Syrian Hamster Glucocorticoid Receptors

CHARACTERISTICS OF BINDING OF PARTIALLY PURIFIED RECEPTOR TO DNA*

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In this paper, we present evidence for three molecular weight forms of Syrian hamster glucocorticoid receptor. Stabilization of the higher molecular weight forms occurs in the presence of 20 mM Na2MoO4. The characteristics of the partially purified receptors are: receptor RB1, \( M_r = 350,000 \pm 5,600 \); Stokes radius, 78.4 Å; \( f/f_0 \) 1.67; and \( s_{20,w} \) 9.5 ± 0.2; receptor RB2, \( M_r = 198,000 \pm 5,500 \); Stokes radius, 53.3 Å; \( f/f_0 \) 1.37; and \( s_{20,w} \) -8.0 ± 0.30; receptor RB3, \( M_r = 84,000 \pm 980 \); Stokes radius, 47.7 Å; \( f/f_0 \) 1.60; and \( s_{20,w} \) 3.5 ± 0.01. Transformation of the receptor was a temperature-dependent process and was most efficient at 30 °C. Common serine protease inhibitors as well as phosphatase inhibitors failed to block transformation. Binding of transformed receptor to DNA-cellulose was examined. The metal salts of Zn2+, Cu2+, Sn2+, Fe2+, and Cd2+ which disrupt hydrogen-bonded regions of duplex DNA blocked binding of receptor to DNA but did not affect receptor transformation. Other mono- and divalent metal salts which interact with DNA phosphates had no effect on either transformation or binding to DNA whereas molybdate ions blocked receptor transformation but left binding unaffected. Single- and double-stranded DNA polymers were tested for their ability to compete for transformed receptor binding to DNA-cellulose and were found to have a lower affinity for receptor than homologous, Escherichia coli, and salmon sperm DNAs. We have demonstrated certain physicochemical parameters of three forms of the hamster glucocorticoid receptor. These studies indicate that the three receptor forms exhibit a precursor-product relationship in their formation. No evidence of enzymatic activity responsible for conversion could be detected. Finally, binding of partially purified receptor to DNA is not by a simple ion exchange mechanism with the DNA phosphate backbone but appears to involve hydrogen-bonded regions on the DNA molecule.

Following the binding of a glucocorticoid to its receptor, a step occurs which alters certain properties of the receptor protein and promotes receptor binding to DNA and chromatin substrates. The nature of the step, variously defined as activation or transformation, is unclear but appears to involve a change in the quaternary structure of the protein (1, 2) which can be induced by heating (3), salt treatment (2), dilution (4), or treatment of the receptor complex with theophylline (5), calcium (6), or basic amines (7). More recently, it has been shown that molybdate and tungstate, both VIIb elements, stabilize the unactivated forms of glucocorticoid and other steroid receptors (8-10). In the original studies on glucocorticoid receptors, the evidence suggested that metals acted as inhibitors of a phosphatase and that the phosphatase inactivated the protein by a dephosphorylation reaction (11-13). In this particular instance, the activation dealt with recovery of steroid-binding capacity as opposed to conversion or transformation of the receptor to a DNA-binding form. Subsequent work demonstrated that transformation was also inhibited (14).

More recently, our laboratory (15) and others (8) have presented strong circumstantial evidence that molybdate acts directly on the receptor, affecting its size and by inference its quaternary structure. A phosphate moiety on the receptor may be involved in this reaction (14).

Metals other than group VIIb elements may also be involved in receptor functions and two reports imply that receptors may be metalloproteins. Lohnar and Toft (16) have performed studies which suggest that metal ions participate in the attachment of receptor to nuclear binding sites. Since most polymerases are zinc-containing enzymes, they suggest that zinc might play a role in the biology of progesterone receptor interaction in the nucleus. Evidence in support of these data is offered by Shyamala and Yeh (17) who suggest that the mouse mammary gland estrogen receptor is a metalloprotein. They performed experiments which indicated that Zn2+ or Mn2+ may be very important in determining the receptor's conformation and, hence, its biological activity. Most recently, Schmidt et al. (18) have extended these observations to include rat hepatic glucocorticoid receptors and suggest that a metal moiety, which can be blocked by 1,10-phenanthroline, is involved in binding of [3H]triamcinolone-acetonide-labeled receptor to DNA.

