Evidence That the 90-kDa Heat Shock Protein Is Necessary for the Steroid Binding Conformation of the L Cell Glucocorticoid Receptor*

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Using L cell glucocorticoid receptors that have been immunopurified by adsorption to protein A Sepharose with a monoclonal antireceptor antibody, we have developed an assay to study the requirements for maintenance of steroid-binding capacity. After rapid purification by immunoabsorption, heteromeric receptor complexes retain the ability to bind glucocorticoid hormone. When the receptor complexes are warmed at 20 °C, steroid-binding capacity is lost, and the 90-kDa heat shock protein (hsp90) dissociates from the receptor. The rates of both temperature- and salt-dependent dissociation of hsp90 parallel the rates of loss of hormone-binding activity. Molybdate and hydrogen peroxide stabilize the hsp90-receptor complex against temperature-dependent dissociation. Molybdate, however, is much more effective in stabilizing steroid-binding capacity than peroxide. Receptors that have been inactivated in the absence of molybdate or peroxide cannot be reactivated. Inactivation of steroid-binding capacity occurs in the presence or absence of reducing agent, and inactivation is not accompanied by receptor cleavage or dephosphorylation. Under no conditions does an hsp90-free receptor bind steroid. Receptor bound to hsp90 can be cleaved to the 27-kDa meroreceptor in the presence of molybdate with retention of both hsp90 and steroid-binding activity. These observations lead us to propose that hsp90 is necessary but not sufficient for maintaining a competent high affinity glucocorticoid-binding site. Although the 27-kDa meroreceptor fragment is not itself sufficient for a competent binding site, it is sufficient when it is associated with hsp90.

The untransformed1 glucocorticoid receptor (GR)2 exists in cytosolic preparations as an 8–9 S (Mr 300,000) heteromeric complex consisting of a single molecule of the steroid-binding protein (1, 2) and probably two molecules (3) of a non-steroid-binding phosphoprotein (4–7), which has been identified as the 90-kDa heat shock protein, hsp90 (8–11). Several studies suggest that this heteromeric receptor-hsp90 complex is derived from the untransformed state of the receptor in intact cells (7, 12–14). When hsp90 dissociates from cytosolic glucocorticoid receptors, the receptors are transformed to the DNA-binding state (7, 8, 15, 16). Although it has been known for some time that conditions which transform steroid-bound receptors in cytosol to the DNA binding state (e.g. heating of cytosol, dilution, increasing ionic strength, or pH) also inactivate the steroid-binding capacity of unliganded receptors (17, 18), the mechanism of inactivation has not been resolved. Both receptor transformation and inactivation of binding capacity are inhibited by molybdate, which also stabilizes the receptor-hsp90 complex (15).

We have recently reported that hydrogen peroxide resembles molybdate in that it inhibits transformation (19) and stabilizes the steroid-binding state (20, 21) of rat liver glucocorticoid receptors. As derivatization of thiol groups prevents peroxide stabilization of the steroid-binding state and as treatment with dithiothreitol reverses the effect, it appears that peroxide acts by promoting the formation of disulfide linkages (21). As both peroxide and molybdate stabilize the association of hsp90 with unliganded receptors and as conditions that disrupt the heteromeric complex inactivate the binding capacity of cytosols, we hypothesized that the physical interaction of hsp90 with GR might be required to generate and/or stabilize a competent steroid binding conformation of the glucocorticoid receptor (21).

In this study, we have utilized immunopurified heteromeric receptor complexes to examine the relationship between the association of hsp90 with the GR and its steroid-binding capacity. Immunopurified receptors retain the ability to bind hormone if they are bound to hsp90, and in no case when hsp90 is separated from the GR is there any steroid-binding capacity. Our results are consistent with the proposal that binding of the glucocorticoid receptor to hsp90 is necessary but not in itself sufficient for a competent steroid-binding site.

