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Progesterone Enhances Target Gene Transcription by Receptor Free of Heat Shock Proteins hsp90, hsp56, and hsp70

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Steroid receptors regulate transcription of target genes in vivo and in vitro in a steroid hormone-dependent manner. Unoccupied progesterone receptor exists in the low-salt homogenates of target cells as a functionally inactive 8 to 10S complex with several nonreceptor components such as two molecules of 90-kDa heat shock protein (hsp90), a 70-kDa heat shock protein (hsp70), and a 56-kDa heat shock protein (hsp56). Ligand-induced dissociation of receptor-associated proteins such as hsp90 has been proposed as the mechanism of receptor activation. Nevertheless, it has not been established whether, beyond release of heat shock proteins, the steroidal ligand plays a role in modulating receptor activity. To examine whether the release of these nonreceptor proteins from receptor complex results in a constitutively active receptor, we isolated an unliganded receptor form essentially free of hsp90, hsp70, and hsp56. Using a recently developed steroid hormone-responsive cell-free transcription system, we demonstrate for the first time that the dissociation of heat shock proteins is not sufficient to generate a functionally active receptor. This purified receptor still requires hormone for high-affinity binding to a progesterone response element and for efficient transcriptional activation of a target gene. When an antiprogestin, Ru486, is bound to the receptor, it fails to promote efficient transcription. We propose that in the cell, in addition to the release of receptor-associated inhibitory proteins, a distinct hormone-mediated activation event must precede efficient gene activation.

Progesterone receptors (PRs) are ligand-induced transcriptional activators which interact with high affinity and specificity with progesterone response elements (PREs) in the regulatory regions of target genes (1, 3, 8, 16, 32, 34, 35). We have demonstrated recently that PR modulates target gene transcription by facilitating the formation of a stable preinitiation complex of general transcription factors at the core promoter (TATA), apparently through interaction with RNA polymerase II and other basic transcription factors (2, 17). Although receptor-mediated gene activation in the cell is absolutely dependent on steroid hormone, the precise role of ligand in this activation process has remained unsolved.

Ligand is widely believed to effect a transformation of the steroid receptor from an inactive 8 to 10S form (unable to bind DNA) to an active 4S form (which binds DNA) (9, 19, 23, 29). In the 8 to 10S oligomeric complex, PR is known to be associated with the 90-Da heat shock protein hsp90 (10, 15, 30), hsp70 (18), and a 56-kDa protein, p56, (28, 33) which has been identified recently as a heat shock protein (hsp56) (27). It has been proposed that hsp90 binds to the hormone-binding domain of unoccupied receptor and maintains it in an inactive state (5, 6, 13, 14, 22, 24). Subsequent binding of ligand triggers the release of hsp90 from the receptor complex and reverses the repression. Implicit in this proposal is the possibility that the principal role of the steroid in receptor activation is to dissociate hsp90 from the receptor; the receptor free of hsp90 would then display constitutive activity (22, 24). Similar mechanisms for activation of receptor by dissociation of other proteins such as hsp70 and hsp56 cannot be ruled out.

We have recently described a cell-free system in which the PR contained in a high-salt nuclear extract of human mammary carcinoma (T47D) cells stimulates transcription from a PRE-linked promoter in a progesterone-dependent manner (3). In an attempt to understand the molecular basis of hormonal activation and the roles of various receptor-associated proteins such as hsp90, hsp70, and hsp56 in regulating receptor activity, we undertook the fractionation of a low-salt cytosolic fraction of T47D cells through several column chromatography steps. In this cytosol, PR is present in an 8 to 10S complex (11) presumably in association with hsp90 and other heat shock proteins. Our aim was to partially purify and dissociate the receptor from known inhibitory proteins and then study the hormonal requirements for biological activity in vitro. In this report we present clear evidence that PR free of heat shock proteins still requires hormone for high-affinity binding to PRE and for efficient transcriptional activation of a target gene.