The glucocorticoid receptor obtained from the DDT, hamster cell line has characteristics that differ somewhat from the rat liver receptors discussed above (15, 19-21). We have demonstrated multiple molecular forms of the receptor, only one of which binds to DNA. In addition, we now present evidence that suggests molybdate ions stabilize a multimeric form of the receptor. Removal of molybdate ions allows the receptor to transform in a temperature-dependent fashion to the lowest molecular weight form which binds to DNA. In addition, while studying the effects of other metal ions on transformation, we determined that certain divalent metal ions which can interrupt hydrogen-bonded regions of duplex DNA (22) prevent DNA-receptor complex formation under conditions in which receptor transformation was unaffected.

We show with present studies that the DDT:MF-2 cell line contains a glucocorticoid receptor existing as a \( M_r = 350,000 \) protein which can be dissociated into a \( M_r = 84,000 \) receptor.

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that, when heated, exposes a DNA-binding site. We now show that molybdate ions block the dissociation step and prevent the exposure of the DNA-binding site. Further, we infer that transformed receptor interacts specifically with hydrogencBonded regions of DNA and not by simple ion exchange mechanisms with phosphate groups exposed on the DNA backbone.

Experimental Procedures

Preparation of DNA-cellulose—DNA-cellulose was prepared by the absorption method described by Alberts and Herrick (23). The final concentration of DNA was adjusted to 100 μg/ml by adding additional cellulose.

Cell Culture—Hamster DDT-MF-28 cells were grown in 15-liter suspension cultures and harvested as previously described (20). Cells not used immediately were stored frozen at -120 °C in 10% glycerol buffered with 20 mM sodium phosphate buffer, pH 6.8. Cells stored in this manner are stable for at least 1 year without demonstrating loss of receptor activity.

Buffers—Sodium phosphate buffer consisting of 20 mM Na2HPO4, NaH2PO4, pH 6.8, was prepared in 10% glycerol. Column chromatography buffers were sodium phosphate buffer, pH 6.8, containing 20 mM sodium molybdate and 300 mM potassium chloride where specified. In certain instances indicated in the text, sodium molybdate was omitted. These buffers were always prepared fresh from stock solutions just prior to use. DEAE-cellulose and phosphocellulose chromatography was performed using sodium phosphate buffer containing 20 mM molybdate for 1 followed by chromatography on Bio-Gel A-5m or A-1.5m agarose (200-400 mesh). Columns (2.6 × 90 cm) were packed by gravity and then thawed at 37 °C. Suspension in sodium phosphate buffer, pH 6.8, and then eluted with 5 ml of sodium phosphate buffer containing 0.18 M potassium chloride was added to the column to achieve 50 or 100 μg in final concentration of DNA (as indicated). Following a 10-mal wash of the packed DNA-cellulose with sodium phosphate buffer containing 0.1 M NaCl, receptor was applied. The columns were washed once with 5 ml of sodium phosphate buffer containing 0.1 M NaCl and then eluted with 5 ml of sodium phosphate buffer containing 0.5 M NaCl. Radioactivity was determined by scintillation counting a 0.5-ml aliquot of eluate as described above. Details concerning the addition of test salts are included in the figures and tables. In all cases, a nonspecific binding to cellulose blanks (average 10%) was subtracted before calculations of specific binding were made (3). Analysis of receptor binding to soluble muscle and synthetic DNA polymers was carried out using a DNA-cellulose column described above. However, 10 min prior to addition of receptor to the column, aliquots of the polymer to be tested were added. All subsequent steps were the same. Concentrations of polymers were determined by optical density determinations and from literature provided by P-L Biochemicals. A second, intermediate form of receptor is detected by incubating cytosol at 20 °C in the presence of 0.6 M KCl and 20 mM molybdate for 1 followed by chromatography on Bio-Gel A-5m in the presence of molybdate and 0.3 M KCl. The Mb, 350,000 form of receptor still seen as peak I under these conditions is in part destabilized and a receptor species with an observed Mb, 288,000 appears (Fig. 1, peak II). The Stokes radius is 53.3 ± 1.4 Å (n = 3) and the s20, w is 8.0 ± 0.3 in salt (Table I). By calculation, Mb, 198,000 ± 5,500. The fractional ratio is 1.37, indicating a more spherical molecular shape when compared with Mb, 350,000 receptor. The intermediate receptor, termed RB2, co-elutes at 0.18 M KCl with the Mb, 350,000 receptor when chromatographed on DEAE-cellulose (Fig. 2, inset). Other Materials—[3H]Triamcinolone acetonide (specific activity, 37.1 Ci/mmol) was obtained from New England Nuclear. Nonradioactive triamcinolone acetonide, activated charcoal, sodium phosphate, glycerol, and thioglycerol were obtained from Sigma Chemical Co. Other chemicals obtained from Sigma included 2,3-dimercaptopropanol, 1,10-phenanthroline, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, EDTA, 8-hydroxyquinoline, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride, diisopropyl phosphorofluoridate, leupeptin, and irononvalent metal salts. Other metal salts were obtained from Aldrich Chemical Co. except sodium vanadate which was obtained from Fisher Scientific. Cellulose, DEAE-cellulose, and phosphocellulose were purchased from Reeve Angel, Clifton, NJ. All reagents were prepared with glass-distilled water. The DNA used to prepare DNA-cellulose was isolated from DDT-MF-28 suspension cultures by the phenol extraction method of Marmur (24), and sheared by sonication followed by alkaline sucrose fractionation. The region of the gradient containing 20-kludase average DNA determined with a [3H]DNA marker set from Bethesda Research Laboratories was pooled and the DNA was precipitated with ethanol. Chromatin was prepared as described previously (21).

