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Subunit Dissociation as a Possible Mechanism of Glucocorticoid Receptor Activation†

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ABSTRACT: For the elucidation of the mechanism of steroid hormone receptor activation, the hydrodynamic properties of the unactivated and activated forms of the nonproteolyzed glucocorticoid receptor from the mouse AtT-20 pituitary tumor cell line were determined. The unactivated, molybdate-stabilized receptor has the following properties: sedimentation coefficient = 9 S; R_s = 8.3 nm; M_r = 317 000; f/f_0 = 1.70; axial ratio (prolate ellipsoid) = 14. The activated monomeric receptor has a sedimentation coefficient of 3.2 S, a Stokes radius of 6 nm, a molecular weight of 81 000, a frictional ratio of 1.93, and an axial ratio (prolate ellipsoid) of 18. A receptor species of intermediate size was detected when the analysis was performed in buffer containing both 0.3 M KCl and 20 mM Na_2MoO_4. Its characteristics are as follows: sedimentation coefficient = 5 S; R_s = 8.3 nm; M_r = 176 000; f/f_0 = 2.06; axial ratio (prolate ellipsoid) = 22. A preliminary study seemed to indicate that this is an activated form of the receptor. On the basis of the molecular weights, it is likely that the unactivated receptor is a tetramer of identical hormone-binding subunits (M_r = 81 000) while the intermediate form is a homodimer. Alternatively, non-hormone-binding components (receptor-binding factors) may be involved in forming the multimeric, nonactivated receptor complex. In either case, the dissociation of a multimeric, nonactivated receptor into subunits appears to be a possible mechanism of receptor activation. Finally, the addition of high concentrations of 1-thioglycerol promoted activation. Thus, sulfhydryl groups may be involved in receptor subunit interaction.

Ever since their discovery, steroid hormone receptor proteins have been the subject of intense investigation. Early studies [reviewed in Milgrom (1981)] demonstrated that the receptor, which is usually localized in the cell cytoplasm, undergoes a process called activation (or transformation) which allows its accumulation in the nucleus. Recent studies have shown that activation does occur in the cell and is not just an in vitro artifact (Munck & Foley, 1979; Markovič & Litwack, 1980). Concomitant with receptor activation, there seems to be some alteration in receptor structure. Results obtained for the estrogen receptor (Notides & Nielsen, 1974; Notides et al., 1981) implicated subunit association (as evidenced by a 4S → 5S transformation) as the structural mechanism for receptor activation. A similar alteration has not been demonstrated

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in other steroid hormone receptor systems (Bailly et al., 1980). Thus, other alterations in receptor structure including subunit dissociation, limited receptor proteolysis (Puca et al., 1972; Sica et al., 1976), and conformational change (Samuels & Tomkins, 1970; Rousseau et al., 1972; Bailly et al., 1980) have been invoked for the molecular mechanism of receptor activation. Any of these mechanism could also involve covalent modification of the receptor, for example, dephosphorylation (Sando et al., 1979; Barnett et al., 1980).

Recent characterization of the glucocorticoid receptor (GC-R)[1] from the mouse AtT-20 pituitary cell line has shown that it is apparently composed of only one hormone-binding moiety (Vedeckis, 1981). Furthermore, the cytosol prepared from this cell line contains little endogenous receptor-cleaving protease activity [see Vedeckis (1983)], which, if present, could complicate the interpretation of results on receptor activation. Finally, the use of sodium molybdate to stabilize the unactivated receptor now allows more definitive studies of the activation process. The study presented below supports subunit dissociation as a possible molecular mechanism of glucocorticoid receptor activation. Additionally, sulfhydryl reduction may play a role in this process.

Materials and Methods

Cell Culture and Preparation of Cytosol. The mouse AtT-20 pituitary tumor cell line was maintained in T flasks and spinner suspension culture as described previously (Vedeckis, 1981). Cytosol was also prepared as described previously (Vedeckis, 1981) by using a stainless-steel Dounce homogenizer, with the following modifications. After the cells were washed with Tris–saline (10 mM Tris-HCl, pH 7.4 at 25 °C, and 0.148 M NaCl), 3 volumes of TETg buffer (20 mM Tris-HCl, pH 7.4 at 25 °C, 1 mM EDTA, and 12 mM 1-thioglycerol) were added to the cellular pellet. However, 20 μL/mL freshly 1:10 diluted 1-thioglycerol was also added to the TETg buffer prior to homogenization. It has been found that long-term storage of TETg buffer results in a marked decrease in the ability of 1-thioglycerol to protect the hormone-binding activity of the receptor (F. Svec, personal communication). Additional modifications included the incorporation of a 30–45-min incubation in TETg buffer to promote cell swelling and improve receptor extraction and a decrease in the number of strokes with the Dounce homogenizer to five. Liver cytosol was prepared as described in the previous paper (Vedeckis, 1983).