EXPERIMENTAL PROCEDURES3

RESULTS

Characterization of a Steroid-binding Assay Using Immunoabsorbed Glucocorticoid Receptor Complexes—Immunoabsorption of L cell cytosol with the BuGR antibody typically

3 Portions of this paper (including “Experimental Procedures,” Tables I and II, and Fig. 3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
results in the recovery of 30–50% (e.g. 36% in the experiment of Table I, in the Miniprint) of the whole cytosol steroid-binding capacity in the protein A-Sepharose pellet. As shown in Table I, somewhat less than half of the binding capacity that is not present in the Sepharose pellet is recovered in the supernatant. Thus, when cytosol is incubated with both BuGR antibody and protein A-Sepharose, 30–40% of the cytosolic binding capacity is not accounted for in the pellet and supernatant together. Resuspension of pellets in either boiled cytosol or receptor-depleted whole cytosol does not improve the recovery of binding capacity in the pellet (data not shown). Immunoabsorbed GR complexes can be washed at least six times with buffer at 0 °C without additional loss of steroid-binding capacity, and the presence of molybdate in the buffer during washing at 0 °C is not required.

**Measurement of Steroid-binding Capacity, Receptor Protein, and Receptor-specific hsp90 in the Same Immunoabsorbed Sample—**Panel A of Fig. 1 shows the specific binding capacity of GR complexes immunoabsorbed from increasing volumes of L cell cytosol. The steroid-binding capacity increases in a linear fashion until 200 µl of cytosol are immunoabsorbed. The curve then turns over because the concentration of free radioligand is depleted at higher concentrations of receptor. In order to remain in the linear portion of the binding curve, all subsequent assays for steroid-binding capacity were performed with immune complexes from 50 to 100 µl of cytosol.

The relative amounts of immunoreactive GR protein (Panel B in Fig. 1) and hsp90 (Panel C) were determined by reacting Western blots of immunoabsorbed complexes with 125I-labeled anti-mouse IgG and directly counting the excised GR or hsp90 bands. In subsequent experiments, immune pellets prepared from 400 to 600 µl of cytosol were used for assay of hsp90 and 100 to 200 µl for assay of the relative amount of GR. Under these conditions, the nonimmune hsp90 value is not more than 15% of the hsp90 value obtained in the BuGR immunoabsorbate (mean 9.2 ± 2.1% S.E., n = 9).

As approximately 30–40% of the GR cannot be accounted for by measurements of binding capacity in the pellet and supernatant following immunoabsorption (Table I), we used the quantitative immunoblotting technique to determine whether this nonbinding receptor fraction is immunoabsorbed to protein A-Sepharose or remains in the supernatant. In the experiment of Table II (in the Miniprint), L cell cytosol was immunoabsorbed with the BuGR antibody or nonimmune mouse IgG, and the unwashed pellets and the supernatants were analyzed for both steroid-binding capacity and immunoreactive GR protein. The data show that the receptor that is inactivated to a nonbinding state during the immunoabsorption procedure is present in the protein A-Sepharose pellet.

**Correlation between Steroid-binding Capacity and Presence of hsp90 in the Immunoabsorbed Receptor Complex—**Fig. 2 presents the results of two experiments in which we compared the rate of hsp90 dissociation from immune complexes to the rate of inactivation of steroid-binding capacity. Panel A shows temperature-dependent inactivation of the complex, and Panel B shows inactivation by salt. In both cases, the rate of hsp90 dissociation is similar to the rate of loss of steroid-binding capacity.

If hsp90 is required for maintaining a competent steroid binding site, stabilization of the GR-hsp90 complex should correlate with stabilization of the steroid binding state. We have previously shown that molybdate and hydrogen peroxide stabilize the association of hsp90 with immunoabsorbed receptors (19, 20) and stabilize steroid-binding capacity in whole cytosol (17, 21). In addition, it was recently reported (27) that fluoride, which has been known for some time to weakly stabilize cytosolic steroid-binding capacity (17), also stabilizes the GR-hsp90 complex. As shown in Fig. 3 (in the

