. The top of each gradient is on the left. 75-μl fractions were resolved on SDS-polyacrylamide gels to determine the sedimentation of the molecular mass markers glucose oxidase (G.O.) and horseradish peroxidase (HRP). Liquid scintillation counting was performed on 50-μl aliquots. Results are expressed as [%estradiol bound in cpm. At least three experiments were performed for each receptor type with similar results.
Following immunoprecipitation, proteins were resolved on SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and probed with monoclonal antibody SPA 835 (raised against hsp90). A 90-kDa protein recognized by SPA 835 was precipitated along with VP16-GAL-ERV, as shown in lane 1 of the figure. Treatment of the extracts with DTT prior to immunoprecipitation to reduce the intermolecular cross-linker strongly decreased the amount of detectable hsp90 that coprecipitated with the chimera, indicating that hsp90 is not being precipitated nonspecifically by the anti-GAL4 polyclonal antibody (Fig. 3, lane 2).

In the presence of 400 mM KCl, very low amounts of hsp90 can be seen to be associated with VP16-GAL-ERV, and this faint band disappeared upon treatment with DTT (Fig. 3, lane 3). This suggests that, in the presence of KCl, only a minute amount of hsp90 may remain associated with the receptor and is consistent with the migration of VP16-GAL-ERV at 4–5 S in glycerol gradients under these conditions (Fig. 2).

While glycerol gradient analyses suggested that VP16-GAL-ERG does not interact stably with hsp90, we cannot rule out the possibility that hsp90 may interact transiently with the chimera in extracts. Chemical cross-linking and immunoprecipitation of extracts expressing VP16-GAL-ERG were therefore performed to trap any transient binding to hsp90. Even under these conditions, no coimmunoprecipitation of hsp90 was detected from extracts incubated in the presence or absence of salt (Fig. 3, lanes 5–8). Taken together, these results indicate that hsp90 selectively recognizes ER chimeras containing the G400V substitution under low salt conditions in the presence of molybdate.

**The ER Ligand-binding Domain Containing the G400V Substitution Is Destabilized by High Salt and the Absence of Sodium Molybdate**—Previous studies have suggested that the G400V substitution destabilizes the ER ligand-binding domain (29). It was therefore of interest to examine the effect of salt and molybdate ions, which modulate the stability of the interaction of the ER with hsp90, on the stability of the G400V ligand-binding domain. Extracts of COS-7 cells transiently expressing either VP16-GAL-ERG or VP16-GAL-ERV were preincubated for varying times in the presence of molybdate under low or high salt conditions prior to performing hormone binding assays. The results (Fig. 4A) indicate that high salt concentrations only slightly affected the stability of the wild-type ligand-binding domain of VP16-GAL-ERV. The VP16-GAL-ERV ligand-binding domain was less stable than that of VP16-GAL-ERG under low salt conditions and was significantly destabilized by high salt concentrations. In addition, the removal of molybdate from the preincubation destabilized the VP16-GAL-ERV ligand-binding domain under low salt conditions (Fig. 4B). A control Western analysis showed that the loss of hormone binding activity in extracts containing VP16-GAL-ERV ligand-binding domain under low salt conditions (Fig. 4C). These results indicate that the VP16-GAL-ERV ligand-binding domain is most stable at low salt concentrations and in the presence of molybdate, conditions under which it interacts most stably with hsp90.
control plasmid pSG5 (lanes 1 and 2). As expected, HEG0 bound a synthetic ERE oligonucleotide in the absence of estradiol, and addition of hormone did not affect the level of DNA binding by the receptor (Fig. 5A, lanes 3 and 4). In contrast, DNA binding by HE0, which contains the G400V mutation, exhibited a clear requirement for estradiol (Fig. 5A, lanes 5 and 6).