Chemicals. Tris and sucrose were "Ultra Pure" grade from Schwarz/Mann. Sodium molybdate and 1-thioglycerol were from Sigma, as were all of the protein standards used except for ferritin, which was from Boehringer/Mannheim. [1,2,4-$^3H_3$]Triamcinolone acetonide was from New England Nuclear (31.3 and 37 Ci/mmol). All other chemicals were reagent grade from J. T. Baker.

Gel Filtration. Gel filtration was performed on a 2.6 cm × 63 cm column of agarose A-1.5 m, 200–400 mesh (Bio-Rad), at a flow rate of 30 mL/h. Samples were treated with 0.3 M KCl for 1–3 h before chromatography. Two milliliters of sample was applied to the column, and 3-mL fractions were collected. The column was equilibrated in TETg buffer containing 0.3 M KCl ± 20 mM Na_2MoO_4.

Ion-Exchange Chromatography. Ion-exchange chromatography using DEAE-cellulose (Whatman DE-52) and phosphocellulose (Whatman P-11) was performed as described previously (Vedeckis, 1981, 1983).

Sucrose Gradient Ultracentrifugation. Five-milliliter 5–20% linear sucrose gradients were prepared in TETg buffer ± 0.3 M KCl and ±20 mM Na_2MoO_4. Two hundred microliters of sample was applied, and the tubes were spun at 190000g_{av} for 16 h at 2 °C. Fractions (200 μL) were collected by puncturing the tube bottom and collecting by gravity flow.

Receptor Activation. The receptor was activated by incubating the cytosol in 0.3 M KCl for 1–3 h (gel filtration experiments) or by warming the cytosol at 25 °C for 1 h.

Liquid Scintillation Counting. Four or eight milliliters of Beckman Ready-Solv EP scintillation cocktail was added to 0.5 or 1 mL of sample, respectively. Counting was done on a Beckman LS 7500 liquid scintillation spectrophotometer at a counting efficiency of about 35%.

Results

Gel Filtration. Sodium molybdate has been found to stabilize the unactivated form of steroid hormone receptors (John & Moudgil, 1979; Leach et al., 1979; Toft & Nishigori, 1979; Grody et al., 1980; Nishigori & Toft, 1980; Schmidt et al., 1980; Barnett et al., 1980; Shyamala & Leonard, 1980; Wolfson et al., 1980; Vedeckis, 1981). Therefore, the cytosol was made 20 mM in Na_2MoO_4 to study the unactivated form of the receptor. In addition, 20 mM Na_2MoO_4 was included in the gel filtration column buffer when the MoO_4^{2-}-stabilized receptor was being chromatographed.

The size of the AtT-20 GC-R has been estimated previously by using Sephadex G-150 gel filtration in TETg buffer containing 0.3 M KCl, with or without 20 mM Na_2MoO_4 (Vedeckis, 1981). Although the high salt, MoO_4^{2-}-stabilized (R_s = 7.7 nm) and the activated (R_s = 6.8 nm) forms could be distinguished, the sizes were too large for an accurate estimate of Stokes radii by using this gel filtration matrix. Therefore, high salt, MoO_4^{2-}-stabilized and activated AtT-20 GC-R's were subjected to Bio-Rad agarose A-1.5 m gel filtration (Figure 1). The Stokes radii for the high salt, MoO_4^{2-}-stabilized and activated receptors were 8.3 and 6.0 nm, respectively. When the MoO_4^{2-}-stabilized receptor was chromatographed in low ionic strength buffer (TETg–0.05 M KCl–20 mM Na_2MoO_4), a Stokes radius of 8.3 nm was also obtained (data not shown). When the high salt, MoO_4^{2-}-stabilized GC-R from mouse liver was analyzed, it had the same Stokes radius (8.3 nm) as this form from the AtT-20 cells (Figure 1). The shoulder seen on the trailing edge of this peak probably represents incomplete MoO_4^{2-} stabilization against activation to the 6-nm form and/or against limited proteolysis. As had been shown previously (Vedeckis, 1983), activation of the liver receptor resulted in limited proteolysis to a 3.9-nm form (Figure 1). Thus, as proposed previously (Vedeckis, 1983), MoO_4^{2-} protects the receptor from proteolysis, even during relatively long procedures such as gel filtration. The average Stokes radii obtained from the experiments performed on both the AtT-20 cell and liver GC-R were 8.3 ± 0.1 nm (n = 5) for the high salt, MoO_4^{2-}-stabilized receptor and 6.0 ± 0.1 nm (n = 4) for the activated (high salt) form.