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**Fig. 1.** Assays of steroid-binding capacity, glucocorticoid receptor, and receptor-specific hsp90. Aliquots of L cell cytosol as indicated in the abscissa were immunoabsorbed to protein A-Sepharose with the BuGR antibody or nonimmune mouse IgG. Panel A, washed pellets were resuspended in 100 µl of Hapes buffer and steroid-binding capacity (■) was assayed with 50 nm [3H]triamcinolone acetonide in the presence or absence of 0.4 M NaCl. Aliquots (700 µl) of L cell cytosol were immunoabsorbed to protein A-Sepharose with the BuGR antibody or nonimmune mouse IgG. Washed pellets were resuspended in 300 µl of Hapes buffer and incubated for 0–180 min at 20 °C. The pellets were washed once with TEGMD buffer (defined under "Methods") and divided into several portions for assay of steroid-binding capacity and quantitative immunoblotting for receptor and receptor-specific hsp90. Values are expressed as percent of zero time values. Binding capacity, receptor, and hsp90 values were corrected for nonspecific radioactivity, which was 3, 1, and 2% of the specific zero time values, respectively. Panel B, inactivation at 0 °C with 0.4 M NaCl. Immunoabsorbed receptor complexes were prepared as described above, and washed pellets were resuspended in Hapes buffer containing 10 mM DTT in the presence or absence of 0.4 M NaCl. Pellets were incubated for 0–120 min at 0 °C and washed with 1 ml of TEGMD buffer. Binding capacity, receptor, and hsp90 values were corrected for nonspecific radioactivity which was 9, 2, and 6% of the specific zero time values, respectively. ▲, receptor; ■, hsp90; •, steroid-binding capacity.
Miniprint), molybdate and fluoride stabilize the binding capacity of immunoadsorbed receptors against thermal inactivation with the same concentration dependence as for stabilization of glucocorticoid-binding capacity in whole cytosol. (For concentration dependence of fluoride effect, see Table VIII in Ref. 17, and for molybdate, Fig. 6 in Ref. 28.) Fluoride apparently acts as AlF₄⁻, with the aluminum in this case being derived from the glassware, the water, and the cytosol (27). This may explain why we have observed rather variable degrees of stabilization with this agent.

In the experiments shown in Fig. 4, the abilities of molybdate, peroxide, and fluoride to stabilize the GR-hsp90 complex are compared with their abilities to stabilize steroid-binding capacity. Molybdate and fluoride stabilize the GR-hsp90 complex and steroid-binding capacity to the same degree, although the fluoride effect in these experiments was rather weak, producing a stabilization of only 2-fold compared to control samples containing buffer alone. Peroxide completely stabilizes the immunoadsorbed GR-hsp90 complex, but in contrast to molybdate, steroid-binding capacity remains low despite the addition of a stoichiometric excess of DTT.

The fact that peroxide stabilized the immunoadsorbed GR-hsp90 complex but did not stabilize steroid-binding capacity suggested the possibility that reactivation of peroxide-stabilized receptor complexes in whole cytosol may require some cytosolic component in addition to DTT. As shown in Table III, when immunoadsorbed receptors have been incubated at 27 °C with 8 mM peroxide, a small degree of reactivation is achieved with DTT alone, and molybdate potentiates this reactivation. At concentrations of peroxide greater than 10 mM, the degree of reactivation that can be obtained decreases markedly (data not shown). The reactivation seen with DTT and molybdate is not enhanced by the addition of GR-depleted whole cytosol, boiled cytosol, ATP, GTP, purified Escherichia coli or rat liver thioredoxin, or large amounts of purified hsp90.

Receptors that have been inactivated by warming in buffer alone cannot be activated to any extent by the addition of DTT and molybdate or by any of the other conditions. We have been unable to find conditions (including readdition of a 1000-fold stoichiometric excess of purified L cell hsp90) that permit any reassociation of hsp90 with the receptor. In addition, purified hsp90 does not have any ability to stabilize the receptor against thermal inactivation of steroid-binding capacity.

Receptor Inactivation Is Not Accompanied by Receptor Dephosphorylation—The inability to reverse either loss of hsp90 or loss of steroid-binding capacity (in the absence of receptor-stabilizing agents) implies that the receptor is not in free equilibrium with the heat shock protein. This raises the possibility that a covalent change occurs during receptor inactivation that prevents reassociation of hsp90. One covalent change that could occur during inactivation is receptor dephosphorylation. The L cell GR contains about four phosphorylated serines (26), and treatment of whole cytosol with purified alkaline phosphatase is known to inactivate its glucocorticoid-binding capacity (29).

To determine if thermal inactivation of immunoadsorbed receptor is accompanied by a change in its phosphorylation state, L cells were labeled with [³²P] orthophosphate, and the phosphorylation state of the GR was examined after incubation of immune complexes at 0 or 20 °C with or without molybdate. The relative amount of receptor phosphate was then determined by calculation of the ratio of ³²P to ¹³¹I as previously described (26). As shown in Fig. 5, the relative amount of receptor phosphate does not change when steroid-binding capacity is reduced by an average of 83%.

