The structure of purified preparations of activated (DNA-binding) glucocorticoid receptor (GR) was analyzed in the presence or absence of DNA. A 35-base pair DNA fragment harboring a strong GR-binding site from the mouse mammary tumor virus promoter (-189/-166) was used for stoichiometric analysis of the GR-DNA complex. Glycerol gradient centrifugation was utilized in order to separate the 6 S GR-DNA complex from the 4 S GR and the 3 S DNA fragment. Synthetic glucocorticoid \([1^3]H\)triamcinolone acetonide bound to GR and \([32P\)-5'-end-labeled DNA fragment were used as probes for quantitation of each component. Such experiments demonstrated that two hormone molecules (two 87.5-kDa GR peptides) are associated with each cognate DNA site. Quantitative DNase I footprinting confirmed this result.

The formation of the GR-DNA complex was ligand-dependent, but once formed the complex remained stable after ligand dissociation. Incubation of GR with 0.01–0.1% (w/v) glutaraldehyde resulted in a shift in its sedimentation rate from 4 to 6 S. Gel filtration chromatography of glutaraldehyde-treated GR resulted in a complex of slightly larger size than the gamma-globulin standard (158 kDa). Gel filtration of GR without glutaraldehyde treatment gave the identical result. This suggests that a GR multimer, probably a homodimer, is stable during gel filtration chromatography but needs to be stabilized by glutaraldehyde cross-linking or DNA during glycerol gradient centrifugation. We conclude that the activated GR exists as a homodimer when unbound as well as when bound to DNA.

The glucocorticoid receptor (GR) is a soluble intracellular protein which is able to bind sequence specifically to DNA and thereby modulate gene expression of a nearby promoter (for review see Ref. 1). GR belongs to a family of ligand-modulated transcription factors which have many features in common (2). The DNA sequences which serve as binding sites for GR are only occupied in the presence of hormone as shown by in vivo footprinting (3). The concomitant appearance of a hormone-dependent DNase I-hypersensitive site in the area of the cognate DNA site supports this concept (4).

Recent reports have described the analysis of steroid receptor mutants in transfected tissue culture cells. These experiments have defined functional domains in GR and other steroid receptors (5–7) and confirmed the presence of a hormone and a DNA-binding domain which were previously identified by use of limited proteolysis (8).

The interaction of the hormone ligand with the receptor initiates an event usually referred to as transformation or activation. The nonliganded steroid receptor monomer (9, 10) seems to be associated with a dimer of a 90-kDa heat shock protein (10, 11). The ligand-induced activation probably involves dissociation of GR from the 90-kDa heat shock protein dimer (12) and an apparent accumulation of GR in the cell nucleus, perhaps as a result of hormone-induced nuclear targeting (13). The ability of GR to bind to DNA is acquired (14), and transcriptional initiation is induced (15) or repressed (16).

Below we present data showing that the purified activated GR is a homodimer in the presence as well as in the absence of DNA. This finding may have important functional implications for the activation process which thus might involve a dimerization step of two 87.5-kDa (17) GR polypeptides in order to form the activated GR.

**MATERIALS AND METHODS**

**Buffers**—The buffers used were all derived from ETG buffer (20 mM Tris-HCl (pH 7.8), 1 mM Na₂EDTA, 10% (w/v) glycerol). Additional components are indicated in each case.