MATERIALS AND METHODS

Fractionation of human PR. Cytosol (3 to 5 mg/ml) was obtained from T47D cells (packed cell volume, 6 ml) cultured in hormone-free growth medium as described previously (3) and treated with 0.5 M NaCl for 1 h at 4°C. The salt-treated cytosol was dialyzed against buffer D containing 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.9), 100 mM NaCl, 20% glycerol, 2 mM dithiothreitol, and 200 μM EDTA for 4 to 5 h with one change of buffer. It was then applied to a 2-ml phosphocellulose (PC) column equilibrated with buffer D and then washed extensively with the same buffer. The bound PR was eluted with a salt gradient of 100 to 500 mM NaCl. The peak of eluted PR was determined to be between 200 and 350 mM salt by binding to [^{3}H]R5020. PC-fractionated PR was adjusted to 100 mM NaCl by dilution with buffer D (minus salt) and applied to a 1-ml double-stranded calf thymus DNA-cellulose column. The column was washed extensively with buffer D, and PR was eluted with 300 mM salt. DNA-cellulose-purified PR was adjusted to 50 mM NaCl and applied to a 2-ml ATP-agarose column. The bound receptor was eluted with buffer

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D containing 0.3 M NaCl. The eluted protein was adjusted to 100 mM salt and reapplied on a 0.5-ml ATP-agarose column. PR eluted from the second ATP-column was immediately assayed for DNA-binding and transcriptional activities. The concentrations of PR in protein fractions were determined by binding to [^{3}H]R5020 followed by charcoal adsorption to remove free hormone as described previously (3). The receptor preparations were typically 10 to 20% pure at the final stage of fractionation.

Western immunoblot. Proteins in cytosol and partially purified PR fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on an 8% acrylamide gel. Separated proteins were electrotransferred to an Immobilon-P (Millipore) membrane for 4 h in a buffer containing 20 mM Tris-HCl, (pH 7.4), 150 mM glycine, and 20% methanol. Strips of membrane containing transferred proteins were incubated with one or a mixture of monoclonal antibodies AB52 (anti-PR), AC88 (anti-hsp90), N27F3-4 (anti-hsp70), or KN382/EC1 (anti-p56) for 4 h at room temperature. Each membrane slice was then briefly washed and treated with rabbit anti-mouse immunoglobulin G and finally reacted with ^{125}I-protein A. Washed filters were then air dried, and the signals were visualized by exposure to Kodak XAR-5 film at −70°C for several hours.

Gel retardation assay. The DNA-binding activity of PR was assayed by gel retardation as described previously by Bagchi et al. (3). PR preparations were preincubated in the presence or absence of 10^{-7} M progesterone at room temperature for 10 min and then added to a binding reaction containing ^{32}P-labeled double-stranded TAT (tyrosine aminotransferase) gene PRE oligonucleotide (0.1 to 0.2 ng, 1×10^8 to 5×10^8 cpm/μg) and 500 ng to 1 μg of *Hin*fI-cut pBR322.

Cell-free transcription assay. The transcription assay was carried out as described previously (2, 17). Receptor fractions were treated with 10^{-7} M progesterone or left untreated, and incubated with template DNA, competitor DNA, and HeLa nuclear extract; finally, the nucleotides and other components were added to initiate RNA synthesis, which was carried out at 30°C for 45 min. A typical transcription reaction mixture contained 10 to 20 nM PR. The construction of test template PRE_2TATA and control template pAdML200 has been described previously (17). Correct initiation of transcription from the test and control promoters generates 360- and 190-nucleotide transcripts respectively. Random initiation of transcription upstream from the G-free cassette results in slightly longer (377- and 200-nucleotide) transcripts after processing by RNase T_1 present in the assay mixture (2).