Sucrose Gradient Ultracentrifugation. Sucrose gradient ultracentrifugation was performed on cytosol from AtT-20 cells under varying buffer conditions. When the receptor was analyzed on a sucrose gradient in high salt buffer (activating conditions), a 3.2S (±0.1 S; n = 7) peak was obtained, while low salt sucrose gradients revealed a 5S (±0.1 S; n = 11) form (Figure 2A). It should be noted that each sample analyzed

[1] Abbreviations: TA, triamcinolone acetonide (9α-fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione 16,17-acetal with acetone); EDTA, ethylenediaminetetraacetate; IAM, iodoacetamide; DEAE, diethylaminoethyl; PC, phosphocellulose; HAP, hydroxylapatite; GC-R, glucocorticoid receptor; RBF, receptor-binding factor; DTT, dithiothreitol; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate.

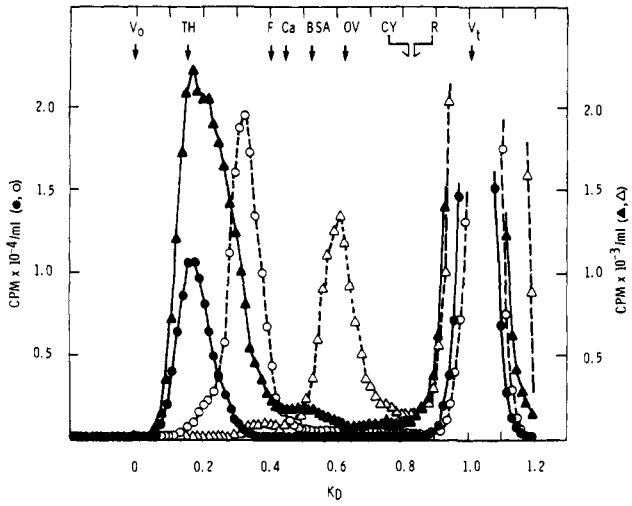


FIGURE 1: Gel filtration of the mouse glucocorticoid receptor. Two milliliters of various samples was pretreated for 1–3 h with 0.3 M KCl prior to chromatography on an agarose A-1.5 m column. When chromatography was performed on MoO_4^{2-}-treated samples, the column buffer included 20 mM Na_2MoO_4. Samples analyzed were MoO_4^{2-}-stabilized AtT-20 cell GC-R (●), salt-activated AtT-20 cell GC-R (○), MoO_4^{2-}-stabilized mouse liver GC-R (▲), and salt-activated mouse liver GC-R (△). The elution positions labeled in the figure are as follows: thyroglobulin (TH), R_s = 8.61 nm; ferritin (F), R_s = 5.75 nm; catalase (Ca), R_s = 5.13 nm; bovine serum albumin (BSA), R_s = 3.59 nm; ovalbumin (OV), R_s = 2.8 nm; cytochrome *c* (CY), R_s = 1.79 nm; ribonuclease A (R), R_s = 1.64 nm; $K_D = (V_e - V_0)/(V_t - V_0)$, where V_e = the elution position of the receptor or standard protein, V_0 = the void volume (117 mL) determined by using blue dextran ($M_r = 2 \times 10^6$), and V_t = the total volume (306 mL) of the column determined by the elution position of KCl. The Stokes radii of the receptor protein forms were determined from linear correlations of $K_D^{1/3}$ and R_s (data not shown).