**GR Preparation**—GR-[\(^3H\)]triamcinolone acetonide complex was purified from rat livers as described previously (18) except that the last DEAE-Sepharose step was replaced by chromatography on a 5-m fast protein liquid chromatography Mono-Q™ column (Pharmacia LKB Biotechnology Inc.) (19). GR-containing fractions, 60–95% pure, were pooled. Insulin, dithiothreitol, and glycerol were added to final concentrations of 0.1 mg/ml, 10 mM, and 40% (w/v), respectively, and stored at -113 °C. GR was quantitated assuming one bound molecule of [\(^3H\)]triamcinolone acetonide (2.25 Ci/mmole specific radioactivity) per 87.5 kilodaltons of polypeptide (17). The validity of this assumption was tested by quantitation of tritium radioactivity, protein (20), and relative purity of a GR preparation, the last by use of a Joyce-Loebl microdensitometer which in one experiment indicated a 60% purity on a silver-stained sodium dodecyl sulfate-polyacrylamide gel. 70 μl of this GR preparation contained 2.8 ± 0.16 μg of protein (mean ± S.D., n = 3) and 1.84 μg of GR protein according to the radioactivity analysis. This suggests a purity of about 65% which is in reasonable agreement with the purity estimated by densitometry. It demonstrates that the quantitation of GR protein by use of the \(^3H\)-ligand is valid.

**Construction and Preparation of DNA Fragments**—A plasmid (pMTV -191/-163) containing 17 copies of a 35-bp direct repeat, each harboring a cognate GR site and an XhoI site, was constructed as follows. Two oligonucleotides coding for the -191/-163 sequence of the MTV long terminal repeat were synthesized on a Pharmacia Gene Assembler™. This sequence includes a strong GR-binding site as shown by a DNase I footprint covering a 24-bp segment -189/-166 (21). The two 35-mer oligonucleotides contained 35 bp of complementary sequence that would form double-stranded DNA with a 6-bp 5'-overhang of asymmetric sequence (position in MTV -180/-175). This allows multimers of direct repeats to be formed during oligomer renaturation and ligation (22). The concatamers were partially restricted with XhoI and separated according to size on low

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The abbreviations used are: GR, glucocorticoid receptor; MTV, murine mammary tumor virus; bp, base pair.
melting agarose (23) followed by ligation with SalI restricted and phosphatase-pGEM-1" (24). The plasmid pMTV -191/-163 was isolated and checked by sequencing (25) and DNase I footprinting with GR which resulted in 17 GR-induced footprints as expected (cf. Fig. 6A). The 640-bp EcoRI/HindIII fragment, labeled at the EcoRI site, was polynucleotide kinase and [γ-32P]ATP (24), was used for quantitative DNase I footprinting (26). The 35-bp XhoI fragment was labeled using the same procedure. It was important to dilute the labeled ATP to be used for end labeling to lower specific activity in order to generate homogeneously labeled DNA fragments of a specific activity suitable for stoichiometric analysis (10–30 cpm/fmol). Labeled DNA fragments were purified by electrophoresis in non-denaturing polyacrylamide gel and electroeluted onto a Schleicher & Schuell DEAE membrane according to recommendations by the manufacturer. The DNA concentration of the purified fragments to be used for stoichiometry measurements was determined by spectrophotometry at 260 nm, assuming that 1 absorbance unit = 50 μg/ml DNA. Its radioactivity was then calculated by liquid scintillation counting (see below).

**Glycerol Gradient Centrifugation**—DNA binding was performed in a total volume of 100 μl of ETG buffer containing 5 mM DL-dithiothreitol, 75 mM NaCl, and 100 μg/ml porcine insulin. GR and/or DNA was incubated for 10 min at 0 °C followed by a 5-min incubation at 25 °C. The samples were kept on ice 10–20 min prior to centrifugation. 4 ml of 12–25% (w/v) glycerol gradients were generated from two solutions by diffusion with the tubes in a horizontal position. They contained ETG buffer with 5 mM DL-dithiothreitol, 100 μg/ml insulin, and 25 mM NaCl. Samples were centrifuged for 18–22 h at 0 °C and 55,000 rpm in a Sorval TST 60.4 rotor followed by fractionation from the bottom of the tube into scintillation vials. 4 ml of Emulsifier-Safe™ (Packard Instrument Co.) was added and followed by radioactivity analysis. The approximate sedimentation coefficients for GR and DNA were determined by use of a 186-bp 32P-labeled DNA fragment (SalI/EcoRI fragment of p5'139) as an external standard having a sedimentation velocity of 6.5 S (18).