Evidence That the 27-kDa Meroreceptor Is Sufficient for a Competent Steroid-Binding Site when hsp90 Is Present—It has been known for several years that glucocorticoid receptors that are prebound with steroid can be cleaved to an approximately 27-kDa meroreceptor fragment without dissociation of steroid (30). This region of the GR also contains the site required for association with hsp90 (12, 13). In the experiment of Fig. 6, we ask if the unoccupied GR can be cleaved to the 27-kDa meroreceptor fragment with retention of steroid-binding capacity. This experiment takes advantage of the observation that glucocorticoid receptors that are cleaved in the presence of molybdate remain in large heteromolecular complexes (1, 31). As shown in lane 4 of Fig. 6, the 27-kDa fragment generated by trypsin cleavage of the molybdate-stabilized receptor is able to bind [¹⁺H]dexamethasone 21-mesylate. However, the meroreceptor fragment prepared from immunoadsorbed receptors that had been washed with high salt and detergent to remove 80–90% of the receptor-associated hsp90 did not bind steroid. The presence of hsp90 in the samples was confirmed by staining with Coomassie Blue. Similar amounts of hsp90 were present in lanes 2 and 4 of Fig. 6, and the low level of binding of intact receptor seen in lane 5 is consistent with the finding that the salt- and detergent-washed receptor still contained 10–20% of the hsp90 that was associated with the receptor in lanes 2 and 4. These results suggest that the 27-kDa meroreceptor is not itself sufficient to form a competent steroid-binding site but that it has an appropriate steroid binding conformation when it is associated with hsp90.

DISCUSSION

The correlation between inactivation of steroid-binding capacity and loss of receptor-associated hsp90 that is shown in Fig. 2 is consistent with the proposal that association of GR with hsp90 may be required for the receptor to maintain

![Graph](image-url)
Glucocorticoid-binding Capacity and hsp90

Reactivation of the steroid binding capacity of peroxide-stabilized immunoadsorbed GR

Washed immunoadsorbed pellets were resuspended in 100 μl of Hepes buffer in the presence or absence of 10 mM molybdate, 10 mM DTT, or 8 mM hydrogen peroxide as indicated. After 10-min preincubation at 0 °C, pellets were incubated for 1 h at 0 or 27 °C. Samples were placed on ice and treated with buffer, 30 mM DTT, or 30 mM DTT premixed with 10 mM molybdate. Steroid-binding capacity was assayed in duplicate samples, and specific binding capacity is expressed as a percent of the zero degree molybdate-stabilized value. The values represent the mean and standard error of determinations from three to five separate experiments.

<table>
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<th>Temperature</th>
<th>First incubation</th>
<th>Second incubation</th>
<th>Specific binding capacity</th>
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<tr>
<td>°C</td>
<td>DTT</td>
<td>MoO₄²⁻</td>
<td>H₂O₂</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>27</td>
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<tr>
<td>27</td>
<td>+</td>
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</table>

FIG. 5. Comparison of the phosphorylation state and steroid-binding capacity of immunoadsorbed L cell glucocorticoid receptors. Parallel aliquots (0.5 ml) of L cell cytosol prepared from unlabeled and ³²P-labeled L cells were immunoadsorbed to protein A-Sepharose with BuGR or nonimmune IgG. The Sepharose pellets were washed four times with TEG buffer, resuspended in 200 μl of Hepes buffer, and incubated for 2.5 h at 0 or 20 °C in the presence of buffer alone or 10 mM molybdate. Steroid-binding capacity was assayed with the unlabeled receptors, and an aliquot of each sample was used to quantitate the amount of receptor protein by ¹²⁵I- immunoblotting. Binding capacity values were normalized for the amount of receptor protein and are expressed as percent of the zero time control values (open bars, mean ± S.E., n = 3). After the incubation at 0 or 20 °C, the ³²P-labeled receptors were washed seven times with TEG buffer containing 0.4% Triton X-100 and 0.5 mM NaCl, followed by one time with TEG buffer. ³²P-labeled receptors were resolved by SDS-polyacrylamide gel electrophoresis, transferred to an Immobilon P membrane, and exposed to x-ray film. After obtaining an autoradiogram, the blot was used to assay the GR by quantitative immunoblotting with the BuGR antibody. Receptor bands were excised and counted for ¹²⁵I and ³²P as described in Dalman et al. (26). The amount of phosphate per receptor (³²P/¹²⁵I) is expressed as percent of the zero time value (stippled bars). With the intact receptor or the meroreceptor when there is no receptor-associated hsp90. To our knowledge, there has never been a report demonstrating that glucocorticoids can be bound to the dissociated 4 S form of the receptor or to any form of the receptor purified in the unliganded state in the absence of molybdate. We certainly have not been able to obtain steroid binding in either case. This stands in marked contrast to the estrogen receptor, which binds hormone when it is in its salt-dissociated form (e.g. 32).