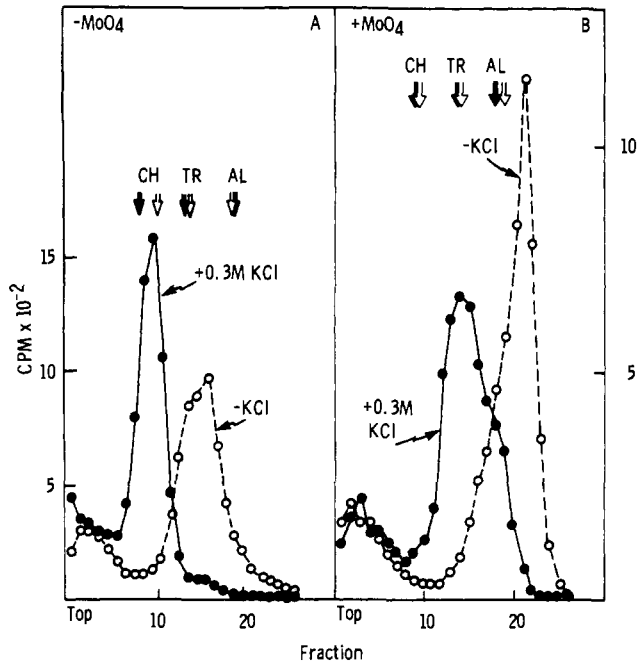


FIGURE 2: Sucrose gradient ultracentrifugation of the glucocorticoid receptor. (A) A sample of AtT-20 cell cytosol was chromatographed on an LH-20 column to remove unbound hormone. Two hundred microliters of the bound fraction was then centrifuged on 5–20% sucrose gradients either in the absence (○) or in the presence (●) of 0.3 M KCl. (B) Molybdate-stabilized AtT-20 cell cytosol was chromatographed on an LH-20 column equilibrated in TETg–20 mM Na_2MoO_4. The bound fraction was then centrifuged on 5–20% sucrose gradients in TETg–20 mM Na_2MoO_4 either in the absence (○) or in the presence (●) of 0.3 M KCl. Standard proteins shown in panels A and B are chymotrypsinogen A (CH = 2.6 S), transferrin (TR = 4.9 S), and aldolase (AL = 7.9 S). Solid arrows represent the sedimentation position of the standards on high salt gradients, while the open arrows are the standards on low salt gradients. Additional standards (not shown) utilized to determine the sedimentation coefficients of the receptor forms were ribonuclease A (1.9 S), ovalbumin (3.5 S), and bovine serum albumin (4.4 S).

here was passed over a Sephadex LH-20 column prior to centrifugation, in order to remove unbound hormone (Sherman et al., 1979; Vedeckis, 1981). This resin utilizes Sephadex G-25 as a support and, thus, results in gel filtration of the sample. Since Sephadex G-25 chromatography causes receptor activation (Cake et al., 1976; Bailly et al., 1977; Goidl et al., 1977; Sato et al., 1980), it is likely that the 5S GC-R is an activated form (see below).

When the unactivated, MoO_4^{2-}-stabilized receptor was analyzed on low salt, MoO_4^{2-}-containing gradients, a sedimentation coefficient of 9 S was obtained (Figure 2B). This is similar to the sedimentation coefficient values obtained for other MoO_4^{2-}-stabilized steroid hormone receptors (Nishigori & Toft, 1980; Shyamala & Leonard, 1980; Wolfson et al., 1980; Hutchens et al., 1981; Miller et al., 1981; Niu et al., 1981; Redeuilh et al., 1981; Sherman et al., 1981, 1982). When the MoO_4^{2-}-stabilized receptor was analyzed on a high salt, MoO_4^{2-}-containing gradient, however, a 5S form was obtained (Figure 2B). Therefore, it appeared that MoO_4^{2-} could partially stabilize the receptor to the salt-induced decrease in the sedimentation coefficient. That is, in 0.3 M KCl, MoO_4^{2-} prevented the formation of the fully activated, 3.2S receptor, although it could not preserve the unactivated, 9S species. Also, because the high salt, MoO_4^{2-}-stabilized receptor had the same sedimentation coefficient (5 S) as the normal cytosolic receptor run on a low salt gradient (Figure 2A), it is likely that the 5S, 8.3-nm receptor is a physiologically relevant form of the receptor. This has recently been confirmed by using vertical tube rotor sucrose gradient ultracentrifugation (S. B. Eastman, C. E. Reker, and W. V. Vedeckis, unpublished experiments). Thus, in vitro activation of the receptor by a variety of treatments [dialysis, Sephadex G-25 and Sephadex LH-20 gel filtration, warming, $(NH_4)_2SO_4$ precipitation] all resulted in the generation of predominantly the 5S GC-R form. Furthermore, when whole cells were incubated at 37 °C with radioactive hormone for 2 h, and cytosol was prepared, the in vivo activated receptor was also 5 S (S. B. Eastman and W. V. Vedeckis, unpublished experiments). Thus, this form may represent the physiologically relevant, activated receptor species.

Characteristics of Mouse Glucocorticoid Receptor Structure. Using the sedimentation coefficients and Stokes radii of the various receptor forms, it was possible to calculate certain physical properties. For the following calculations, it was assumed that the partial specific volume and the degree of solvation of the receptors were 0.735 cm^3/g and 0.2 g/g of protein, respectively. Molecular weights were calculated by using *s* and R_s according to the method of Siegel & Monty (1966):

$$M_r = 424 s R_s$$

while the frictional ratios were obtained by using the equation (Sherman, 1975)

$$f/f_0 = 1.393(R_s/M_r^{1/3})$$

The axial ratio was determined by assuming the shape of a prolate ellipsoid (Schachman, 1959).

