Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10434–10438, November 1993
Biochemistry

Interaction of glucocorticosteroid receptor and wild-type or mutated 90-kDa heat shock protein coexpressed in baculovirus-infected Sf9 cells

(steroid receptor/hsp90 interaction)

F. CADEPOND, N. BINART, B. CHAMBRAUD, N. JIBARD, G. SCHWEIZER-GROYER, I. SEGARD-MAUREL, AND E. E. BAULIEU*

Institut National de la Santé et de la Recherche Médicale U33, Lab Hormones, 94276 Le Kremlin-Bicêtre Cedex France

Contributed by E. E. Baulieu, July 16, 1993

ABSTRACT **Coexpression of the human glucocorticosteroid receptor (hGR) and chicken 90-kDa heat shock protein α (chsp90) in recombinant baculovirus-infected Sf9 cells is a system that provides a large quantity of wild-type chsp90–hGR complexes able to bind hormone ([^{3}H]triamcinolone acetonide; TA), sedimenting at 8 S, and displaceable to 11 S by BF4 and D7α anti-chsp90 monoclonal antibodies. Thus, we were able to examine the effects of selective chsp90 mutations on heterooligomeric complex formation. Two deletions involved hydrophilic regions, A between amino acids 221 and 290 and B between amino acids 530 and 581, and the third, Z, removed a central leucine heptad repeat region (amino acids 392–419). When these chsp90 mutants were expressed, the lack of displacement of [^{3}H]TA receptor complexes on sucrose gradient by specific chsp90 antibodies was consistent with the formation of [^{3}H]TA receptor complexes containing only endogenous insect hsp90. By using an immunoadsorption method and sedimentation analysis, we found that the deletion of region A precluded the interaction of chsp90 with the hGR, while B and Z deletions led to formation of abnormal complexes with the hGR, which displayed large forms (>10 S), were unable to bind hormone, and apparently formed only small amounts of tightly bound nuclei hGR upon *in vivo* hormone treatment. As a whole, the data are consistent with distinct roles of hsp90 regions in hGR function.**

Heat shock proteins (hsp) are polypeptide chain binding proteins or molecular chaperones (1). One of the main family of hsp proteins is the 90-kDa heat shock protein (hsp90) class, a family that is highly conserved and crucial for cell viability (tested in yeast; see ref. 2). Their role in the stress response was described initially, but progressively their intervention in cellular processes such as protein folding, assembly, disassembly, and intracellular transport has been brought to light (1, 3–5). hsp90s associate with various cellular proteins, including steroid hormone receptors (6, 7), and frequently maintain these proteins in a nonfunctional state (8–12). Cytosol steroid receptors, in the absence of hormone, form dimeric hsp90-containing complexes. Hormone binding triggers complex dissociation and allows the receptor to acquire DNA binding capacity and transcriptional activity (7, 13–15). For the glucocorticosteroid receptor (GR), high-affinity steroid binding occurs only in the heterooligomeric form, and it is believed that hsp90 is involved in maintaining the appropriate conformation for hormone binding (16, 17). The requirement *in vivo* of adequate levels of hsp90 for biological activity of steroid receptors in yeast has also been reported (18).

Biochemical analysis (19) and studies of mutated steroid receptors have shown that the ligand binding domain (LBD) is directly involved in their interaction with hsp90 (20–22). The absence of a discrete interacting motif in this domain may reflect the propensity of the hsp90 dimer to participate in the folding of the LBD, in agreement with its potential chaperonage role (18, 20–23). No mutagenesis analysis of hsp90 has yet been reported. hsp90 is a very abundant and well-conserved protein and the *in vivo* efficient competition of the endogenous hsp90 by a foreign protein is very difficult to carry out. The *in vitro* experiments, using reticulocyte lysates, have not yet allowed examination of the effects of mutated hsp90 (24–27).

In this work, we describe the formation of heterooligomeric complexes including chicken hsp90α (chsp90) and human GR (hGR) when they were coexpressed in Sf9 cells. This system allowed us to study three mutated chsp90s: two derivatives were deleted in highly charged regions, which were good candidates for interaction with the steroid receptor (28, 29); a third chsp90 devoid of a leucine heptad repeat could be involved in protein–protein contacts. Overexpression of exogenous chsp90 relative to the endogenous hsp90 and the use of specific antibodies permitted (*i*) analysis of the effects of mutations on the sedimentation profiles of free hsp90s, (*ii*) demonstration of the *in vivo* formation of hGR heterooligomeric complexes with an exogenous hsp90, and (*iii*) investigation of the role of some hsp90 regions in the interaction with hGR and the modulation of its biological properties.