**Radioactivity Analysis**—Tritium and 32P were quantitated in fractions after glycerol gradient centrifugation and gel filtration chromatography using a Packard Tri-Carb™ 300. An efficiency of 31% for tritium and 100% for 32P was determined by the use of tritiated toluene (Du Pont) and 32P-labeled DNA as internal standards. The GR and DNA in the peak fraction were estimated by subtracting the radioactivity background, correcting for spillover of 32P into the γ channel (1.2–2.5%), and finally quench correcting by use of internal standards. Figures showing radioactivity diagrams are corrected for spillover. Correction was made for radioactive decay of the labeled DNA assuming a 32P half-life of 14.3 days.

**Quantitative DNase I Footprinting**—DNase I footprinting was performed as described previously (18), and the recommendations concerning quantitative footprinting were followed (26). However, we also determined the amount of DNA in the reactions. Autoradiograms were obtained using Fuji RX film which was exposed without screen. Several exposures were made in order to obtain film density which was directly proportional to the radioactivity. Two-dimensional densitometry of autoradiograms (cf. Fig. 6A) was conducted using a Phosphorimager system P-1000 (Optimics International Inc.) connected to a VAX 11/750 computer (Digital Equipment Corp., Marlboro, MA). The autoradiogram was thus digitalized, and indicated bands (cf. Fig. 6A) were selected for densitometry using a Spectragraphics 1500 display.

**RESULTS**

**Stoichiometry of the GR-DNA Complex**—Purified GR containing the synthetic glucocorticoid [3H]triamcinolone acetonide was used for DNA-binding experiments. One GR polypeptide having a molecular mass of 87.5 kDa harbors one ligand-binding site (see "Materials and Methods") thus allowing the quantitation of the hormone receptor complex by analysis of triitated hormone. A 32P-labeled 35-bp DNA fragment harboring a strong GR-binding site, -189/-166 in the MTV promoter, was used for formation of the GR-DNA complex. It harbors the conserved GR-associated motif TGTTCTC arranged in an imperfect palindrome (21).

The GR and DNA were incubated at 75 mM NaCl and subsequently analyzed by glycerol gradient centrifugation. Fig. 1A shows that GR in the absence of DNA sediments as...
one main peak with an additional small peak at the frontal base. The large and small peaks have sedimentation coefficients of 4 and 6 S, respectively. The tritium at the top of the gradient consists of dissociated hormone ligand. The small 6 S peak was a reproducible finding which routinely constituted 5–10% of total bound hormone. The fraction of bound hormone in these experiments varied between 40 and 60% in different GR preparations. Fig. 1B shows that the 35-bp DNA fragment sediments as a 3 S peak. Fig. 1, C–E, shows the result of incubating a constant amount of GR with an increasing amount of DNA. The GR-DNA complex forms a 6 S peak which thus sediments significantly faster than the main GR peak and the DNA peak alone. This allows the quantitation of tritiated hormone (GR) and DNA in the peak fraction of the 6 S complex without interference from free GR or free DNA. The molar ratios of hormone to DNA in the 6 S peak fraction of gradients C, D, and E are 2.0, 2.2, and 2.1, respectively. The DNA-GR interaction is clearly saturable, and all of the hormone receptor complex, detected as a tritium peak, can be shifted from 4 to 6 S by the addition of DNA. The results of six different gradients from three different experiments show that 2.17 ± 0.09 (mean ± S.D., n = 6) mol of hormone ligand is cosedimenting with each mol of 35-bp XhoI DNA fragment. This indicates that two polypeptides of 87.5-kDa GR interact with each cognate DNA site.

The observed 4 to 6 S shift of GR was dependent on sequence-specific DNA binding. This shift was not observed when GR was incubated with either of two nonspecific DNA fragments containing 42 and 45 bp, respectively. On the other hand, an 85-bp DNA fragment harboring identical ends as the 45-bp nonspecific fragment and a cognate GR site was able to shift GR from 4 to 6 S (data not shown).