When these values were determined for the unactivated, MoO_4^{2-}-stabilized GC-R, the molecular weight, frictional ratio, and axial ratio were 317 000, 1.70, and 14, respectively. The

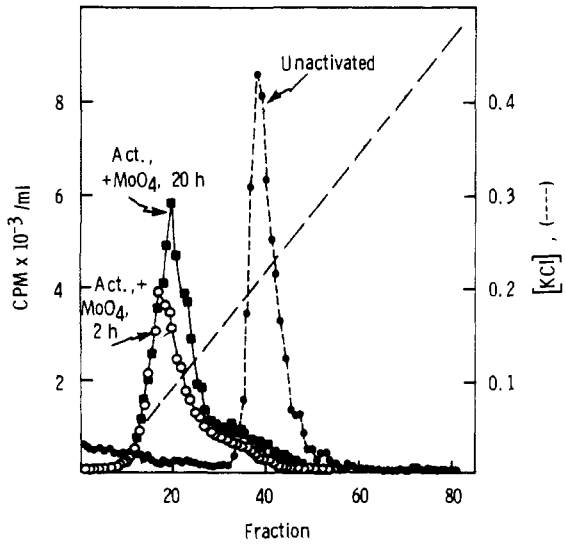


FIGURE 3: Inability to demonstrate reversal of glucocorticoid receptor activation. Either AtT-20 cell cytosol was chromatographed on DEAE-cellulose directly (unactivated) (●) or the glucocorticoid receptor was activated as described under Results. Na_2MoO_4 (20 mM) was then added to the activated receptor, and the samples were allowed to sit for 2 (○) and (■) 20 h. These samples were then chromatographed on DEAE-cellulose.

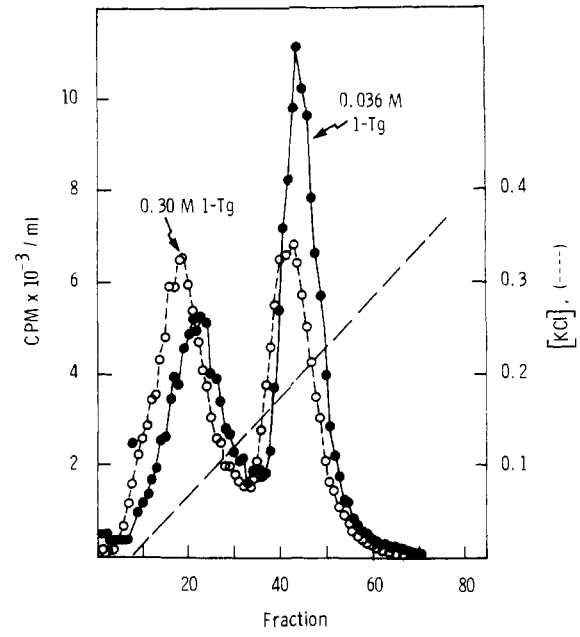


FIGURE 4: Promotion of glucocorticoid receptor activation by 1-thioglycerol. Cytosol (1 mL) from AtT-20 cells was made 0.036 (●) or 0.30 M (○) in 1-thioglycerol and incubated for 18 h at 0–4 °C. These samples were then chromatographed on 4-mL DEAE-cellulose columns and aliquots counted for radioactivity. The amount of total applied radioactivity which adsorbed to DEAE-cellulose was 25% for the 0.036 M 1-thioglycerol-treated sample and 22% for the sample in 0.30 M 1-thioglycerol.

high salt, MoO_4^{2-}-stabilized (hereafter called intermediate) form had a molecular weight of 176 000 (close to half that of the unactivated receptor) and was more asymmetric than the unactivated receptor, with a frictional ratio of 2.06 and an axial ratio of 22. The fully activated receptor had a molecular weight of 81 000, close to half that of the intermediate form and one-fourth that of the unactivated GC-R. Its fractional ratio and axial ratio were 1.93 and 18, respectively. Thus, glucocorticoid receptor activation results in a decrease in molecular weight, and subunit dissociation is proposed as the mechanism of activation. Whether a homotetramer–homodimer–monomer relationship is involved, or other protein components (receptor binding factors, RBFs) comprise the unactivated and intermediate forms, remains to be determined. It should be emphasized that protein subunit dissociation is not the only possible explanation for the observed molecular weight decrease. In view of some recent results showing the stabilization of the unactivated receptor with leupeptin, a protease inhibitor (Sherman et al., 1979, 1980, 1981), it is possible that a very specific, limited receptor proteolysis is involved. Furthermore, although RBFs are generally thought to be proteinaceous, an RNA component in the unactivated complex has also been suggested (Costello & Sherman, 1980; Hutchens et al., 1982).

Inability To Demonstrate Reversal of in Vitro Activation. If subunit dissociation is involved in receptor activation, it should be possible to reverse this effect. This was attempted as follows. The receptor was activated via warming at 25 °C for 1 h and passage over a Sephadex G-25 column. Aliquots were then incubated with or without 20 mM Na_2MoO_4 at 0–4 °C for 2 or 20 h. These samples were then analyzed via DEAE-cellulose chromatography.