MATERIALS AND METHODS

Generation of Recombinant Baculoviruses. The full-length hGR cDNA, between *Kpn* I and *Xho* I restriction sites of pRShGRα (30), was introduced into the *Nhe* I site of the pBlueBac vector (Invitrogen) and the full-length chsp90α cDNA (28, 31) was inserted into the *Bam*HI site of the baculovirus transfer vector PVL941 (32) after filling in all protruding ends. Deletions of the A region (amino acids 221–290) and B region (amino acids 530–581) or Z region (amino acids 392–419) were obtained by oligonucleotide-directed mutagenesis (N.B. and B.C., unpublished data). Recombinants from wild-type baculovirus (*Autographa californica* nuclear polyhedrosis virus or AcMNPV) and vector plasmids were obtained in *Spodoptera frugiperda* (Sf9) cells as described (32, 33). In all cases, expressed proteins were analyzed for their molecular size and the hGR was analyzed for its ability to bind steroid.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LBD, ligand binding domain; chsp90, 90-kDa chicken heat shock protein α; hGR, human glucocorticosteroid receptor; TA, triamcinolone acetonide.

*To whom reprint requests should be addressed.

Sf9 Culture and Infection. Sf9 cells were grown in spinner flasks containing TNM-FH medium (Sigma) including 10% steroid-depleted fetal calf serum (34). Stock virus suspensions (multiplicity of infection, 1–10) were obtained as described (32). Coinfection was performed by adding 3 ml of chsp90-expressing virus suspension to 6×10^7 Sf9 cells in 6 ml of culture medium and 2 h later 3 ml of the hGR-expressing virus suspension. After 2 more h, the cells were added to 60 ml of culture medium in a spinner flask. They were harvested at different times after infection. An infection time of ≥36 h allowed enough hGR and chsp90 to accumulate (data not shown).

For the metabolic labeling of Sf9 cells, 6×10^7 cells were carefully washed in Grace's medium without methionine (Sigma) 24 h after coinfection and then cultured for 6 h in Grace's medium containing 1 mCi (1 Ci = 37 GBq) of [^{35}S]methionine.

Hormonal treatment was performed by introduction of [^{3}H]triamcinolone acetonide (TA; 1 μM) into the cell suspension 1 h before harvesting.

Preparation of Cellular Extracts. High-salt extracts were prepared as described (35). Steroid binding was measured by the hydroxylapatite method (36).

Cytosols were prepared in GTED buffer (21) containing 15 mM sodium molybdate in an all-glass motorized homogenizer. Steroid binding capacity was determined by a 2-h incubation with 200 nM [^{3}H]TA and treatment by the dextran-coated charcoal technique (21).

Sedimentation Analysis on Sucrose Gradients. Aliquots were layered on 5–25% sucrose gradients (5 ml) prepared in TED buffer (20 mM Tris·HCl/1 mM EDTA/1 mM dithioerythritol, pH 7.4 at 4°C) containing 10% (vol/vol) glycerol. Centrifugation at 65,000 rpm was performed for 2 h in a VTI 65.2 rotor in the presence of internal standards (21). Fractions (three drops) were assayed and/or used for Western blots.

Immunodetection of hGR and hsp90. Western blots were prepared as described (21). Briefly, saturation with nonfat dry milk (10%) was followed by incubation with either anti-hsp90 antibodies (BF4 or AC19) or a polyclonal anti-hGR antibody raised in the rabbit against the hGR sequence of amino acids 149–168. The latter can interact with both the native 8 S and 4 S hGR and with the SDS-denatured form of the hGR on Western blot. Bound antibody on Western blot was revealed by the immunoperoxidase technique (21) (Vectastain ABC) using a chemiluminescent substrate (Amersham, ECL system).

Immunoadsorption of hGR-containing complexes was carried out on an anti-hGR or control IgG protein A-Sepharose resin (4–5 mg of antibody per ml) prepared and cross-linked as described (37, 38). Cytosols were cleared of free hsp90 and nonspecific contaminants by two successive adsorption runs on control IgG resin. Cytosol supernatants were immunoadsorbed on 0.1 ml of control IgG or specific resin. After washing, adsorbed proteins were eluted by 50 mM Tris·HCl (pH 10.9), precipitated (39), and then analyzed by Western blot using the AC19 antibody. When [^{35}S]methionine-labeled cytosols were used, SDS gels were enhanced with Amplify (Amersham), dried, and autoradiographed.

RESULTS

Hormone-Labeled, 8 S Heterooligomeric hGR Complexes Are Formed with Wild-Type chsp90 but Not with Deleted chsp90s. Sedimentation analysis on sucrose gradients of cytosol aliquots of hGR-expressing Sf9 after *in vitro* labeling with [^{3}H]TA gave the usual hGR profile with a major peak at 8 S, corresponding to the heterooligomeric complex formed with the endogenous insect hsp90. After incubation with the anti-chsp90 monoclonal antibodies BF4 or D7α (6, 7, 40–42), no displacement of this 8 S peak was observed (data not shown).