VP16-GAL-ERV, like HEG0, binds to cognate DNA sequences both in the presence and absence of estradiol (Fig. 5B, compare lanes 1 and 2). In this respect, it is indistinguishable from the constitutive activator VP16-GAL (Fig. 5B, lanes 7 and 8). In contrast, significant DNA binding by VP16-GAL-ERV was observed only in the presence of estradiol (Fig. 5B, lanes 5 and 6). These results show that the chimeric ERs display DNA binding characteristics that are very similar to those of their respective full-length receptors and that the G400V mutation disrupts DNA binding in the absence of hormone by both the full-length ER HE0 and the corresponding chimera VP16-GAL-ERV.

In the presence of HEG0, low but consistent levels of transactivation of an ERE-containing reporter gene were observed in the absence of estradiol. Hormone induced transactivation by 10-fold (Fig. 6, A and B). In contrast, no hormone-independent transactivation over background levels was observed in the presence of HE0, consistent with previous reports (29, 30). Transactivation by HE0 was induced at least 20-fold by hormone. VP16-GAL-ERV activated a 17-mer-containing reporter transcription to levels ~40% of those seen with VP16-GAL-ERV (Fig. 6C). Thus, in both the full-length receptor HE0 and in VP16-GAL-ERV, the G400V substitution abolished hormone-independent transactivation (Fig. 6, B and D). Given the highly synergistic transactivation potential of VP16–ERV, transactivation over background levels was observed in the absence of estradiol with extracts of cells transfected with the expression vector for VP16-GAL-ERV, (V-G-ERV, lanes 1 and 2) or VP16-GAL-ERV (V-G-ERV, lanes 5 and 6). As a negative control, extracts of cells transfected with pSG5 were used (lanes 3 and 4). As a control for ligand-independent DNA binding, extracts expressing VP16-GAL were used (lanes 7 and 8; V-G). Assays were performed with a synthetic 17-mer oligonucleotide recognized by the GALA DNA-binding domain.

**Discussion**

Introduction of the G400V mutation into the ER ligand-binding domain profoundly affected its interaction with hsp90. Unlike VP16-GAL-ERV, the VP16-GAL-ERV chimera formed salt-sensitive 8 S complexes in glycerol gradients and interacted with hsp90 in cross-linking/coimmunoprecipitation experiments (Figs. 2 and 3). This is consistent with previous observations that hsp90 could be coimmunoprecipitated with β-galactosidase-ER ligand-binding domain chimeras derived from HE0 (21). In this respect, the isolated ligand-binding domain of the ER mutant behaved similarly to that of the wild-type GR, which interacts with hsp90 in *vitro* (21).

The G400V substitution also significantly affected ER ligand-binding domain function. Previous results indicated that the HE0 form of the ER has a reduced affinity for ligand in *vitro* and that this reduction may be at least partially responsible for the observed alterations in receptor function (29). Our gene transfer experiments (28) and those of others (29, 32, 33) indicate that the wild-type ER ligand-binding domain is able to dimerize in the absence of hormone both in *vitro* and in *vivo*. The G400V mutation alters the conformation of the ER ligand-binding domain to such a degree that the full-length receptor is rendered hormone-dependent for dimerization and hence DNA binding (Fig. 5) (29, 34). Our results with chimeric receptors show that the equivalent mutation can render a heterologous DNA-binding domain, which is capable of dimerizing independently, hormone-dependent for DNA binding. This suggests that the mutated ligand-binding domain sterically hinders the function of the GALA dimerization function or masks the amino acids responsible for DNA recognition. The effect of the G400V mutation on the interaction with hsp90, coupled with inhibition of DNA binding in the absence of hormone, provides an explanation for the observations that both HE0 and VP16-GAL-ERV are more strongly dependent on hormone for transactivation than their wild-type counterparts. These results would be consistent with mutated receptors and their chimeric homologues being maintained more stably in a cytoplasmic complex with hsp90 than their wild-type counterparts. Moreover, any dissociated receptors would homodimerize poorly and hence not bind DNA efficiently.