As can be seen in Figure 3, the unactivated GC-R eluted at about 0.20 M KCl, as had been found previously (Vedeckis, 1981), while the activated form eluted at 0.08 M KCl. However, no significant conversion back to the unactivated form occurred after incubation either with MoO_4^{2-} present (Figure 3) or with no additions (data not shown). Thus, at least under these specific conditions, a reversal of receptor activation could not be demonstrated. However, some very recent, preliminary experiments may indicate that a reversal of activation is, indeed, possible (S. B. Eastman and W. V. Vedeckis, unpublished experiments). Whole AtT-20 cells were incubated with radioactive hormone at 37 °C to allow in vivo activation to occur. Cytosol was then prepared, and aliquots either were left untreated or were treated with 20 mM Na_2MoO_4. An apparent conversion of the 5S form to the 9S form occurred in the MoO_4^{2-}-treated sample. This may indicate that in vitro thermal activation (25 °C, 1 h) might destroy some component necessary for the reassociation reaction, while this component is not destroyed when in vivo activation is performed. More detailed experiments are currently in progress to resolve this question.

Promotion of Activation by 1-Thioglycerol. A recent report has shown that progesterone receptor activation (as analyzed by DNA–cellulose adsorption) can be promoted by thiol-protecting reagents, such as dithiothreitol and 1-thioglycerol (MacDonald & Leavitt, 1982). Since we were adding 1-thioglycerol to our homogenization buffer to protect hormone-binding activity, we wished to determine if a similar situation existed for the mouse GC-R. In addition, MoO_4^{2-} has been shown to interact with cysteine thiols on certain proteins (Weathers et al., 1979), and this could be involved in the mechanism of MoO_4^{2-} stabilization of the hormone-binding activity and unactivated physical state.

Cytosol from AtT-20 cells was incubated overnight with increasing concentrations of 1-thioglycerol. Receptor activation was assessed by the conversion of the 0.2 M KCl-eluting peak to the 0.08 M KCl-eluting moiety upon DEAE-cellulose chromatography. For example, as can be seen in Figure 4, the addition of an 8-fold increased concentration of 1-thioglycerol caused a substantial activation of the GC-R, as evidenced in a shift of the receptor from the later eluting (unactivated) peak to the earlier eluting (activated) form. In a more complete study, a progressive activation of the receptor occurred with increased 1-thioglycerol concentrations (data

not shown).[2] However, at the highest concentration of 1-thioglycerol used (0.588 M), a decrease in total hormone-binding activity occurred to half the normal level. This could have been due to a partial denaturation of the receptor structure via intrachain disulfide reduction or a disruption of thiols in the hormone-binding site.

Additional experiments were performed to determine if other thiol-protecting reagents (dithiothreitol, 2-mercaptoethanol) could promote glucocorticoid receptor activation. The results obtained were very dependent upon the length of treatment time, the concentration of agents, and the dilution of the cytosol. Conditions could not be found in which dithiothreitol (DTT) would promote activation, whereas 2-mercaptoethanol gave a consistent, but low, level of activation (5–10%) under certain conditions. Both of these results were partially due to dramatic effects of these agents on the total hormone-binding capacity of the cytosol. Thus, an increase in hormone-binding activity was observed in 1:20 diluted cytosol when concentrations of DTT were increased up to 10 mM. A dramatic decrease in hormone-binding activity was observed in samples treated with concentrations greater than 10 mM for DTT and 50 mM for 2-mercaptoethanol. These types of effects were noted previously for the progesterone receptor (MacDonald & Leavitt, 1982) and make the interpretation of the effects on receptor activation difficult. For example, if both the total hormone-binding activity and the percent of activated receptor are increased, it is difficult to distinguish if this represents a conversion of the unactivated receptor to the activated form, or if cryptic, activated receptor sites are being exposed by thiol-protecting agent. The moderate (5–10%) activation with 2-mercaptoethanol was achieved under conditions where the total amount of bound hormone did not change significantly over the concentration range tested (0.5–50 mM) and, thus, represents a true promotion of activation. The loss of hormone-binding activity prevented the use of higher concentrations of these agents, which might be necessary for the activation process to be stimulated optimally. Finally, both the loss of hormone-binding activity by high concentrations of these agents and the promotion of activation by 2-mercaptoethanol were completely blocked by 20 mM Na_2MoO_4. Thus, it appears that, at least in the AtT-20 GC-R system, 1-thioglycerol is the reagent of choice for future studies. This may be because the hormone-binding activity is protected by the glycerol, while allowing the attainment of high concentrations of reducing equivalents necessary for the promotion of receptor activation.