When Sf9 cells were coinfected with both hGR and chsp90 expression viruses for 36 h, the [^{3}H]TA 8 S peak was totally displaced to the 11 S position by BF4 or D7α antibody, indicating that the ligand-bound receptor interacts preferentially with chsp90 (Fig. 1*a*). Control ascites fluid or mouse and rat IgG gave negative results. Therefore, analysis of the chsp90 subregion functions by mutagenesis techniques was undertaken.

The three deletions introduced into chsp90 are illustrated schematically in Fig. 1*e*. Region A corresponds to the most negatively charged region of the hsp90 molecule. Region B is also a charged segment, while region Z is characterized by a leucine heptad repeat at the center of the molecule (28, 29). None of the deleted chsp90s led to the formation of [^{3}H]TA-bound 8 S complexes, which could be displaced by specific anti-chsp90 antibodies (Fig. 1 *b–d*). Since we checked that epitopes of the native, free (not included in the receptor complex), wild-type, and mutated chsp90s remain accessible to antibodies (data not shown), this result was consistent with the formation of ligand-bound hGR complexes with only the endogenous insect hsp90.

Two distinct situations may account for the lack of reactivity of the antibodies; either deletions simply abolish the capacity of chsp90 to interact with the hGR, or, if interaction

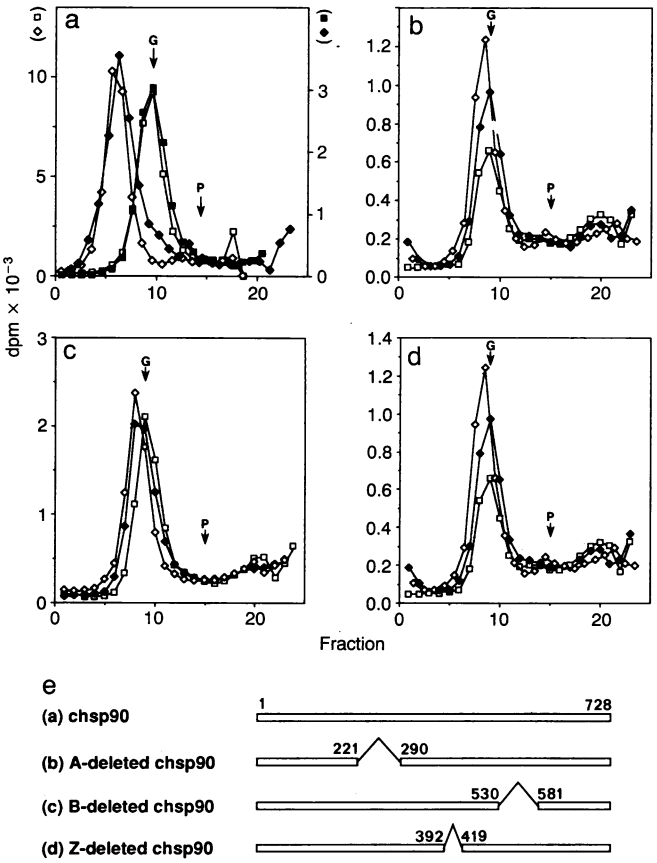

FIG. 1. Sedimentation analysis of [^{3}H]TA GR coexpressed with wild-type or mutated chsp90s. Effect of D7α and BF4 antibodies. Cytosol aliquots from Sf9 cells coinfected with the hGR and wild-type chsp90 (60 and 20 μg) (*a*), with A-deleted chsp90 (37 μg) (*b*), with B-deleted chsp90 (63 μg) (*c*), or with Z-deleted chsp90 (37 μg) (*d*) were diluted to 100–200 μl with GTED buffer containing protease inhibitors and incubated for 2 h in the absence (□, ■) or presence of 0.2–0.4 mg of D7α (◆) or 0.25–0.62 mg of BF4 (◇) antibodies (ascites fluids), depending on the chsp90 expression levels. They were layered on sucrose/glycerol gradients and analyzed as described. Internal standards: glucose oxidase, 7.9 S (G); horseradish peroxidase, 3.6 S (P). (*e*) Schematic representation of wild-type and mutated chsp90s.