The conformational change induced by the G400V mutation significantly destabilized the ER ligand-binding domain. The ligand-binding domain of VP16-GAL-ERV was somewhat less
stabilized ER ligand-binding domain. These results are consistent with this hypothesis (26, 27). Although cross-linking analysis suggests that the ER also forms complexes with hsp90 in vivo (35), several experiments have suggested that at least a portion of the hormone-free ER is nuclear and capable of binding DNA (32, 33, 40–43) and that hsp90 may not be essential for ER function (26). Taken together, these observations suggest that HEG0 interacts with hsp90 in vitro, but that this interaction is transient, resulting in dissociation, homodimerization, and nuclear transport of aporeceptors.

Glycerol gradient and combined cross-linking/coimmunoprecipitation experiments (Figs. 2 and 3) did not provide any evidence that the wild-type ER ligand-binding domain interacts transiently or stably with hsp90. Previous results have shown that the isolated wild-type GR ligand-binding domain can interact with hsp90 in vitro (21). These results provide support for the idea that the ER binds less stably to hsp90 than the GR.

While our present and previous results (28) and those of others (26, 32, 33) suggest that hsp90 may not be essential for controlling the function of the native wild-type ER, it may be important for correct folding of the receptor. Genetic studies in yeast showed that reduced hsp90 levels compromised ER function (26). Taken together, these observations suggest that HEG0 interacts with hsp90 in vivo, but that this interaction is transient, resulting in dissociation, homodimerization, and nuclear transport of aporeceptors.

Yeast GAL4 fusion proteins showed that hsp90 is required for the GR to attain a functional, ligand-inducible state. Furthermore, to remain in such a state, continuous interaction of the GR with hsp90 is required (27). The apparent instability of the GR ligand-binding domain further emphasizes its similarity to that of the HE0 form of the ER.

It is noteworthy that the ER ligand-binding domain has been used to confer hormone-dependent function on the nuclear protein Fos (38) and the cytoplasmic kinase Raf-1 (39). In both cases, the ER ligand-binding domain was derived from HEG0. Based on our results, one can speculate that chimeras derived from HEG0 would be more nuclear in the absence of hormone than those derived from HE0, which would fundamentally affect the function of the resulting fusion proteins.

Current models of steroid receptor action suggest that aporeceptors form high molecular mass complexes with hsp90 and other proteins. These complexes are thought to keep the receptor in a stable inactive state until ligand binding frees it from hsp90. Indeed, both genetic and biochemical studies using the GR are consistent with this hypothesis (26, 27). Although cross-linking analysis suggests that the ER also forms complexes with hsp90 in vitro (35), several experiments have suggested that at least a portion of the hormone-free ER is nuclear and capable of binding DNA (32, 33, 40–43) and that hsp90 may not be essential for ER function (26). Taken together, these observations suggest that HEG0 interacts with hsp90 in vitro, but that this interaction is transient, resulting in dissociation, homodimerization, and nuclear transport of aporeceptors. Glycerol gradient and combined cross-linking/coimmunoprecipitation experiments (Figs. 2 and 3) did not provide any evidence that the wild-type ER ligand-binding domain interacts transiently or stably with hsp90. Previous results have shown that the isolated wild-type GR ligand-binding domain can interact with hsp90 in vitro (21). These results provide support for the idea that the ER binds less stably to hsp90 than the GR.

While our present and previous results (28) and those of others (26, 32, 33) suggest that hsp90 may not be essential for controlling the function of the native wild-type ER, it may be important for correct folding of the receptor. Genetic studies in yeast showed that reduced hsp90 levels compromised ER function, although not to the same degree as GR function. hsp90 may therefore be necessary to reduce the levels of partially folded or misfolded ER, which, once correctly folded, dissociates from the chaperone and translocates to the nucleus.

Acknowledgments—We are grateful to Drs. P. Chambron, M. Featherstone, and R. Kothary for the gifts of ER cDNAs and 17M5-TATA-Luc and p610AZ recombinants, respectively; and Drs. P. Chambron and Y. Lutz for anti-GAL antibodies.

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Selective Interaction of hsp90 with an Estrogen Receptor Ligand-binding Domain Containing a Point Mutation

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J. Biol. Chem. 1997, 272:12229-12235.
doi: 10.1074/jbc.272.18.12229

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