Activation State of the Intermediate Receptor Form. Preliminary studies have been performed to determine if the 5S intermediate receptor form is unactivated or activated. The GC-R from AtT-20 cells was thermally activated (25 °C, 60 min) and applied to a phosphocellulose column as described previously (Vedeckis, 1981). The absorbed receptor was step eluted with TETg–0.3 M KCl and collected in fractions, and the peak fractions were pooled (data not shown). The salt concentration was reduced to 0.15 M KCl by the addition of an equal volume of TETg buffer, and an aliquot was run on a low salt sucrose gradient. The majority of the receptor eluted as a 5S peak, although a considerable amount of receptor aggregation also occurred (data not shown).[2] Since the receptor which adsorbs to phosphocellulose is, by definition, activated, and since a reversal of activation is not facile (Figure 3), the intermediate, 5S receptor is tentatively designated as an activated form. This is supported by the studies described above involving the use of various methods of in vitro activation, all of which resulted in the formation of the 5S receptor.

Model for Mouse Glucocorticoid Receptor Structure. A hypothetical model for mouse liver and AtT-20 cell glucocorticoid receptor structure and activation is presented in Figure 5. Also included are the physicochemical and chromatographic properties of all receptor forms studied here and in the previous paper (Vedeckis, 1983). The implications of this model are discussed below.

Discussion

The molecular mechanism of steroid hormone receptor activation has been investigated for many years. However, it was still unclear what physicochemical changes occurred during the activation process. The results presented here point toward subunit dissociation as a possible mechanism of glucocorticoid receptor activation in mouse liver and AtT-20 cells.

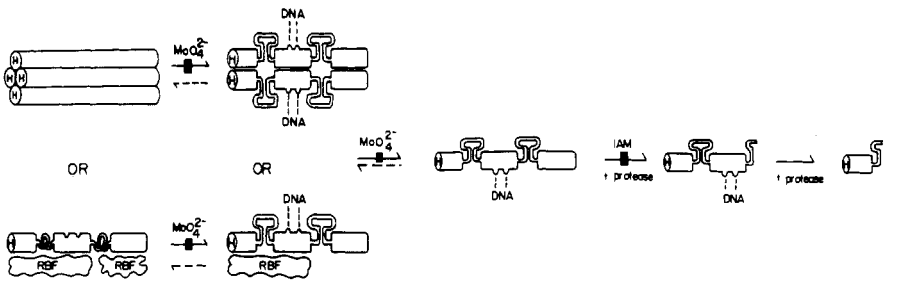
Sodium molybdate has recently been used to study receptor structure because of its ability to stabilize the unactivated form (John & Moudgil, 1979; Leach et al., 1979; Nishigori & Toft, 1980; Schmidt et al., 1980; Barnett et al., 1980; Shyamala & Leonard, 1980; Wolfson et al., 1980; Vedeckis, 1981). In recent comparative studies on various steroid receptors, it was shown that the MoO_4^{2-}-stabilized, unactivated forms have the same basic characteristics (Niu et al., 1981; Sherman et al., 1982). That is, they have a Stokes radius of 7.7–8.2 nm, a sedimentation coefficient of 9.2–9.9 S, and a molecular weight of 293 000–323 000. The results obtained here for the molybdate-stabilized mouse GC-R, in hypotonic buffer, are in good agreement with these values.

Upon receptor activation, a decrease in size is obtained. The 5S intermediate form, in the preliminary analysis performed here, appears to be an activated species. In addition, the 3.2S form also has the characteristics of an activated receptor. It is not yet known which of these forms, or if both, are involved in regulating gene expression. If the designation of the intermediate 5S form as activated is confirmed, it would be of considerable significance. For instance, the estrogen receptor is the only steroid receptor shown to undergo a 4S to 5S transformation upon activation. These studies involved the use of high salt sucrose gradients to analyze this process, although an 8–10S form has also been observed on low salt gradients and after molybdate stabilization. In our hands, the GC-R 5S form is not stable on high salt sucrose gradients, with dissociation into 3.2S subunits occurring (unless molybdate is present). Thus, it may be that the 5S activated estrogen receptor is much more stable in high salt sucrose gradients than comparable 5S activated forms for other steroid hormone receptors.

A dimeric structure for the receptor, as is proposed here, would allow cooperative interactions of the subunits with DNA. It is interesting to note that other gene regulatory proteins, such as the λ repressor (Johnson et al., 1979), bind cooperatively to DNA. In addition, many of these proteins are composed of two domains, separated by a protease-sensitive region. For the λ repressor, the amino-terminal domains interact with the operator DNA, while the carboxyl-terminal domains participate in subunit interactions. It will be interesting to see if more detailed analyses on steroid receptors reveal a similar situation.