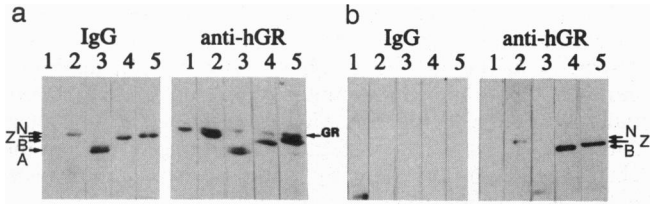

FIG. 2. Immunoadsorption of hsp90s on an anti-GR matrix. (*a*) Various [^{35}S]methionine Sf9 cell cytosols (0.6 ml; 0.9–2.4 mg) were incubated twice with 0.2 ml of control IgG matrix. Half of the resulting supernatant was incubated with 0.1 ml of IgG control resin; the other half was incubated with anti-hGR resin. Eluates obtained from washed resins were analyzed by SDS/PAGE as described. hsp90s were detected by autoradiography. (*b*) Unlabeled cytosol aliquots (0.7–1.5 mg) were treated as described in *a*. The chsp90s were detected by Western blot analysis using AC19 antibody. Lanes: 1, hGR expressed alone; 2, hGR expressed with wild-type chsp90; 3, hGR expressed with A-deleted chsp90; 4, hGR expressed with B-deleted chsp90; 5, hGR expressed with Z-deleted chsp90. N, Z, B, and A, positions of native (or wild type), Z-, B-, and A-deleted chsp90s, respectively.

still occurs, they alter the ability of chsp90 to maintain hormone binding of the hGR LBD. Therefore, the next step was to analyze the possible formation of complexes between chsp90 and hGR devoid of steroid binding activity.

B- and Z-Deleted chsp90 Mutants, but Not the A-Deleted Mutant, Interact with the hGR. A rabbit polyclonal anti-hGR antibody was used to prepare a protein A-Sepharose resin able to adsorb hGR-containing complexes. Fig. 2 shows the hsp90s immunoadsorbed in [^{35}S]methionine (24–30 h)-labeled cytosols (Fig. 2*a*) and in unlabeled cytosols from cells infected for 48 h (Fig. 2*b*).

In [^{35}S]methionine cytosols, chsp90 was preferentially labeled with respect to other cellular components, consistent with its very high synthesis rate beyond 24 h after infection; thus [^{35}S]GR was expected to interact predominantly with chsp90. We observed that wild-type, B-, and Z-deleted chsp90s were significantly retained on the anti-hGR matrix (less efficiently for the B-deleted mutant) (Fig. 2*a*, lanes 2, 4, and 5). In contrast, no significant adsorption was recorded for the A-deleted mutant (lanes 3). We quantified the intensities of the hGR and hsp90 bands obtained on the gel autoradiogram. Determination of hsp90/hGR ratios (after subtraction of IgG-adsorbed hsp90) gave values close to 2 for B- and Z-deleted chsp90, in agreement with the reported stoichiometry of two molecules of hsp90 per hGR molecule in the heterooligomeric complexes (43–45). A smaller value of 1.54 was obtained for wild-type chsp90, suggesting that under our experimental conditions some wild-type chsp90 dissociation may have occurred. Alternatively, a small amount of GR interacted with unlabeled insect hsp90. This implies that B- and Z-deleted chsp90 may form some complexes containing more than two chsp90 molecules per GR. This assumption is compatible with the detection of both GR and B- or Z-deleted chsp90 in the high molecular weight fractions of glycerol/sucrose gradients (described below). Consistent with the low level of [^{35}S]methionine labeling of endogenous Sf9 proteins, insect hsp90 was not detected (lane 1).

Results obtained with unlabeled cytosols agreed with that found with [^{35}S]methionine cytosols. For the wild-type chsp90, a faint band was detected while strong bands were observed for the Z- and B-deleted chsp90s (Fig. 2*b*), consistent with the formation of Z- and B-deleted chsp90 complexes with chsp90/GR ratios >2. Insect hsp90 was not detected (lane 1), a result that could be related to its limited immunoreactivity to AC19 antibody (data not shown).

Consistent with these data, complementary cross-linking experiments with dimethylpimelimidate (46) did not detect receptor complexes formed with the A-deleted chsp90 but revealed several high molecular weight hGR complexes containing Z- and B-deleted and wild-type chsp90s (data not shown).

Cosedimentation of the Overexpressed hGR and B- or Z-Deleted chsp90. To determine the size of complexes formed by the hGR and B- and Z-deleted chsp90s under high-expression