Immunoprecipitation reaction. Aliquots of ATP-agarose column-purified PR preparation and HeLa nuclear extract were combined in a 100-μl reaction mixture containing all components of a typical cell-free transcription reaction (17) except nonspecific and template DNAs. Incubations were carried out for 45 min at 30°C. Control reactions contained either the PR fraction or HeLa nuclear extract alone. Excess of anti-PR antibody AB52 (50 μg/ml) was added to these reactions at the end of the incubation, and the tubes were incubated further for 2 h at 4°C. Rabbit anti-mouse antibody (200 μg/ml) was then added to these tubes, and the incubation was continued for 2 h at 4°C. A 100-μl suspension of protein A-Sepharose (1:1, vol/vol) was then added, and the tubes were incubated with gentle agitation for 1 h at 4°C. The Sepharose beads were collected by centrifugation and gently washed three times with 1 ml of a buffer containing 20 mM Tris-HCl (pH 7.4) and 100 mM NaCl. The proteins were then extracted from the beads with SDS-PAGE loading buffer and analyzed by Western immunoblotting as described above.

RESULTS

PR devoid of hsp90 and hsp56 displays hormone-dependent DNA-binding and transcriptional activities. An earlier study indicated that a 4S form of PR activated transcription from a PRE-linked promoter in a hormone-dependent manner (3). Since crude receptor preparations were used which contained significant amounts of all heat shock proteins, it was not possible to exclude a possible role of these proteins in the hormone-dependent activities displayed by PR. To rule out this possibility, we purified the crude receptor preparations to remove contaminating heat shock proteins.

Cytosol from T47D cells was treated with high salt, adjusted to 100 mM NaCl, and subjected to PC chromatography as described in Materials and Methods. The PC-purified receptor fraction was then analyzed for the presence of various heat shock proteins by Western immunoblotting with monoclonal antibodies against hsp90, hsp70, and hsp56. The results shown in Fig. 1A, lanes 3 and 4, indicate that although the cytosol fraction displayed a major 90-kDa band upon probing with the anti-hsp90 monoclonal antibody, AC88 (26), which is known to recognize human hsp90, no such signal was detectable in the PC fraction containing receptor, even when 10 times more receptor protein was used for immunoblotting (Fig. 1A, lanes 1 and 2). The hsp90 in the cytosol fraction does not bind to a PC column and was recovered in the flowthrough fraction (data not shown). Figure 1A, lanes 5 and 6, also indicates that no detectable hsp56 was seen in the PC-purified fraction upon analysis with anti-p56 antibody, KN382/EC1 (33), whereas a major 56-kDa band was observed in the cytosol fraction. A recent report by Renoir et al. (25) suggests that in the untransformed receptor complex, hsp56 is bound directly to hsp90. Removal of hsp90 from the receptor complex would then be expected to cause an accompanying loss of hsp56, which is consistent with our present results.

The PC-purified PR was then tested for DNA binding and transcriptional activities. As shown in Fig. 1B (left panel), in the absence of hormone, PR failed to generate any specific DNA-protein complex with PRE. Treatment with hormone resulted in a marked induction of specific high-affinity binding of both A and B forms of human PR to PRE. In another experiment whose results are shown in Fig. 1B (right panel), PC-purified receptor was added to a transcription reaction containing a PRE-linked promoter and HeLa nuclear extract, which served as a source of general transcription factors. Addition of hormone-free PR to this reaction did not result in the synthesis of any correctly initiated RNA. Addition of receptor which was preincubated with hormone, however, resulted in a significant induction (about five- to eightfold) in the synthesis of accurately initiated gene transcript (solid triangle). As expected, no change in correctly initiated transcription of an adenovirus promoter was noted (open triangle).

Our results establish that unoccupied PR, free of hsp90 and hsp56, neither binds to PRE with high affinity nor transactivates a PRE-linked promoter. Treatment with hormone creates an active receptor which can now perform both functions. The occurrence of a reassociation during the in vitro transcription reaction of ligand-free PR with hsp90 contaminants that may be present in the HeLa nuclear extract is highly unlikely because (i) our previous studies with sucrose gradient sedimentation analysis indicated that