RESULTS

If [3H]triamcinolone acetonide-labeled receptor is prepared in the presence of 20 mM sodium molybdate and chromatographed on A-5m agarose, a single peak of radioactivity (not shown) is recovered and chromatographs exactly like peak I (Fig. 1). The observed molecular weight of this form of the receptor is 575,000 and the Stokes radius is 78.4 Å. The observed molecular weight of this form of the receptor is 575,000 and the Stokes radius is 78.4 Å. This is constant.

\[ M_b = \frac{4\pi N_r \eta^2}{3\eta} \]

where \( M_b \) is the molecular weight of the receptor, \( N_r \) is the number of residues, \( \eta \) is the viscosity of water at 20 °C, and \( \rho \) is the degree of solution. Further, we infer that transformed receptor interacts specifically with hydrogen-bonded regions of DNA and not by simple ion exchange mechanisms with phosphate groups exposed on the DNA backbone.

**Proteins used for column calibration were**

| Protein          | Mb   | ra |
|------------------|------|----|
| Thyroglobulin    | 670,000 | 85 |
| Pyruvate kinase  | 235,000 | 54 |
| Fumarase         | 190,000 | 51 |
| Bovine serum albumin | 65,200 | 35.5 |
| Ovalbumin        | 45,000  | 27.3 |
| α-Chymotrypsin   | 22,500  | 20.9 |
| Myoglobin        | 12,800  | 20.7 |

**Other Materials—**[3H]Triamcinolone acetonide (specific activity, 37.1 Ci/mmol) was obtained from New England Nuclear. Nonradioactive triamcinolone acetonide, activated charcoal, sodium phosphate, glycerol, and thioglycerol were obtained from Sigma Chemical Co. Other chemicals obtained from Sigma included 2,3-dimercaptopropanol, 1,10-phenanthroline, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, EDTA, 8-hydroxyquinoline, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride, diisopropyl phosphorofluoridate, leupeptin, and irononvalent metal salts. Other metal salts were obtained from Aldrich Chemical Co. except sodium vanadate which was obtained from Fisher Scientific. Cellulose, DEAE-cellulose, and phosphocellulose were purchased from Reeve Angel, Clifton, NJ. All reagents were prepared with glass-distilled water. The DNA used to prepare DNA-cellulose was isolated from DDT-MF-28 suspension cultures by the phenol extraction method of Marmur (24), and sheared by sonication followed by alkaline sucrose fractionation. The region of the gradient containing 20-kludase average DNA determined with a [3H]DNA marker set from Bethesda Research Laboratories was pooled and the DNA was precipitated with ethanol. Chromatin was prepared as described previously (21).
The resulting peak represented by the receptor labeled with 20 mM sodium molybdate is present throughout all chromatographic procedures. If receptor is treated with 0.6 M KC1, it is not possible under any of our experimental conditions to affect transformation. However, if the receptor is dialyzed against phosphate buffer to remove molybdate, it subsequently can be transformed by heating (Fig. 3). In the absence of molybdate, spontaneous activation occurs very slowly at 4°C. At higher concentrations of receptor, both spontaneous and heat-induced transformation occurs more slowly than in dilute solution.1

We have examined receptor transformation in the presence of several different types of enzyme inhibitors in order to establish if serine proteases or phosphodiesterases were involved in the steps which produce the DNA-binding RA1 receptor. Transformation occurred under all conditions (Table II) except in the presence of Na2MoO4 which was used as the negative control.