The experiments with the stabilizing agents molybdate and fluoride (Fig. 4) also suggest a correlation between the presence of receptor-associated hsp90 and steroid-binding capacity. As peroxide can completely stabilize the complex between the GR and hsp90 (Fig. 4) without producing a similar degree of stabilization of binding capacity, it seems likely that the presence of hsp90 is not the only thing that is required for the receptor to have a competent steroid-binding site. It is known, for example, that oxidized glucocorticoid receptors do not bind hormone (21, 33), and we have previously shown that thioredoxin, an endogenous protein-reducing agent in cytosol, generates hormone-binding activity (33). However, in this work, under the best conditions we were able to regenerate only 30% of the binding capacity of peroxide-stabilized, immunopurified GR-hsp90 complexes after heating (Table III).

Despite the fact that several laboratories have tried to reassociate hsp90 with steroid receptors, there are as yet no reports of success. The avian viral transforming protein pp60vsrc is also found in cytosol in association with hsp90 (34), and that complex has not yet been reconstituted either. One can hypothesize that failure to reassociate is due to the fact that the receptor has undergone a covalent change. Both previous experiments with liganded glucocorticoid receptors (35, 36) and the experiments of Fig. 5 with unliganded receptor indicate that the receptor is not dephosphorylated during temperature-mediated hsp90 dissociation and that failure to reassociate cannot be explained by that particular covalent change. It is possible that dissociation of hsp90 is accompanied by a conformational change of both steroid receptors and pp60vsrc such that reassociation with the heat shock protein cannot occur. It has been speculated that a short highly conserved region (a potential "transducing domain") located within the steroid-binding domains of the steroid receptors may be directly involved in the binding of the receptor to hsp90 (12, 37). This potential transducing domain contains 55% hydrophobic amino acids, and it is possible that when hsp90 dissociates, this region of the receptor folds in from the

an appropriate steroid binding conformation. From the experiment of Fig. 6, it seems clear that portions of the receptor other than the carboxyl-terminal 27-kDa fragment are not required for the steroid binding conformation. The experiment of Fig. 6 also suggests that the 27-kDa fragment of the receptor is sufficient to form a binding site only if hsp90 is present. Under no conditions do we obtain steroid binding with the intact receptor or the meroreceptor when there is no receptor-associated hsp90. To our knowledge, there has never been a report demonstrating that glucocorticoids can be bound to the dissociated 4 S form of the receptor or to any form of the receptor purified in the unliganded state in the absence of molybdate. We certainly have not been able to obtain steroid binding in either case. This stands in marked contrast to the estrogen receptor, which binds hormone when it is in its salt-dissociated form (e.g. 32).

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Glucocorticoid-binding Capacity and hsp90

receptor surface making reassociation impossible.

Glucocorticoid receptors that are translated from mRNA in vitro are able to bind steroid. If our model regarding the necessity of hsp90 for steroid binding is correct, then hsp90 must associate with the receptor in the in vitro translation system. The reticulocyte lysate that is commonly used to promote such in vitro translation contains hsp90. It may be possible to test the hypothesis by determining if steroid-binding capacity is generated only by whole reticulocyte lysate and not by lysate that has been depleted of hsp90.