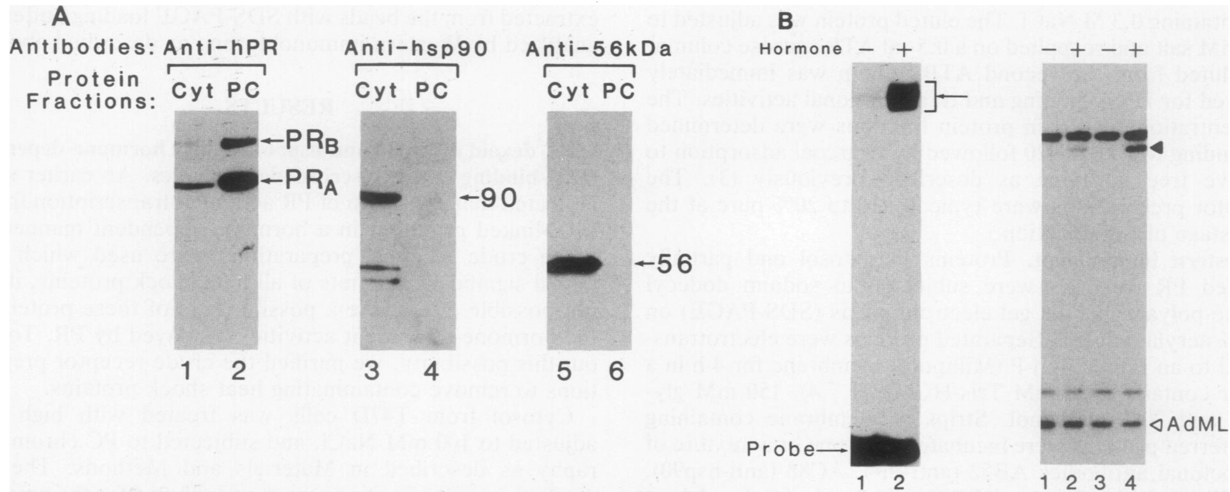

FIG. 1. Progesterone receptor free of hsp90 and hsp56 displays hormone-dependent functional activities. (A) Immunoblot assays. Aliquots of cytosol and PC fractions containing 0.2 and 2.0 pmol of PR, respectively, were analyzed by Western blotting as described in Materials and Methods. Antibodies: AB52 (lanes 1 and 2), AC88 (lanes 3 and 4), and KN382/EC1 (lanes 5 and 6). (B) The left panel shows DNA-binding activity of PC-purified PR (0.1 pmol), determined by a gel retardation assay as described in Materials and Methods. The right panel shows results of the cell-free transcription assay, performed as described in Materials and Methods. PC fractions containing 0.1 pmol (lanes 1 and 2) and 0.3 pmol (lanes 3 and 4) of PR were used. Solid and open triangles indicate correctly initiated transcripts from the test and control templates, respectively.

in the transcription extracts of T47D cells PR remains in a 4S form, presumably dissociated from hsp90 (3), and (ii) the ligand-dependent DNA-binding activity displayed by the purified receptor (Fig. 1B, left panel) in the complete absence of hsp90 in the reaction mixture strongly suggests that a hormonal activation of the receptor can occur irrespective of its association with hsp90.

The receptor was next fractionated by DNA-cellulose chromatography as described in Materials and Methods. The receptor preparation thus obtained consisted predominantly of PR_A with only a small amount of PR_B. This partially purified PR bound to PRE and enhanced transcription from PRE-linked promoters in a ligand-dependent manner (data not shown).

Ligand binding enhances DNA-binding and transcriptional activities of hsp70-free PR. A significant amount of a 70-kDa protein identified by immunoreaction as the hsp70 protein was detected in the DNA-cellulose-purified receptor fraction (Fig. 2A, lanes 3 and 4). The relative content of hsp70 in the receptor fraction diminished during fractionation of the cytosol through PC and DNA columns (Fig. 2A). This may be due to the partial removal of hsp70 associated with other cellular proteins during purification of PR. Estes et al. (12) have reported the copurification of human PR and hsp70