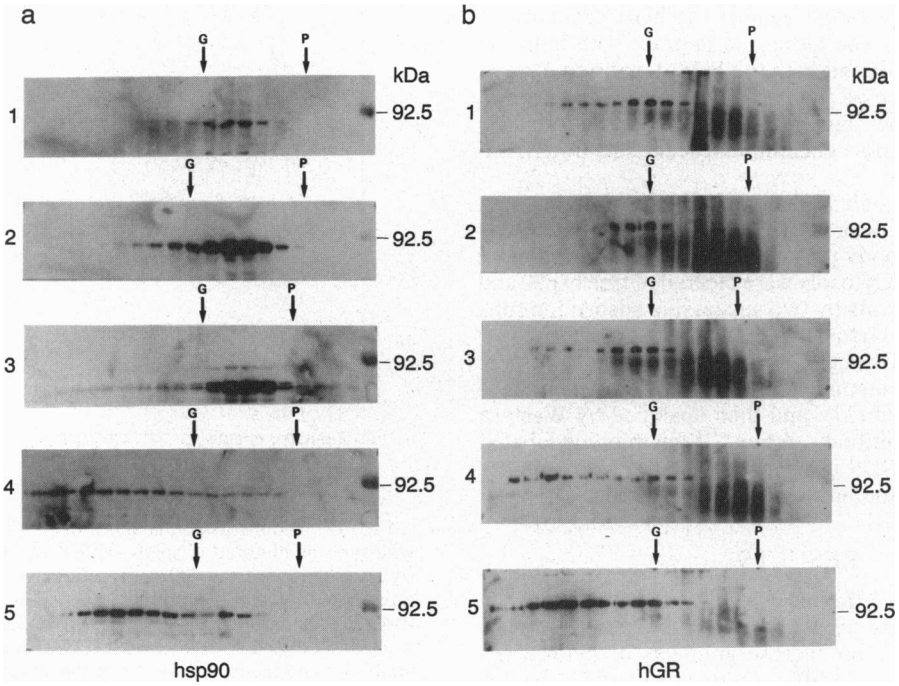

FIG. 3. Sedimentation analysis of endogenous insect hsp90 or wild-type or mutated chsp90s and hGR when overexpressed in Sf9 cells. Cytosol aliquots (130 μg for hGR expressed alone or coexpressed with B-deleted chsp90 and 260 μg for hGR coexpressed with wild-type or A- or Z-deleted chsp90) were layered on sucrose/glycerol gradients. Sedimentation analysis was performed by SDS/PAGE and Western blotting of gradient fractions 1–19 with the AC19 antibody for hsp90 detection (*a*) and anti-hGR antibody for receptor detection (*b*). Internal standards were the same as in Fig. 1. Numbers 1–5 are the same as lane designations in Fig. 2.

conditions, we analyzed the distribution of both the hGR and each hsp90 in sucrose/glycerol gradient fractions. Results are shown in Fig. 3. Fig. 3*a* shows that the insect hsp90, wild-type chsp90, and the A-deleted mutant (lanes 1–3) migrated as 6.5–7 S, as expected for the dimer. The B- and Z-deleted mutants formed heavier complexes in addition to the dimer (lanes 4 and 5).

When the receptor was expressed either alone or with wild-type or A-deleted chsp90 (Fig. 3*b*, lanes 1–3), a major peak was observed at 8 S, representing the hGR complexed with endogenous insect hsp90 or with wild-type chsp90. When the hGR was coexpressed with B- or Z-deleted mutants (lanes 4 and 5), it exhibited a bimodal distribution. One peak was centered at 8 S and the second was located at 11–12 S for the Z-deleted mutant and at 12–13 S for the B-deleted mutant. These last data are consistent with the formation of polymeric or aggregated forms of overexpressed B- and Z-deleted chsp90s, able to interact with the hGR.

Deficient Nuclear Accumulation of hGR Complexes Coexpressed with B- and Z-Deleted chsp90s. *In vivo* treatment with hormone triggers hsp90 dissociation and tight receptor binding to nuclear structures. The extraction of the activated receptor then requires high-salt treatment. To investigate the activation ability of the hGR coexpressed with mutated hsp90s, we systematically quantified the hGR content in total, fractionated extracts and pellets by steroid binding and/or Western blot techniques. The same cell batch was divided into two parts. One part was not exposed to the hormone and the cytosol was prepared in low ionic strength buffer and then labeled with [^{3}H]TA. The other was treated with 1 μM [^{3}H]TA 1 h before cell collection. Cells were then lysed by low ionic strength washing, before high-salt extraction of the receptor.

No significant differences were observed in terms of steroid binding between the different cytosols and high-salt nuclear extracts (≈31 and ≈190 pmol per mg of protein, respectively). However, the total hormone binding capacity of cytosols (57–73 pmol) was largely inferior to the nuclear hGR labeling obtained after [^{3}H]TA cell exposure (710–940 pmol).

Fig. 4 shows the proportions of high-salt nuclear hGR, estimated by quantification of the band intensities on Western blots, with respect to total cellular immunoreactive hGR.

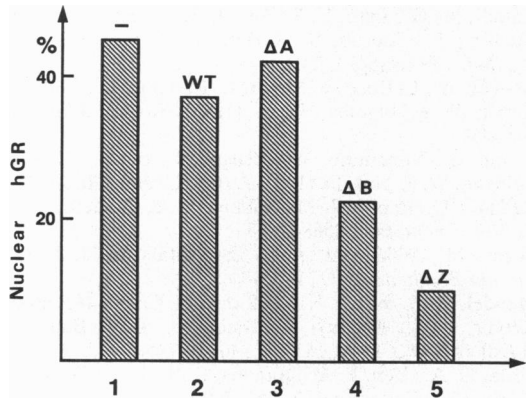

FIG. 4. *In vivo* hormonal activation of GR. Percentage of nuclear high-salt extractable hGR expressed alone or coexpressed with wild-type and mutated chsp90s. Sf9 cells were treated with 1 μM [^{3}H]TA before collection and fractionation (35). Immunoreactive hGR content was estimated by quantification of enhanced chemiluminescence Western blots with a Millipore bioimage electrophoresis analyzer. Data, expressed as percentages of nuclear hGR with respect to total cellular hGR, are mean values of two independent experiments. Numbers are the same as lane designations in Fig. 2. –, WT, ΔA, ΔB, and ΔZ, hGR expressed alone, with wild-type, or with A-, B-, and Z-deleted chsp90s, respectively.