Effects of Ligand on the Formation of a GR-DNA Complex—As already mentioned, 40–60% of the [3H]triamcinolone acetonide ligand was bound to GR, and the rest was recovered at the top of the gradient. In our experience only insignificant amounts of ligand dissociate from GR at 0 °C, and the main portion of free ligand has dissociated during the last step of GR purification. We were concerned as to whether the fraction of GR which had lost its ligand would still be able to bind DNA. If this occurred it would introduce an error into our stoichiometric analysis which is based on a 1:1 relationship of GR and the tritiated ligand. For this reason we designed experiments where we could monitor the dissociation of ligand and correlate the remaining portion of the ligand-receptor complex to DNA-binding activity. GR in the presence or absence of the 35-bp XhoI DNA fragment was exposed to 30 °C which resulted in a time-dependent dissociation of the GR-DNA complex. Glycerol gradient analysis showed a decreasing 6 S peak with increasing time of incubation at 30 °C but with a constant ratio of tritium and 32P in the 6 S peak with a molar ratio of about 2:1 (not shown). However, the tritium ligand which was lost from the GR peak was only partly recovered (5–10%) at the top of the gradient, while the rest formed precipitates at the bottom of the tube (data not shown). Consequently, this experiment did not tell us whether the loss of ligand or the formation of the precipitate was the primary cause for the loss of DNA binding.

In order to suppress precipitation of GR during temperature-induced ligand dissociation we introduced the detergent Triton X-100 into the incubation buffer. GR was incubated at 30 °C for 0–50 min with buffer containing 0.002% (w/v) Triton X-100 followed by cooling on ice. [32P]DNA was added, and the incubation was prolonged for 30 min at 0 °C. Glycerol gradient centrifugation (Fig. 2, A–D) demonstrated a gradual dissociation of hormone ligand and a concomitant loss of DNA binding as seen by the redistribution of [32P]DNA from the 6 to 3 S region. The GR to DNA ratios in the 6 S peak fraction in Fig. 2, A, B, and C were 2.1, 2.0, and 2.1, respectively. Furthermore, the presence of 0.002% detergent during the ligand dissociation resulted in 50–68% of the dissociated ligand being recovered at the top of the gradient. This result strongly correlates the loss of bound ligand with the loss of DNA binding. We conclude that DNA binding is indeed
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ligand-dependent under these conditions and that unliganded GR does not influence our stoichiometric analysis.

We then tested whether the ligand was required for maintaining the GR-DNA complex. After preincubation of GR and DNA, increasing concentrations (0, 0.1, and 0.5%) of Triton X-100 were added. We had previously established that the exposure of GR to 0.05% Triton X-100 causes complete ligand dissociation, while 0.002% of the detergent had no effect (not shown). Fig. 3 demonstrates that the preformed GR-DNA complex is also stable in the absence of ligand which was dissociated in 0.1% Triton X-100. The 6 S 32P peak representing the GR-DNA complex has the same amplitude in the presence as well as in the absence of any tritiated ligand (Fig. 3, A and B). The same result was obtained with 0.5% detergent (not shown).

Glutaraldehyde Cross-linking of GR—As shown in Fig. 4A, glycerol gradient centrifugation analysis of GR results in a main 4 S peak and a small peak around 6 S. This suggests the presence of a larger but unstable GR complex. In order to stabilize any such complex we exposed GR to increasing concentrations of glutaraldehyde (0–1% (w/v)) prior to glycerol gradient centrifugation. High concentrations of glutaraldehyde resulted in dissociation of the hormone ligand and/or aggregation of GR. However, low concentrations (0.01–0.1%) of the cross-linking agent resulted in a switch in the sedimentation rate of GR from 4 to about 6 S (compare Fig. 4, A and B), thus resulting in the same sedimentation rate as seen with the GR-DNA complex (compare Fig. 4, B and C). The addition of 0.01% glutaraldehyde to the preformed GR-DNA complex resulted in dissociation of the DNA, leaving the 6 S GR complex intact (compare Fig. 4, C and D). 50 mM glycine in the buffer completely quenched the glutaraldehyde

The components were preincubated for 10 min at 0°C and 5 min at 25°C. Then a 0.10-volume of 1 % Triton X-100 was added to B and the same volume of water to A, and the incubation was continued for 30 min at 0°C.