The molecular weight of the GC-R monomer from mouse liver and AtT-20 cells is about 81 000. This is in good agreement with values determined previously by using similar techniques. Importantly, this also agrees well with values obtained by running photoaffinity-labeled glucocorticoid re-

[2] These data were submitted to the scrutiny of the reviewers but were deleted due to space considerations. These will by furnished by the author upon request.



	LOW SALT, UNACTIVATED	INTERMEDIATE, ACTIVATED	ACTIVATED	PARTIALLY PROTEOLYZED	MERO-RECEPTOR
R_S (nm)	8.3	8.3	6.0	3.9	2.4
S value	9.0	5.0	3.2	3.2	2.4
M_r (g/mole)	317,000	176,000	81,000	53,000	24,000
f/f_0	1.70	2.06	1.93	1.45	1.15
Axial Ratio	14	22	18	8	3
DEAE [KCl]	0.20	0.08	0.08	0.03	DT
PC [KCl]	DT	0.17	0.17	0.30	DT
DNA-C [KCl]	DT	0.14	0.14	0.20	DT
HAP [PO_4]	0.11	0.11	0.11	0.17	0.02

FIGURE 5: Proposed model for mouse liver and AtT-20 cell glucocorticoid receptor structure and mechanism of activation. The unactivated receptor exists as a homotetramer of identical hormone-binding subunits or, alternatively, as a complex of the receptor subunit with receptor binding factor(s) [RBF(s); see Discussion]. For the sake of clarity only, the detailed structure for the four subunits is omitted in the homotetramer shown. The DNA-binding region is occluded in the unactivated form and becomes exposed when activation occurs. Activation involves the dissociation of the receptor into a homodimer [or a receptor subunit plus RBF(s)] and/or the activated monomeric subunit. Both of these processes are inhibited by Na_2MoO_4. Activation also results in the conversion of the protease-sensitive regions from a protected to a sensitive configuration (Vedeckis, 1983). If the appropriate protease is present, the receptor is cleaved to a partially proteolyzed form (3.9 nm) and/or the mero-receptor (2.4 nm). The native receptor monomer is composed of three domains (hormone-binding, DNA-binding, specifier) of approximately equal sizes. Further details of this model are dealt with under Discussion. H = steroid hormone; IAM = iodoacetamide; DT = dropthrough (nonadsorbed) fraction.

ceptors on $NaDodSO_4$–polyacrylamide gel electrophoresis. Values obtained for rat liver (Eisen et al., 1981), rat HTC cell (Simons & Thompson, 1981), and mouse S49 cell (Nordeen et al., 1981) GC-R were 90 000, 85 000, and 87 000, respectively. In the study with S49 cells, it was claimed that the molecular weight obtained was for the unactivated receptor, since sodium molybdate was included in the homogenization buffer. However, since these gels are run under denaturing conditions, protein subunit interactions would be abolished. Thus, the molecular weight obtained in this study was probably that of the activated, monomeric receptor subunit.

We propose that glucocorticoid receptor activation involves the dissociation of a tetrameric, unactivated receptor (M_r = 317 000) into a dimeric (M_r = 176 000) and/or a monomeric (M_r = 81 000), activated form. This implies that the larger molecular weight complexes are composed solely of identical, hormone-binding subunits. However, on the basis of some recent findings, an alternative model is possible (shown in Figure 5). Cytosolic, non-hormone-binding proteins have been described, which cause a conversion of receptor proteins to higher sedimenting forms. These "receptor-binding factors" (RBFs) can convert the 4.5S estrogen receptor subunit to 5S, 6S, 7S, and 8S forms (Murayama et al., 1980a–d; Fukai & Murayama, 1981; Murayama & Fukai, 1981). Similar results were also found for the hen oviduct progesterone receptor (Murayama et al., 1980e). In addition, an "8S androgen receptor promoting factor" has been described in the rat prostate Dunning tumor (Colvard & Wilson, 1981). Finally, the possibility that RNA is involved in the structure of the unactivated (9S) receptor (Costello & Sherman, 1980; Hutchens et al., 1982) or that a very specific, leupeptin-inhibitable proteolysis is involved (Sherman et al., 1979, 1980, 1981) cannot be excluded. Further experiments are required to determine which situation exists for the glucocorticoid receptor from the mouse AtT-20 cell line.

Finally, a role for sulfhydryl reduction in receptor activation may be indicated in this study, as was previously shown for the progesterone receptor (MacDonald & Leavitt, 1982). The interaction of molybdate with sulfhydryls of certain proteins may have important implications for the process of molybdate stabilization of the unactivated receptor. For example, molybdate has been shown to form a dimeric bridge between cysteine side chains on the insulin A chain (Weathers et al., 1979). A similar interaction of molybdate with the receptor subunits in the 9S, unactivated receptor species is possible. Obviously, a good deal more experimentation is required to further clarify the molecular mechanism of steroid hormone receptor activation.

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