When no chsp90 was synthesized in Sf9 cells or when wild-type chsp90 or A-deleted derivative was expressed, ≈40% of the hGR was found in the nuclear high-salt fraction. On the contrary, significant reduction in this level was observed when the Z- or B-deleted chsp90s were overexpressed (10% and 25%, respectively). These data suggest that the loss of steroid binding resulting from Z and B deletions maintains the receptor in a nontransformed form, insensitive to hormone.

DISCUSSION

The data presented here define a system to investigate hsp90 function. By overexpressing an exogenous hsp90, such as chsp90, in baculovirus-infected Sf9 cells together with the hGR, we obtained a great amount of hGR heterooligomeric complexes containing chsp90 that were able to bind steroid ligand.

This led us to test the effect of selective deletions in the chsp90 molecule on heteromeric complex formation and hGR properties. Interpretation of the results is restricted by the possible conformational changes resulting from sequence modifications, which may interfere with functions of other part(s) of the molecule.

Each of the three deletions studied, corresponding to separate regions of the chsp90 molecule noticeable for their charged amino acid content (A and B) or leucine heptad repeat (Z), impeded the formation of hormone-bound hGR heterooligomeric complexes. This indicates the importance of hsp90 structural integrity in the hGR steroid binding property, in agreement with previous observations (16–18) and the concept of a chaperonage role (3–5).

The fact that multiple regions of chsp90 may be involved in receptor interaction is consistent with our previous data, suggesting multiple contact points in the steroid receptor LBD (21–23), and for the estrogen receptor a possible participation of the nuclear localization region 1 (NL1) (22). Although the GR NL1 did not participate in hsp90–GR complex formation, the interaction of anti-NL1 antibody occurs only with the dissociated form of GR, but not when the receptor is interacting with hsp90 (47, 48). Altogether, these data confirm that hsp90 may interact in a complex manner to regulate receptor activity.

Role of the A Region in hsp90 Structure and Function. From the primary sequence of chsp90 and predictive secondary structure examination, we suggested previously that region A could be involved in steroid receptor interaction (28, 29). Its high content of negatively charged amino acid residues would allow ionic interactions with positively charged subregions of the LBD of the GR.

Different observations, using the *Escherichia coli* expression system, are consistent with that hypothesis. Expression of the LBD of the hGR in this microorganism did not generate a steroid binding GR molecule (49), a result that could be related to the absence of the major part of the A region (between amino acids 236 and 285 in chsp90) in the *E. coli* hsp90 analogue (HtpG) (28).

In the present work, our immunoadsorption and cross-linking data suggest that the A region is involved in interaction of chsp90 with the hGR, although we cannot completely rule out that our experimental procedures would cause dissociation of very unstable hGR–chsp90 complexes. The sedimentation coefficient observed for the A-deleted chsp90 (6.5–7 S) supports the hypothesis that the A region is not involved in interdimeric contact, consistent with the reported dimeric form of the *E. coli* HtpG (4).

Influence of B and Z Deletions on hsp90 Properties. The modifications observed in the polymerization state of overexpressed B- and Z-deleted chsp90s suggest that the B and Z regions may be involved in formation or stabilization of the

dimer structure. Our data converge to demonstrate the interaction of these chsp90 derivatives with the hGR without maintaining the proper folding of the hGR LBD for hormone binding. The lack of hormone binding is probably involved in the deficient nuclear accumulation of the hGR in the presence of hormone, as has been described when deletions were introduced into the hGR LBD or when antagonist ligand was used (RU 486) (13, 21, 34).

GR Hormone Binding and hsp90 Expression. We reproducibly obtained a dramatically higher steroid binding capacity of Sf9 cells after *in vivo* hormonal treatment than after *in vitro* hormone labeling of cytosols (in both cases, low levels of hGR remained in pellets). This suggests that a significant amount of cytosol receptors cannot bind steroid. However, the overexpression of a wild-type chsp90 molecule did not increase the steroid binding capacity of the cytosol or of the nuclear extract, indicating that increasing levels of hsp90 were not sufficient for formation of the appropriate conformation of the LBD of the hGR for hormone binding. These data are consistent with reports that deal with the involvement of other associated proteins and ATP (4, 14, 50) in heterooligomeric complex formation.