Fig. 3. Glycerol gradient centrifugation analysis of 1.07 pmol of GR (filled squares) and 1.1 pmol of 32P-labeled XhoI DNA (empty squares). The components were preincubated for 10 min at 0°C and 5 min at 25°C. Then a 0.10-volume of 1% Triton X-100 was added to B and the same volume of water to A, and the incubation was continued for 30 min at 0°C.

Fig. 4. Glutaraldehyde cross-linking of GR analyzed by glycerol gradient centrifugation. A and B, 0.86 pmol of GR was incubated for 5 min at 25°C followed by the addition of a 0.01-volume of 0.1% (v/v) glutaraldehyde to B and water to A followed by another 25°C incubation for 5 min. C and D represent incubations containing 0.86 pmol of GR and about 0.3 pmol of the 32P-labeled 35-bp XhoI DNA fragment which was kept at 25°C for 5 min followed by the addition of a 0.10-volume of 0.1% (v/v) glutaraldehyde to D and water to C and another 5-min incubation at 25°C. GR, filled squares; DNA, empty squares.
effect (not shown). This allowed us to test whether high salt
would inhibit the glutaraldehyde-induced 4 to 6 S switch. GR
was adjusted to a final concentration of 1 M NaCl and preincu-
bated for 10 min at 0 °C followed by the addition of glutara-
aldehyde to 0.01% (w/v) and an additional incubation at 25 °C
for 5 min. This was then followed by the addition of glycine
to 50 mM in order to inactivate any remaining glutaraldehyde
prior to glycerol gradient centrifugation. The 4 to 6 S shift
was still seen (not shown). The identical glutaraldehyde-
duced 4 to 6 S shift was observed in GR preparations of
various purities (60–95%) and also with GR from different
regions of the GR-containing peak eluted from the last puri-
fication step, a Mono-Q™ column (not shown). We conclude
that the observed phenomenon represents the stabilization of
a GR oligomer by glutaraldehyde cross-linking and that this
GR oligomer is stable in 1 M NaCl.

Gel filtration experiments were conducted in order to in-
vestigate the effect on the complex size by glutaraldehyde
cross-linking. Glutaraldehyde-treated GR was distributed
as a complex of slightly larger size than γ-globulin (158 kDa).
The same distribution was obtained in 150 mM NaCl (Fig. 5)
and in 25 mM NaCl (not shown). GR net exposed to glu-
taraldehyde was distributed as a plateau of undefined size when
chromatographed in 25 and 50 mM NaCl (not shown). The
untreated GR oligomer was, however, stable during gel fil-
tration in 150 mM NaCl and to our surprise was distributed
exactly as the glutaraldehyde-treated GR (Fig. 5), indicating
that the 6 S GR complex is stable during gel filtration without
prior glutaraldehyde treatment. We did not obtain an in-
creased amount of 6 S complex by increasing the NaCl con-
centration in the absence of glutaraldehyde during glycerol
gradient centrifugation.