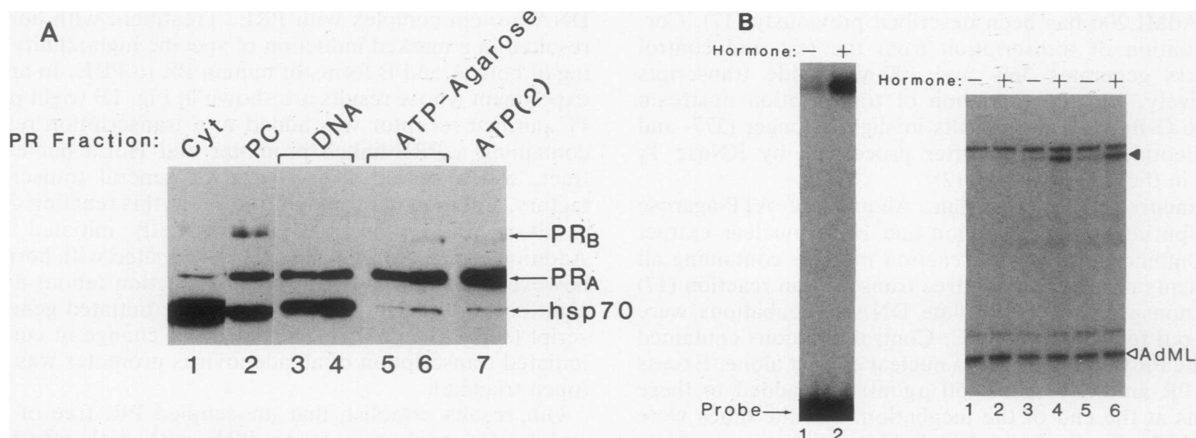

FIG. 2. Purified PR free of hsp70 requires progesterone for functional activities. (A) Immunoblot analyses of various PR fractions were carried out as described in the legend to Fig. 1. The amounts of PR applied to different lanes were as follows: cytosol, 0.1 pmol; PC fraction, 1 pmol; DNA-cellulose fraction, 0.5 and 1 pmol; ATP-agarose fraction, 1 and 1.5 pmol; ATP-agarose (second cycle) fraction, 2.5 pmol. The blots were probed with monoclonal antibodies AB52 and N27F3-4 (anti-hsp70). The amounts of hsp70 present in the PR fractions after ATP-agarose chromatography were determined by comparative immunoblots performed with a homogeneous hsp70 preparation of known concentration. The homogeneous hsp70 was obtained by the method of Welch and Feramisco (36). (B) The left panel shows results of a DNA-binding assay (0.05 pmol of PR and 150 ng of pBR322 were used). The right panel shows results of an in vitro transcription assay. Lanes: 1 and 2, no PR; 3 and 4, 0.35 pmol of PR; 5 and 6, 0.4 pmol of PR.

through several ion-exchange chromatographic steps. Kost et al. (18) have demonstrated by immunoprecipitation studies that PR remains associated with hsp70 even after high-salt treatment. We have observed also that significant amounts of hsp70 remain associated during immunoprecipitation of PR from cytosol or partially purified PC or DNA column fractions. These observations suggest that a substantial portion of the total pool of PR in the cell is tightly associated with hsp70.

To examine whether the association of hsp70 with PR contributes to the hormone responsiveness of the receptor, we sought to purify the receptor free of hsp70 and then test its biological activity. We took advantage of the observation by Kost et al. (18) that ATP binding triggers the release of hsp70 from the receptor complex. We passed our DNA-cellulose-purified receptor preparation through an ATP-agarose column at low salt. The bound PR was eluted at high salt. The results of an immunoblot experiment with this fraction (Fig. 2A, lanes 5 and 6) show that the vast majority of hsp70 was removed from the receptor fraction by this procedure. We believe that interaction of hsp70-PR complex with ATP in the column resulted in disruption of their association. The receptor was then released from the column by 0.3 M salt while the hsp70 remained bound to the column. To reduce the amount of hsp70 that was still present in the receptor preparation after the first ATP chromatography, we performed a second cycle. The PR fraction which eluted from the second ATP column showed only trace amounts of hsp70 by immunoreaction (Fig. 2A, lane 7). When we quantitated the actual molar concentrations of PR and hsp70 in the receptor fraction after the final ATP chromatography, as described in Materials and Methods, we found that more than 95% of the PR in this preparation was essentially free of hsp70.