In conclusion, this study describes a tool for analyzing the functions of subregions of hsp90 with respect to its association with various proteins such as steroid receptors, the dioxin receptor (51), p59/HBI (50), and others.

We gratefully thank Dr. D. Toft (Mayo Clinic, Rochester, MN) for the anti-hsp90 antibodies (AC19 and others), Dr. J. Brugge for D7α antibody, and Dr. M. G. Catelli for helpful discussions. We acknowledge Dr. R. Fiddes, J. C. Lambert, L. Outin, and C. Rajkowski for preparation of the manuscript and figures.

1. Gething, M. J. & Sambrook, J. (1992) *Nature (London)* **355,** 33–45.
2. Borkovich, K. A., Farrelly, F. W., Finkelstein, D. B., Taulien, J. & Lindquist, S. (1989) *Mol. Cell. Biol.* **9,** 3919–3930.
3. Wiech, H., Buchner, J., Zimmermann, R. & Jakob, U. (1992) *Nature (London)* **358,** 169–170.
4. Nadeau, K., Das, A. & Walsh, C. T. (1993) *J. Biol. Chem.* **268,** 1479–1487.
5. Csermely, P., Kajtar, J., Hollosi, M., Jalsovszky, G., Holly, S., Kahn, C. R., Gergely, P., Söti, C., Mihaly, K. & Somogyi, J. (1993) *J. Biol. Chem.* **268,** 1901–1907.
6. Joab, I., Radanyi, C., Renoir, J. M., Buchou, T., Catelli, M. G., Binart, N., Mester, J. & Baulieu, E. E. (1984) *Nature (London)* **308,** 850–853.
7. Catelli, M. G., Binart, N., Jung-Testas, I., Renoir, J. M., Baulieu, E. E., Feramisco, J. R. & Welch, W. J. (1985) *EMBO J.* **4,** 3131–3135.
8. Brugge, S. J., Erikson, E. & Erikson, R. (1981) *Cell* **25,** 363–372.
9. Oppermann, H., Levinson, W. & Bishop, J. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1067–1071.
10. Miyata, Y. & Yahara, I. (1992) *J. Biol. Chem.* **267,** 7042–7047.
11. Matts, R. L. & Hurst, R. (1989) *J. Biol. Chem.* **264,** 15,542–15,547.
12. Lindquist, S. L. & Craig, E. A. (1988) *Annu. Rev. Genet.* **22,** 631–677.
13. Groyer, A., Schweizer-Groyer, G., Cadepond, F., Mariller, M. & Baulieu, E. E. (1987) *Nature (London)* **328,** 624–626.
14. Pratt, W. B., Hutchison, K. A. & Scherrer, L. (1992) *Trends Endocrinol. Metab.* **3,** 326–333.
15. Smith, D. F. & Toft, D. O. (1993) *Mol. Endocrinol.* **7,** 4–11.
16. Bresnick, E. H., Dalman, F. C., Sanchez, E. R. & Pratt, W. B. (1989) *J. Biol. Chem.* **264,** 4992–4997.
17. Nemoto, T., Ohara-Nemoto, Y., Denis, M. & Gustafsson, J. Å. (1990) *Biochemistry* **29,** 1880–1886.
18. Picard, D., Khursheed, B., Garabeddian, M. J., Fortin, M. J., Lindquist, S. & Yamamoto, K. (1990) *Nature (London)* **348,** 166–168.
19. Denis, M., Gustafsson, J. A. & Wikström, A. C. (1988) *J. Biol. Chem.* **263,** 18520–18523.
20. Pratt, W. B., Jolly, D. J., Pratt, D. V., Hollenberg, S. M., Giguere, V., Cadepond, F. M., Schweizer-Groyer, G., Catelli, M. G., Evans, R. & Baulieu, E. E. (1988) *J. Biol. Chem.* **263,** 267–273.
21. Cadepond, F., Schweizer-Groyer, G., Segard-Maurel, I., Jibard, N., Hollenberg, S. M., Giguere, V., Evans, R. M. & Baulieu, E. E. (1991) *J. Biol. Chem.* **266,** 5834–5841.
22. Chambraud, B., Berry, M., Redeuilh, G., Chambon, P. & Baulieu, E. E. (1990) *J. Biol. Chem.* **265,** 20,686–20,691.
23. Schowalter, D. B., Sullivan, W. O., Maihle, N. J., Dobson, A. D., Conneely, O. M., O'Malley, B. W. & Toft, D. O. (1991) *J. Biol. Chem.* **266,** 21,165–21,173.
24. Smith, D. F., Schowalter, D. B., Kost, S. L. & Toft, D. O. (1990) *Mol. Endocrinol.* **4,** 1704–1711.