Quantitative DNase I Footprinting—The stoichiometry of
the GR-DNA complex was also measured by DNase I foot-
print ing (21). The 640-bp EcoRI/HindIII fragment of the
PMTV −191/−163 (22) was 5′-end labeled at the EcoRI site and
incubated with increasing amounts of GR and a constant
amount of DNA (Fig. 6A). The development of a receptor
footprint was utilized in monitoring the binding of GR to
DNA. The level of GR protection was quantitated by two-
dimensional densitometry of the autoradiogram after electro-
phoresis on 6% polyacrylamide sequencing gel. A reference
band, not protected by GR, was used for correction of varia-
tions in DNase I treatment and amounts of radioactivity
applied on the gel (20). The data were used to construct
saturation diagrams (cf. Fig. 6B). The displayed experiment
involved three different GR titrations with 0.9, 1.9, and 3.9
nm concentration of GR-binding DNA sites, respectively. The
concentration of GR required for 50% saturation of the DNA
sites was determined from these diagrams. We assumed a
constant fraction of added GR to be active in DNA binding
(activity factor a = active GR/total GR). This fraction was
determined by glycerol gradient centrifugation analysis of the
GR-DNA complex in excess of DNA as described in Fig. 1, D
and E. An activity factor (fraction of total titrated ligand
which formed a 6 S GR-DNA complex) of 0.48 ± 0.01 (mean
± S.D., n = 3) was thus established and was used for calcu-
lation of the concentration of active GR since

\[
[\text{GR}_{\text{total}}] \times a = [\text{GR}_{\text{active}}]
\]

The concentration of free active GR ([GR_{\text{active}}]) at 50% satu-
ration of the DNA sites corresponds to the dissociation con-
stant and is identical for each titration series. From this
follows that [GR_{\text{active}}], which has to be added to reach 50% saturation,
is the sum of free active GR and the GR which is
bound by DNA. Bound GR at half-maximal saturation is
calculated from the product of the concentration of GR-DNA
complex (half the input DNA at 50% saturation) and the
stoichiometry factor F (the number of GR entities interacting
each DNA site). This is described by the function

\[
[\text{GR}_{\text{active}}] = [\text{GR}_{\text{total}}] + [\text{GR}_{\text{DNA}}] \times F
\]

The result from the titration series (Fig. 6B) was used to plot
this function (Fig. 6B, inset). The stoichiometry factor F
is given by the slope of the line and was found to be 2.11, which
is in agreement with the stoichiometry determined by glycerol
gradient centrifugation. The concentration of free GR at 50% saturation
(the dissociation constant K_0) is given by the
intercept on the ordinate and is 1.37 nm. This is in the same
order of affinity as was observed for the −189/−166 GR-
binding site in the wild type MTV promoter.2

DISCUSSION

The stoichiometry of the GR-DNA complex was previously
analyzed by techniques similar to those above but with the
difference that another DNA fragment, the 186-bp StuI/EcoRI
fragment of the pS139 and derivatives thereof was used (18).
The previous report concluded that only one hormone ligand
(one 87.5 GR polypeptide) was bound to each DNA site. The
discrepancy with the results presented above is probably due

2 T. Perlmann, P. Eriksson, and Ö. Wrange, unpublished observa-
stions.
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FIG. 6. A, autoradiogram of the DNase I footprints generated by the titration of 0.1 pmol of specific DNA with increasing amounts of GR (see "Results"). The reference band and the protected band used for two-dimensional densitometry are indicated. B, DNA binding of GR as a function of added GR, titration with 0.09 pmol of DNA sites (filled squares), 0.19 pmol of DNA sites (empty squares), and 0.39 pmol of DNA sites (filled triangles). Inset, plot for determination of the stoichiometry factor $F$ and the dissociation constant ($K_d$) by least squares linear regression analysis (correlation coefficient $r = 0.99$).

to the choice of a DNA fragment for stoichiometric analysis in the previous study. The previously used p5′139-derived SstI/EcoRI fragment contains MTV promoter sequences from -264 to -107 relative to the cap site. This segment of DNA harbors three (21) or two (28) GR-induced DNase I footprints according to two different reports. The middle footprint, called "1.4" and covering the -159/-135 segment, has a less distinct appearance (19, 21) and lacks regulatory capacity according to linker-scanning mutagenesis (29). Regardless of whether or not there is a distinct GR-binding site at 1.4 it is clearly preferable to use one strong and well defined binding site on a small DNA fragment for stoichiometric analysis.