Because Na2MoO4 blocked the transformation step, we examined a series of other metals for similar activity. Our assay measured the binding of activated receptor to DNA. We discovered several groups of metals that seemed to block transformation (Table III, Group I, Column A) while another series of metals had minimal or no inhibitory activity in our assay measured the binding of activated receptor to DNA. We discovered several groups of metals that seemed to block transformation (Table III, Group I, Column A) while another series of metals had minimal or no inhibitory activity in our

1. J. S. Norris, unpublished observations.
values which were considered to be the DNA-cellulose-binding assay. Results were adjusted to control of M, as the amount of transformed receptor formed at column. Chromatography and subsequently dialyzed to remove indicated. As a control, receptor was treated at Na2MoO4. DNA-cellulose was used to measure the percentage of DNA binding in the presence of increasing concentration of metal ions that competed for the DNA-cellulose matrix under our experimental conditions. To examine this possibility, we performed an experiment to determine if the effect of the metal salt was reversible. Receptor was activated and metal salts were added and allowed to incubate for 30 min. One-half of each aliquot was then dialyzed against a 1000-fold excess of phosphate buffer for 1 h. Both fractions were subsequently analyzed for DNA binding versus identically processed controls. The results (Table IV) indicate that DNA binding capacity returns, although to slightly lower levels than expected. Presumably, this indicates incomplete dialysis or enhanced receptor degradation. Although not presented graphically, we have shown, with the exception of 1,10-phenanthroline, that chelating agents do not enhance activation of receptor nor do they promote DNA binding. 1,10-Phenanthroline is a special exception in that apparent activation and DNA binding are promoted in its presence. However, 1,10-phenanthroline directly attacks DNA in the presence of Cu2+ ions and its

![Fig. 3. The effect of temperature on transformation of the M, = 350,000 receptor. Receptor was partially purified by A-5m column chromatography and subsequently dialyzed to remove Na2MoO4. DNA-cellulose was used to measure the percentage of receptor transformed as a function of time at the various temperatures indicated. As a control, receptor was treated at 30 °C in the presence of 20 mM Na2MoO4 (— — —).](image)

**TABLE II**

**Effect of enzyme inhibitors on transformation**

| Inhibitor                      | % control receptor bound to DNA |
|--------------------------------|--------------------------------|
| Control                        | 100 ± 4.8                      |
| Soybean trypsin inhibitor (1 mg/ml) | 90.6 ± 6.7                     |
| Diisopropyl fluorophosphate (1 μM) | 95.8 ± 1.2                     |
| Phenylmethylsulfonyl fluoride (1 mM) | 108.8 ± 1.9                    |
| Leupeptin (1 mg/ml)            | 101.7 ± 3.8                    |
| NaF (2 mM)                     | 100.1 ± 2.4                    |
| Na2MoO4 (20 mM)                | 14.6 ± 7.0                     |

When re-examined, DNA binding in the presence of increasing concentrations of metal ions was depressed in a concentration-dependent manner (Fig. 4A) or unaffected (Fig. 4B) (see also Table III). As can be seen, the metals Sn2+, Cu2+, Cd2+, and Fe3+ are by far the most potent inhibitors of DNA binding. Conversely, Na+, K+, Mg2+, and Ca2+ have essentially no effect on receptor binding over a 1000-fold range in concentration. Binding of receptor to DNA preincubated with metal ions resulted in similar competition curves shifted to the right an order of magnitude for the metal ions that competed for the DNA-binding site (data not shown). Since the volume of DNA-cellulose was roughly 10% of the receptor volume (3-5 ml), the magnitude of the rightward shift of the competition curves is approximately equivalent to the expected metal ion concentration remaining in the DNA-cellulose matrix under our experimental conditions.