25. Scherrer, L. C., Dalman, F. C., Massa, E., Meshinchi, S. & Pratt, W. B. (1990) *J. Biol. Chem.* **265,** 21,397–21,400.
26. Smith, D. F., Stensgard, B. A., Welch, W. J. & Toft, D. O. (1992) *J. Biol. Chem.* **267,** 1350–1356.
27. Hutchison, K. A., Stancato, L. F., Jove, R. & Pratt, W. B. (1992) *J. Biol. Chem.* **267,** 14,047–14,053.
28. Binart, N., Chambraud, B., Dumas, B., Rowlands, D. A., Bigogne, C., Levin, J. M., Garnier, J., Baulieu, E. E. & Catelli, M. G. (1989) *Biochem. Biophys. Res. Commun.* **159,** 140–146.
29. Binart, N., Chambraud, B., Levin, J. M., Garnier, J. & Baulieu, E. E. (1989) *J. Steroid Biochem.* **34,** 369–374.
30. Giguère, V., Hollenberg, S. M., Rosenfeld, M. G. & Evans, R. M. (1986) *Cell* **46,** 645–652.
31. Catelli, M. G., Binart, N., Feramisco, J. R. & Helfman, D. M. (1985) *Nucleic Acids Res.* **13,** 6035–6047.
32. Summers, M. D. & Smith, G. E. (1987) *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedure* (Texas Agricultural Experiment Station and Texas A&M Univ., College Station), Bull. 1555.
33. Vialard, J., Lalumiere, M., Vernet, T., Briedis, D., Alkhatib, G., Henning, D., Levin, D. & Richardson, C. (1990) *J. Virol.* **64,** 37–50.
34. Cadepond, F., Gasc, J. M., Delahaye, F., Jibard, N., Schweizer-Groyer, G., Segard-Maurel, I., Evans, R. & Baulieu, E. E. (1992) *Exp. Cell Res.* **201,** 99–108.
35. Elliston, J. F., Beekman, J. M., Tsai, S. Y., O'Malley, B. W. & Tsai, M. J. (1992) *J. Biol. Chem.* **267,** 5193–5198.
36. Erdos, T., Best-Belpomme, M. & Bessada, R. (1970) *Anal. Biochem.* **72,** 244–252.
37. Hutchison, K. A., Csar, M. J. & Pratt, W. B. (1992) *J. Biol. Chem.* **267,** 3190–3195.
38. Schneider, C., Newman, R. A., Sutherland, D. R., Asser, U. & Greaves, M. F. (1982) *J. Biol. Chem.* **257,** 10,766–10,769.
39. Perdew, G. H. & Whitelaw, M. L. (1991) *J. Biol. Chem.* **266,** 6708–6713.
40. Catelli, M. G., Joab, I., Renoir, J. M., Mester, J., Binart, N., Buchou, T., Zoorob, R. & Baulieu, E. E. (1983) *J. Steroid Biochem.* **19** (Suppl.), 34S.
41. Groyer, A., LeBouc, Y., Joab, I., Radanyi, C., Renoir, J. M., Robel, P. & Baulieu, E. E. (1985) *Eur. J. Biochem.* **149,** 445–451.
42. Schuh, S., Yonemoto, W., Brugge, J., Bauer, V., Riehl, R., Sullivan, W. & Toft, D. (1985) *J. Biol. Chem.* **260,** 14292–14296.
43. Radanyi, C., Renoir, J. M., Sabbah, M. & Baulieu, E. E. (1989) *J. Biol. Chem.* **264,** 2568–2573.
44. Denis, M., Wikström, A. C. & Gustafsson, J. A. (1988) *J. Steroid Biochem.* **30,** 271–276.
45. Mendel, D. B. & Orti, E. (1988) *J. Biol. Chem.* **263,** 6695–6702.
46. Renoir, J. M., Radanyi, C., Faber, L. E. & Baulieu, E. E. (1990) *J. Biol. Chem.* **265,** 10,740–10,745.
47. Urda, L. A., Yen, P. M., Simons, S. S., Jr., & Harmon, J. M. (1989) *Mol. Endocrinol.* **2,** 251–260.
48. Scherrer, L. C., Picard, D., Massa, E., Harmon, J. M., Simons, S. S., Jr., Yamamoto, K. R. & Pratt, W. B. (1993) *Biochemistry* **32,** 5381–5386.
49. Ohara-Nemoto, Y., Strömstedt, P. E., Dahlman-Wright, K., Nemoto, T., Gustafsson, J. A. & Carlstedt-duke, J. (1990) *J. Steroid Biochem. Mol. Biol.* **37,** 481–490.
50. Callebaut, I., Renoir, J. M., Lebeau, M. C., Massol, N., Burny, A., Baulieu, E. E. & Mornon, J. P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6270–6274.
51. Pongratz, I., Mason, G. G. & Poellinger, L. (1992) *J. Biol. Chem.* **267,** 13,728–13,734.