We then tested this PR preparation, which was devoid of hsp90, hsp70, and hsp56, in DNA-binding and cell-free transcription assays. As shown in Fig. 2B (left panel), the PRE-binding activity of this PR was enhanced in the presence of progesterone. Similarly, the results of the transcription experiment described in Fig. 2B (right panel) indicate that very little transcriptional initiation occurred in the absence of hormone upon addition of the ATP-agarose-purified PR to the transcription reaction. In contrast, significant enhancement (about fivefold above the background level) in accurate RNA synthesis was observed when progesterone-occupied receptor was added.

We conclude from these results that dissociation of hsp90, hsp70, and hsp56 from the receptor does not render it constitutively functional in gene activation. A ligand-dependent activation event after the removal of these receptor-associated proteins is clearly necessary for both efficient binding of the receptor to the hormone response element and productive interaction with the transcriptional apparatus at the target promoter. The 5% of the receptor population which may remain complexed with hsp70 clearly cannot account for the hormone-dependent activity displayed by the purified PR if one considers the facts that (i) in the crude cytosol the vast majority of receptor molecules are complexed with hsp70 and (ii) the activity of the purified receptor compares well with that of the unfractionated PR in the cytosol (data not shown). Therefore, the trace contamination of hsp70 does not interfere with our interpretation that the activity of the purified receptor is that of the hsp70-free species.

We next investigated the possibility that during the in vitro transcription reaction, purified, ligand-free PR could reassociate with hsp70 present in the HeLa nuclear extract. Numerous attempts by Toft and coworkers to reassociate receptors with heat shock proteins in either crude extracts or purified preparations have proved to be futile (18). This group, however, has recently reported that a successful reconstitution of chicken oviduct PR with hsp70 and hsp90 can be performed in rabbit reticulocyte lysates but not in oviduct extracts (31). Since 4S PR complexes are known to be associated with hsp70 (18), migration in sucrose density gradients could not be used to assess the ressociation of hsp70 with PR. This prompted us to design an immunoprecipitation protocol to test whether a reassociation between PR and hsp70 takes place in our in vitro transcription extracts. We incubated purified hormone-free PR either in the presence or in the absence of HeLa nuclear extracts under transcription reaction conditions. A monospecific anti-PR antibody was then added to these reactions, and the antigen-antibody complexes thus formed were isolated, washed rapidly under mild conditions, and subjected to Western analysis. The results of this experiment (Fig. 3) clearly indicate that although the HeLa nuclear extract contains a large amount of hsp70 (lane 1), purified PR does not reassociate with hsp70 in these transcriptional extracts (lane 4).

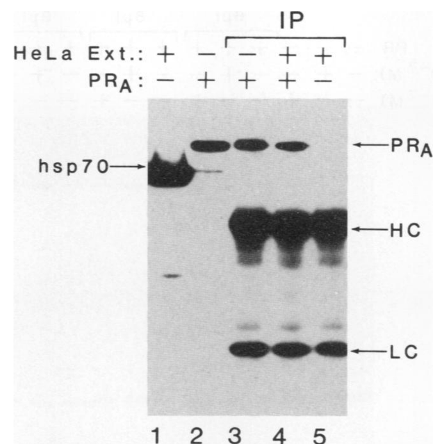

FIG. 3. Purified hormone-free PR does not reassociate with hsp70 in transcriptional extracts. Western blots and immunoprecipitation reactions were performed as described in Materials and Methods. In lanes 1 and 2, 20 μl (200 μg) of HeLa nuclear extract and 20 μl (1 pmol) of ATP-agarose-purified PR, respectively, were immunoblotted with a mixture of AB52 and N27 antibodies. Lanes labeled IP represent results of immunoprecipitation reactions. Lane 3 contains PR (1 pmol); lane 4 contains PR (1 pmol) plus HeLa extract (200 μg); lane 5 contains HeLa extract (200 μg). HC and LC represent antibody heavy chain and light chain, respectively.