Several mechanisms were possible for the observed inhibition of receptor DNA complex formation. Reports in the literature (6) and personal observations indicated the possibility that the metal ions might be complexing with receptor, causing precipitation of the complexes. To examine this question, A-5m partially purified receptor in phosphate buffer was incubated with varying concentrations of salts followed by centrifugation at 10,000 × g to pellet any precipitates. A negligible number of counts were recovered as precipitates regardless of receptor concentration (data not shown). Receptor suspended in Tris- or N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid-buffered systems demonstrated varying but greater degrees of precipitation formation.

Since receptor precipitation didn't seem to be cause for ion inhibition of receptor DNA complex formation, we began to examine additional mechanisms. One possibility was that the metals caused a rearrangement of the receptor-binding site. To begin our examination of this possibility, we performed an experiment to determine if the effect of the metal salt was reversible. Receptor was activated and metal salts were added and allowed to incubate for 30 min. One-half of each aliquot was then dialyzed against a 1000-fold excess of phosphate buffer for 1 h. Both fractions were subsequently analyzed for DNA binding versus identically processed controls. The results (Table IV) indicate that DNA binding capacity returns, although to slightly lower levels than expected. Presumably, this indicates incomplete dialysis or enhanced receptor degradation. Although not presented graphically, we have shown, with the exception of 1,10-phenanthroline, that chelating agents do not enhance activation of receptor nor do they promote DNA binding. 1,10-Phenanthroline is a special exception in that apparent activation and DNA binding are promoted in its presence. However, 1,10-phenanthroline directly attacks DNA in the presence of Cu2+ ions and its

| Concentration | Bound receptor | A | B |
|---------------|----------------|---|---|
| mM            |                |   |   |
| Control       | 90.2 ± 3.6     | 100 ± 1.8 |
| Group I       |                |   |   |
| CuCl2         | 7.3 ± 1.0      | 5.6 ± 2.2 |
| ZnCl2         | 31.2 ± 3.6     | 13.2 ± 2.5 |
| SnCl2         | 17.5 ± 1.8     | 12.6 ± 3.3 |
| FeCl2         | 2              | 21.6 ± 1.6 |
| CdCl2         | 2              | 14.8 ± 4.3 |
| Group II      |                |   |   |
| NaVO4         | 0.5            | 83.1 ± 5.3 | 94.3 ± 2.9 |
| MgCl2         | 2              | 84.1 ± 4.3 | 92.6 ± 4.1 |
| CaCl2         | 2              | 71.4 ± 1.8 | 93.0 ± 6.8 |
| NaF           | 2              | 99.0 ± 4.4 | 93.3 ± 7.3 |
| CsCl          | 2              | 90.6 ± 4.0 | 91.4 ± 4.9 |
| LiCl          | 2              | 100.6 ± 1.6 |
| KCl           | 2              | 96.1 ± 6.1 | 92.3 ± 3.2 |
| NaCl          | 2              | 98.8 ± 3.9 | 96.2 ± 1.7 |
mechanism in promoting DNA-receptor complex formation may be unrelated to the subject of this paper (26), although alternative possibilities have been suggested (18).

We have also examined receptor-DNA complex formation from a different standpoint by performing competition studies using synthetic DNA polymers. Homologous DNA (DDT, MF-2S cells) linked ionically to cellulose was incubated with receptor previously mixed with the various nucleic acid competitors as shown (Fig. 5). Bound receptor was measured and we determined that homologous DNA, sheared to approximately 20,000 base pairs in length, was the most effective competitor followed by poly(dA-dT) (approximately 700 base pairs in length). The single-stranded homopolymers poly(dG) and poly(dC) did not inhibit receptor DNA binding at the indicated concentrations. It should be noted that the DNA-cellulose matrix contained 50 μg of bound DNA whereas 50% inhibition of binding was achieved by only 10 μg of soluble DDT-MF-2S DNA. These data are not surprising in view of the fact that preincubation of the soluble homopolymers with the labeled receptor was carried out prior to the assessment of specific binding to the DNA-cellulose matrix.