In this report synthetic oligonucleotides were used for the construction of a 35-bp DNA fragment harboring a strong GR-binding site detectable as a distinct 24-bp footprint covering the -189/-166 segment in MTV (21). The formation of a GR-DNA complex with a sedimentation coefficient of 6 S, well separated from each of the free components, allowed the quantitation of each component in the 6 S peak fraction. The use of the tritium ligand as a tag for receptor quantitation was valid for several reasons. (i) The purified GR preparation contained one hormone per 87.5-kDa receptor peptide according to correlation of purity determined either by densitometry or by quantitation of GR protein and total protein (see "Materials and Methods"). (ii) Studies involving in vitro mutagenesis (5) and affinity labeling of GR with a hormone analog (27) demonstrated the presence of one ligand-binding domain on the protein and one affinity-labeled amino acid, respectively. (iii) The formation of a GR-DNA complex was ligand-dependent.

DNA fragments lacking a cognate GR site were unable to generate the 6 S complex which demonstrates that this phenomenon requires a sequence-specific interaction.

The glutaraldehyde-induced 4 to 6 S shift of purified GR in the absence of DNA demonstrates that the cross-linking agent stabilizes a GR multimer of defined size. Gel filtration at low ionic strength demonstrated a glutaraldehyde-induced stabilization since untreated GR was distributed as a broad plateau. Gel filtration at high ionic strength showed no difference in the distribution of GR with or without glutaralde-
hyde treatment suggesting that the GR oligomer exists as a stable complex also in the absence of DNA. It is, however, necessary to stabilize the GR oligomer by glucaraldehyde or a specific DNA fragment during glyceral gradient centrifugation. The discrepancy between the results obtained by glyceral gradient centrifugation and gel filtration is probably caused by subtle differences in the conditions employed. However, we cannot exclude the possibility that the formation of the GR oligomer which is detectable by glyceral gradient centrifugation fails to increase the size (Stokes radius) of the complex.

We conclude that there are two 87.5-kDa receptor polypeptides in the GR-DNA complex since stoichiometric analysis of the 6 S GR-DNA complex by glyceral gradient centrifugation as well as quantitative DNase I footprinting analysis resulted in 2.17 and 2.11 hormone molecules per DNA site, respectively. Furthermore, the observed GR oligomer occurring in the absence of DNA is in fact a homodimer as demonstrated by two observations. (i) The glucaraldehyde-induced complex has the same sedimentation velocity as the specific GR-DNA complex shown to harbor two GR polypeptides. The molecular mass of the DNA component is 23.1 kDa. This is probably too small an addition in mass to the putative 175-kDa GR homodimer to affect the sedimentation rate significantly. (ii) Gel filtration shows the distribution of a complex of slightly larger size than γ-globulin (158 kDa).

The data presented above are in agreement with a report showing that the GR consensus hexanucleotide TGTTCTC organized as a perfect inverted repeat flanking three central bp will serve as a strong GR-responsive element (30). It is a common finding that DNA-binding proteins occur as homodimers and have cognate DNA sites arranged as inverted repeats (e.g. Refs. 31-33).

The rat uterine estrogen receptor was previously suggested to form a homodimer as a result of ligand-dependent thermal activation, an event that could be monitored as a 4 to 5 S shift in the sedimentation rate (34) and which resulted in a significantly increased affinity for DNA (35). In a recent report (36) the use of the gel retardation assay has shown that estrogen receptor is indeed a homodimer which is formed in a ligand-dependent manner.

If the above statement is correct, a monomer (9, 10) associated with a 90-kDa heat shock protein dimer (10, 11) in the absence of hormone ligand. The first step in the ligand-induced activation process is probably the dissociation of the GR monomer from the 90-kDa heat shock protein dimer (11, 37, 38). The second step, as suggested from the findings presented above, is a dimerization of two identical 87.5-kDa GR entities. The homodimer obtained may thus be suitable for the interaction with the palindromic DNA site.

The many structural and functional similarities among different members of the steroid receptor family (2) imply that a dimerization similar to the findings concerning estrogen receptor (36) and GR plays a role in the ligand-induced activation process in other steroid receptors as well.

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