We also note with interest that the trace amounts of hsp70 present in the ATP-agarose purified PR (Fig. 3, lane 2) did not coprecipitate with the receptor during immunoprecipitation (lane 3), although the precipitation of PR was almost quantitative. This result strongly suggests that the small amount of contaminating hsp70 in the final purified PR preparation is not directly associated with the receptor but most probably is carried along with other protein(s) present in this fraction.

PR complexed with antiprogestin Ru486 fails to promote efficient transcription. It has been suggested previously that the antiprogestin Ru486 acts by preventing dissociation of heat shock proteins from native aporeceptor (6). Conse-

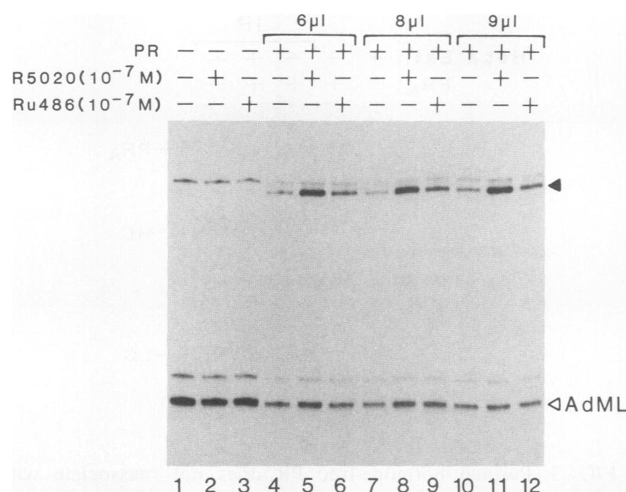

FIG. 4. Effect of antiprogestin Ru486 on the activity of PR free of heat shock proteins. ATP-agarose-purified PR (0.05 pmol/μl) was preincubated with or without 10^{-7} M R5020 or Ru486 and then assayed for cell-free transcriptional activity as described in the legend to Fig. 1. Lanes: 1, 2, and 3 no PR; 4, 5, and 6, 0.3 pmol of PR; 7, 8, 9, 0.35 pmol of PR; 10, 11, and 12, 0.4 pmol of PR.

quently, we tested the effect of Ru486 on the transcriptional activity of PR which was devoid of heat shock proteins and compared it with that of R5020-PR. Our results indicated (Fig. 4) that Ru486-PR promoted transcription much less efficiently (ca. 25 to 30%) than did R5020-PR. We reported previously that RU486-complexed PR can bind to PRE with high affinity (1, 3). Taken together, these results strongly imply that the inhibitory activity of Ru486 is exerted at least in part at a step beyond the release of heat shock proteins and DNA binding of the receptor and most probably affects the transactivation properties of the receptor. Although we cannot rule out the possibility that Ru486 also inhibits an earlier step such as receptor transformation, as proposed by Baulieu and coworkers (6, 13), our results strongly suggest that impairment of the transcriptional activity of the receptor through an alteration of its structure would be a plausible and attractive explanation of the antihormone properties of this compound.

DISCUSSION

In this paper we have explored the question of how a steroid hormone modulates the gene-regulatory activity of its cognate receptor. The multistep model described in Fig. 5 is consistent with our present understanding of the mechanism of steroid receptor activation. Unoccupied receptor is widely believed to exist in the cell in a functionally inactive oligomeric complex with a variety of nonreceptor proteins, of which hsp90, hsp70, and hsp56 are the best characterized. Strong circumstantial evidence has given rise to the proposal that hsp90 somehow blocks the biological activity of the receptor. Hormone binding to receptor results in the release of the associated proteins and leads to activation of receptor. The activated receptor, presumably still ligand bound, undergoes dimerization, binds to target PREs, and brings about gene activation by recruiting core transcription machinery to the target promoter.