The last series of experiments we performed was carried out to determine if receptor demonstrated a specificity for homologous DNA. DDT, MF-2S DNA approximately 20,000 base pairs in length was linked ionically to cellulose and adjusted to 100 μg/ml packed volume. Receptor partially purified as described using A-5m column chromatography was dialyzed to remove Na₂MoO₄ and activated at 30 °C for 15 min. DNAs to be tested were all adjusted to approximately 20,000 base pairs in length by sonication and added to 100,000 cpn (2–3 μg) of receptor in approximately 2.5 ml of phosphate buffer. Following a half-hour incubation, receptor-binding assays using DNA-cellulose columns were performed. Using this protocol, there was little detectable difference in the DNAs, a finding in agreement with previously published observations using rat HTC cell DNA and glucocorticoid receptor complexes reacted under more rigorously defined competition conditions (27). However, Escherichia coli DNA which had the highest C-G content was slightly more effective in competing for the receptor than MF-2 or salmon sperm DNA (Fig. 6). It was clear that single-stranded tRNA at the concentrations used failed to inhibit binding.

### Table IV

| Compound | Concentration (mM) | Transformed receptor bound to DNA (%) Before dialysis | Following dialysis |
|----------|--------------------|-----------------------------------------------------|--------------------|
| Control  | 100                | 100                                                 | 19.9               |
| Control  | (not heated)       | 100                                                 | 14.1               |
| CuCl₂    | 0.1                | 29.3                                                | 64.9               |
|          | 1.0                | 22.8                                                | 56.3               |
| CdCl₂    | 0.1                | 31.8                                                | 57.7               |
|          | 1.0                | 26.3                                                | 73.6               |
| LiCl     | 0.1                | 101.2                                               | 99.0               |
|          | 1.6                | 98.4                                                | 111.2              |
| NaF      | 0.1                | 93.8                                                | 106.4              |
|          | 1.0                | 104.2                                               | 89.0               |

### Figure 4

**Reversal of inhibition by dialysis**

Receptors were heated in the absence of Na₂MoO₄, to 30 °C for 15 min and then cooled to 0-4 °C and the indicated metal salts were added. DNA-cellulose chromatography was performed upon one-half of each sample 30 min later. The remainder of each sample was dialyzed for 1 h against phosphate buffer before being applied to DNA. The second dialyzed samples were corrected for loss of radioactivity during dialysis by adjusting the controls upward 12.2%. Each number represents the average of two determinations.

### Figure 5

**Binding of transformed receptors to DNA-cellulose in the presence of synthetic polynucleotides.** Poly(dG) (▪), poly(dC) (■), poly(dG-C) (□), poly(dA-T) (□) and control MF-2 DNA (○) were added to transformed receptors at the indicated concentration 10 min prior to addition of receptors to the DNA-cellulose columns. Other details are given under "Experimental Procedures."
FIG. 6. Binding of transformed receptors to DNA-cellulose in the presence of homologous and heterologous DNA. Receptor was incubated 30 min with DNA from salmon sperm (○), DDT, MF-2 cells (■), E. coli (□) or single-stranded tRNA as a control (△). Receptor-binding assays using DNA-cellulose (100 μg/column) were carried out as described under "Experimental Procedures." All DNA species are sheared by sonication to an average length of 20 kilobases and isolated as the major peaks from alkaline sucrose gradients. Other details are described under "Experimental Procedures."

DISCUSSION

Numerous studies have been carried out designed to dissect the nature of cytoplasmic glucocorticoid steroid-receptor complex formation, the subsequent steps involved in receptor translocation to the nuclear compartment, and the final events involved in steroid-directed gene expression (28, 36). We have presented in this paper data which provide information concerning these mechanisms with regard to the hamster glucocorticoid receptor. Our studies on determining the molecular weights of the glucocorticoid receptors suggest a subunit relationship. The cytoplasmic receptor (M₀ = 350,000) under appropriate conditions appears to form an intermediate receptor (Mᵢ = 198,000). Both high molecular weight forms of the receptor bind in an identical fashion to DEAE-cellulose and appear to be untransformed. However, when the cytoplasmic receptors are prepared under conditions allowing transformation to occur, a new receptor species of Mᵢ = 84,000 appears. This receptor-steroid complex elutes from DEAE-cellulose at significantly lower KCl concentrations, suggesting a difference in charge from the larger receptor complexes. Furthermore, this form of receptor exhibits binding to DNA-cellulose (21).