On the basis of functional studies of glucocorticoid receptor fusion proteins which demonstrate that regulation by the steroid binding domain is relatively independent of protein structure, Picard et al. (38) have proposed that hsp90 causes the receptor to assume an “unfolded” conformation that is readily reversed upon hormone binding and transformation. Such a proposal would fit well with the concept that hsp90 binds to a hydrophobic site like the proposed transducing domain during receptor synthesis. The observations presented here support the proposal (20) that binding of hsp90 to its binding site on the unliganded receptor is necessary to generate the high affinity conformation of the glucocorticoid-binding site. It is possible that binding of hsp90 to the hydrophobic transducing domain of the unliganded receptor somehow keeps the hydrophobic steroid-binding “pocket” open and available for steroid occupancy. Our working model of the initial event in steroid-mediated signal transduction is that the free energy involved in the binding of steroid to its high affinity binding site is transduced through a change in the conformation of the conserved transducing domain to yield a substantial increase in the dissociation rate of the hsp90-transducing domain complex (8, 12, 15). The conformation change that occurs in the receptor as a result of the hsp90 dissociation leaves the receptor unable to reassociate with the heat shock protein in cytosol. It is important to note that the methionine that becomes covalently bound to the A ring of triamcinolone acetonide on UV irradiation of the steroid-bound receptor (39) lies immediately to the carboxyl-terminal side of the most highly conserved portion of the transducing domain. Thus a collapse of this hydrophobic region from the receptor surface when hsp90 dissociates might also destroy the steroid binding conformation of the hormone binding site. Once the steroid is bound to the receptor in a high affinity manner, however, it clearly remains bound despite the dissociation of hsp90. Some of these concepts may be applicable to signal transduction by other members of the steroid/thyroid hormone receptor family.

Acknowledgments—We wish to thank Drs. Robert W. Harrison and David O. Toft for generously providing the BuGR2 anti-receptor and the AC88 anti-hsp90 monoclonal antibodies, respectively, and Dr. Arne Holmgren for providing purified rat liver thioredoxin.

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SUPPLEMENTAL MATERIAL TO EVIDENCE THAT THE 90-kDa HEAT SHOCK PROTEIN IS NECESSARY FOR THE STEROID BINDING CONFORMATION OF THE L. CELL GLUCOCORTICOID RECEPTOR

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EXPERIMENTAL PROCEDURES

MATERIALS

[46x455]EVIDENCE THAT THE 90-kDa HEAT SHOCK PROTEIN IS NECESSARY FOR THE STEROID BINDING CONFORMATION OF THE L. CELL GLUCOCORTICOID RECEPTOR

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EXPERIMENTAL PROCEDURES

Materials

1,7-Phenanthroline mononitrate (42.5 mg/mole) and [125I]-labelled conjugate of ovine/muscle immunoglobulin (10,000 d) were from New England Nuclear (Boston, Mass.). Nonradioactive phosphate-buffered saline was from Sigma Chemical Co. (St. Louis, Mo.). Implanted polyacrylamide gel electrophoresis slab gels were purchased from Bio-Rad Laboratories (Richmond, Calif.). Rabbit anti-human IgG (Fab') 2 antibody purified prepared against the rat glucocorticoid receptor (22) was kindly provided by Dr. Robert Harrison, and the AcG monoclonal antibody against the 90-kDa heat shock protein (23) was kindly provided by Dr. David Toft.

Methods

Cell source and preparation - 1,292 murine fibroblasts were grown in monolayer culture in DMEM's modified eagle's medium supplemented with 10% calf serum at 37°C. Cells were harvested by scraping into Earle's saline and centrifuged at 400 g for 5 min. After a week in regeneration and Earle's saline, cells were resuspended in 1.5 ml of DMEM supplemented with 10% calf serum at 37°C. Cells were harvested by scraping into Earle's saline and centrifuged at 400 g for 5 min. Following harvest, cells were resuspended in 1.5 ml of phosphate-free medium containing 10% dialyzed ovalbumin, Mr 44,000. For all experiments, cells in log phase of growth were washed with phosphate-free medium containing 10% dialyzed ovalbumin, Mr 44,000, and sediments were aliquoted in located in the immunoadsorbed pellet.

Recruitment of steroid-binding capacity during immunoadsorption of heteromeric CR complexes

All aliquots of L. cell cytosol (70 µl) were immunoadsorbed to protein-A-Sepharose with an equivalent amount of nonimmune mouse IgG. Steroid-binding capacity and immunoreactive CR protein were assayed in the unadsorbed pellets and the supernatants by ligand binding and quantitative immunoblot assay, respectively. Immunoreactive CR protein values are expressed as % of total.

Table I

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Figure 1: Concentration of L. cell cytosol (70 µl) and fluoride to stabilize the steroid-binding capacity of immunoadsorbed receptors against thermal denaturation. After 2 h at 60°C, pellets were reconstituted with a 70-µl aliquot of 10 mM HCl in the presence or absence of the indicated concentrations of sodium molybdate or sodium fluoride. After a 15-min incubation, steroid-binding capacity was assayed. Values represent duplicate determinations and are expressed as % of zero time specific binding capacity.

Table I

Recovery of steroid-binding capacity during immunoadsorption of heteromeric CR complexes

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