The cell-free transcription system that we have used in the present study has allowed us to isolate discrete intermediates in receptor activation. We have shown that high-salt treatment followed by fractionation yields an inactive receptor form free of hsp90 and hsp56. Dissociation of hsp70 from the receptor occurs in the presence of ATP, but the vast majority of the receptors still remain functionally inactive. There appears to be little doubt that heat shock proteins must be dissociated from the receptor prior to its functioning as a transactivator of transcription. Nevertheless, we have established that an additional ligand-dependent activation step beyond the release of the heat shock proteins is essential to achieve a biologically active receptor form. It is conceivable that in an in vivo setting, ligand binding, release of inhibitory proteins from the receptor complex, and the hormone-dependent structural activation event may occur concurrently.

The physiological relevance of the association of heat shock proteins with certain of the steroid receptors remains speculative. Recent evidence suggests that hsp70 associates with a variety of cellular proteins and holds them in an "unfolded" state, which presumably facilitates their translocation across membranes (7, 20). Very little is known about the mechanism that promotes the release of hsp70 and the proper folding of the protein after it emerges through the nuclear membrane.

In a recent study, Picard et al. (21) investigated the role of hsp90 in the ligand-dependent activation of glucocorticoid receptor expressed in a mutant yeast strain in which the levels of endogenous hsp82 (the yeast equivalent of hsp90) could be altered by manipulations of growth conditions. They observed that under conditions in which the concentration of hsp82 within the cell is drastically reduced (less than 5% of wild-type levels), hsp82-free receptor does not exhibit ligand-independent activity, which is consistent with our results presented in this paper. These authors have proposed, however, that an interaction of hsp90 with nascent glucocorticoid receptor determines the latter's ability to maintain a "hormone activatable" conformation. Although it is possible that the association of heat shock proteins with the cellular glucocorticoid receptor may serve some physiological role such as facilitating correct folding of newly synthesized receptor proteins, stabilizing receptor

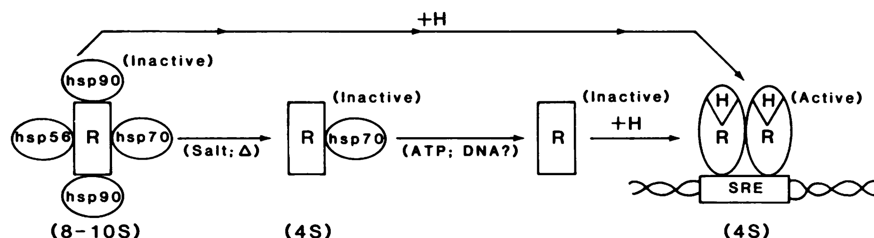

FIG. 5. Hypothetical kinetic intermediates in steroid hormone activation of receptor.

structure, or helping membrane transport as discussed above, our present data do not reveal any significant alteration in ligand binding or ligand-dependent transactivation properties of progesterone receptors whose prior association with heat shock proteins has been disrupted.

Hormone-independent DNA-binding and transcriptional activities of steroid receptors in vitro have been described previously (4, 17, 37). Highly purified chicken progesterone receptor in a ligand-free form stimulates transcription from a PRE-linked promoter in HeLa nuclear extracts (17). Although the basis of this constitutive activity remains unclear, one can speculate that these receptors have undergone structural alteration and activation by various in vitro manipulations during isolation from tissues. The fact that a part (ca. 10%) of our final purified receptor pool acquires hormone-independent transcriptional activity (Fig. 4, lanes 4, 7, and 10) following ATP-agarose chromatography is consistent with this hypothesis. In vivo and at earlier stages of purification, the receptor would not have undergone such a conformational change and hormone ligand would be absolutely essential for receptor activation. It is possible that removal of hsp70 from the receptor complex during purification renders the receptor susceptible to denaturation and creates a small population of structurally altered receptors which display the observed low level of constitutive activity. The vast majority of heat shock protein-free receptors in our purified preparation, however, exist in a conformation which responds to hormonal activation.

The precise molecular nature of the hormone-induced structural activation step is unclear. It is still possible that hormone causes a dissociation of some as yet undefined repressor(s) from the receptor. Perhaps more likely, the hormone causes a direct allosteric modulation of receptor structure and/or a subsequent covalent modification of the receptor, such as phosphorylation. This sequence of events would provide the molecule with a conformation which is optimal for association with the transcriptional apparatus.

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