Although no direct evidence is available, or data are consistent with a receptor model existing as a tetramer (M₀ = 350,000), composed of two dimers (Mᵢ = 198,000) which, upon heating, dissociate into two monomers (Mᵢ = 84,000) with exposed DNA-binding sites. The fractional ratios of the three receptor forms tend to support our model in that the largest and smallest receptor molecules are elongated, with the intermediate receptor being more globular. This suggests that the monomer may be a prolate ellipsoid similar to the chick oviduct progesterone receptor B subunit (29). When two monomers are combined, they form the more spherical dimer. The dimer interacts in a manner that forms the more elongated glucocorticoid receptor tetramer.

In order to study the interaction of receptor with DNA substrates, we needed to establish conditions for promoting receptor transformation. Early evidence suggested enzyme activity was involved in the transformation process. However, we believe for the glucocorticoid receptor that none of the enzymes suggested by others (11, 30, 31) were involved. We did observe that Na₂MoO₄ appeared to prevent the process of transformation. This led us to the discovery that several metal chlorides (and acetates) were capable of blocking receptor binding to DNA. At first, our working model envisioned the receptor as a metalloprotein (also proposed by others) (16, 17) in which the metal blocked the receptor's DNA-binding site. This suggested that receptor transformation involved the loss of a metal (possibly Cu²⁺ or Zn²⁺). However, we were unable to effect the transformation of receptor with chelating agents. Dialysis of receptor incubated previously with the metal restored receptor DNA binding capacity (Table IV), demonstrating that the transformation step if it involves the removal of a metal ion appears to be irreversible. Although we cannot eliminate the possibility that loss of a metal ion constitutes the transformation step, evidence in the literature indicated that at least some of the metals that inhibited receptor binding may act by interrupting hydrogen-bonded regions of duplex DNA (22). Other metals such as Na⁺, K⁺, and Mg²⁺ that failed to inhibit receptor binding are generally thought to interact with phosphate groups in duplex DNA (22), stabilizing the duplex structure.

To determine if any specificity for receptor by DNA existed as had been reported for estrogen receptors (32, 33) and glucocorticoid receptors (35), we examined homologous, heterologous, and synthetic DNA polymers. Our conclusions based on these experiments are that no clearly defined specificity exists. However, E. coli DNA (high G-C content) was more effective at competing for receptor than DDT, MF-2 or salmon sperm DNAs which are more A-T rich. The synthetic poly(dA-dT) and poly(dG-dC) polymers both failed to demonstrate specificity and were much less effective inhibitors when compared to the natural DNAs tested. No binding to single-stranded polynucleotides was evident. These data suggest that receptor binds only to the duplex form of total cellular DNA and fails to bind to single-stranded forms, observations consistent with the existing literature (33).

In conclusion, we have demonstrated that the DDT, MF-2 cell line contains a glucocorticoid receptor which exists in at least three forms. Our data are consistent with these receptors having a precursor-product relationship. Transformation of the receptor to the DNA binding form is inhibited by Na₂MoO₄; however, in the absence of molybdate ions, receptor can be transformed to a molecule with a fractional ratio of 1.60 (elongated) which binds to DNA. The exact mechanism of binding is unknown but our data strongly suggest that the receptor inserts into hydrogen-bonded regions of DNA. It is possible that receptors bind preferentially to A-T-rich regions of DNA since the A-T-rich regions are the most easily destabilized by the metal salts and receptor appears to bind more avidly to poly(dA-dT) than to poly(dG-dC). We draw this conclusion because inhibition of receptor binding occurs only with metal salts that, under our conditions, interrupt hydrogen-bonded regions altering the topography of the DNA duplex. Metal salts which act to stack DNA phosphates, hence stabilizing the topography of the DNA duplex, fail to block binding. Finally, the elongated DNA-binding form of the receptor facilitates insertion into the major groove of DNA in a manner which may be similar to the lac repressor interaction with Lac operator (37). In fact, recent evidence suggests that the λ bacteriophage cro repressor protein recognizes its specific DNA-binding site, which occurs in the major groove of right-handed B-form DNA, by amino acid side chain interaction with "multidentate hydrogen bonds" formed with DNA base pair atoms (35). These interactions are sequence specific and involve the α-helical structure of the cro protein.

We interpret our data to suggest that hamster glucocorticoid receptor exists as a multimeric molecule until exposed to hormone and temperature which effects transformation of the receptor to its DNA-binding form. This form of the receptor subsequently acts on glucocorticoid-regulated transcriptionally controlled sites by binding to DNA, perhaps at a few specific base pairs (34) with exposed hydrogen-bonded regions.
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