Separation of the Hormone- and DNA-binding Sites of the Hepatic Glucocorticoid Receptor by Means of Proteolysis*

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The dexamethasone receptor complex in rat liver cytosol has a Stokes radius of 61 Å. It may be converted to smaller complexes with Stokes radii of 36 Å and 19 Å by proteolytic digestion with trypsin, a-chymotrypsin (that only gives rise to 36 Å complex), papain, or an extract of the 1,000 x g to 10,000 x g pellet of liver homogenate. The 61- and 36-Å complexes have dissociation constants of 6 to 8 x 10^-9 M and 6 to 9 x 10^-9 M, respectively, for dexamethasone. The 19-Å complex is very labile in the absence of bound ligand and therefore its affinity to dexamethasone could not be determined.

Both the 61- and 36-Å complexes are bound to nuclei and DNA-cellulose following heat activation in the presence of ligand. The 61-Å complex is eluted from a DNA-cellulose column with a linear KCI gradient at 0.11 to 0.13 M KCl, whereas the 36-Å complex is eluted at 0.15 to 0.20 M KCl.

The 19-Å complex binds neither to DNA-cellulose nor to nuclei. When nuclei or DNA-cellulose containing bound dexamethasone receptor complex were digested with trypsin and subsequently extracted or eluted in low ionic strength, the recovered dexamethasone receptor complex had a Stokes radius of approximately 19 Å.

We conclude that the glucocorticoid receptor in liver cytosol may be divided into three parts separable by proteolytic digestion: (a) the steroid-binding site (retained on the 19-Å and 36-Å receptor fragments); (b) the DNA-binding site (retained on the 36-Å, but absent from the 19-Å receptor fragment); and (c) a region with an as yet unknown function and only present on the 61-Å receptor.

In all biological systems studied so far the physiological action of steroid hormones seems to involve binding of the hormone to a specific soluble receptor protein present in the cells of the target organ (for review see Refs. 1 to 5).

The glucocorticoid receptor has been studied in several systems, such as hepatoma cells (3, 6-8), rat liver (9-11), and rabbit lung (12). The physical characteristics of the glucocorticoid receptor in these different tissues appear to be similar and also conform to the characteristics reported for other steroid hormone receptors.

In a previous paper (13) we have reported that the glucocorticoid receptor complex in rat liver cytosol has a Stokes radius of 61 Å. In contrast, the 0.4 M KCl extract of liver cell nuclei contains a steroid-receptor complex with a Stokes radius of 30 to 36 Å. The 61-Å dexamethasone receptor complex in cytosol is converted to a 36-Å complex by incubation with a hypotonic extract of a fraction of rat liver homogenate (the 1,000 x g to 10,000 x g pellet) or by limited proteolysis with trypsin. The latter treatment also results in further digestion to a complex with a Stokes radius of 19 Å (cf. Fig. 1). The trypsin-dependent decrease of the Stokes radius of the dexamethasone receptor complex from 61 Å to 36 Å and 19 Å is irreversible. The molecular weights of these complexes calculated according to Siegel and Monty (14) are 102,000, 46,000, and 19,000, respectively. The 36-Å complex formed after proteolysis of the 61-Å complex has a similar sedimentation coefficient and isoelectric point as the nuclear dexamethasone receptor complex. The nuclear form, however, has a more variable Stokes radius (30 to 36 Å). We suggest that the nuclear 30- to 36-Å complex is formed by nuclear protease digestion of the 61-Å complex.

In the present investigation we have studied the proteolytic fragmentation of the dexamethasone receptor complex using papain and a-chymotrypsin. Furthermore, we have investigated the binding of the different forms of the dexamethasone receptor complex to DNA and nuclei as well as some kinetic characteristics of dexamethasone binding to the different receptor forms.

MATERIALS AND METHODS

Steroids and Chemicals—[1,2,4,6]-H-Dexamethasone and [6,7-3H]Dexamethasone (specific radioactivity 27 Ci/mmol and 57 Ci/mmol, respectively) were purchased from New England Nuclear. The isotopic compounds were more than 98% pure after purification as described previously (13) and were stored in a concentration of 5 μM in 25% (v/v) ethanol in water at 0-2°C; aliquots of this solution were added to biological samples. Unlabeled dexamethasone, Tris, 2-mercaptoethanol (crystallized twice), 2-hydroxyethylmercaptoethanol, and papain, crystallized twice (10.8 units/mg), were obtained from Sigma. Dextran T 500, blue dextran, Sephadex G-25, G-150, and G-200 were obtained from Pharmacia. Activated charcoal, glycerol, disodium EDTA, and sodium azide were purchased from Chem. Trypsin, crystallized twice, dialyzed and lyophilized (211 units/mg), a-chymotrypsin, crystallized three times (81 units/mg), calf thymus DNA, soybean trypsin inhibitor, and lima bean trypsin inhibitor were purchased from Worthington. Cellulose Munktell 410 was obtained from Bio-Rad. Water used for prepara-
Fig. 1. Sephadex G-150 chromatography of $[^{3}H]$dexamethasone-labeled cytosol subjected to partial proteolysis with trypsin. Three pools of rat liver cytosol were incubated for 30 min at 10$^\circ$C with 0 pg ($x-x$), 0.1 pg ($O-O$), or 0.5 pg ($O-O$) of trypsin/A$^{190-210}$ nm of cytosol. Ferritin ($Fe$) and hemoglobin ($Hb$) were used as internal standards. $V_e$ signifies the exclusion volume of the column. The chromatograms from three identical Sephadex G-150 columns were superimposed on each other by use of the internal standards.

Fig. 2. Sephadex G-150 chromatography of $[^{3}H]$dexamethasone-labeled cytosol subjected to partial proteolysis with papain. Cytosol labeled with $[^{3}H]$dexamethasone (see "Materials and Methods") was incubated for 30 min at 10$^\circ$C with 0 pg ($x-x$), 0.5 pg ($O-O$), or 2 pg ($O-O$) of papain/A$^{190-210}$ nm of cytosol. Ferritin ($Fe$) and hemoglobin ($Hb$) were used as internal standards. $V_e$ signifies the exclusion volume of the column. The chromatograms from three identical Sephadex G-150 columns were superimposed on each other by use of the internal standards.
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FIG. 3. Sephadex G-150 chromatography of (A) 61-Å [3H]dexamethasone-receptor complex incubated with (○ --- ○) or without (○ — ○) trypsin and of (B) α-chymotrypsin-induced 36-Å [3H]dexamethasone-receptor complex incubated with (○ --- ○) or without (○ — ○) trypsin. Cytosol prepared in Buffer B was divided in two halves and α-chymotrypsin (0.2 μg/A280 nm of cytosol) was added to one-half. Both samples were then incubated at 10°C for 30 min. Subsequently, each receptor preparation was labeled with [3H]dexamethasone (incubation at 0°C for 1 h with 50 nM steroid). Three-milliliter aliquots were taken from each incubation mixture and trypsin (0.1 μg/A280 nm of cytosol) was added to one of the two aliquots. All four aliquots were incubated at 10°C for 30 min. Following addition of lima bean trypsin inhibitor (4 μg/A280 nm) to each incubation mixture, these were analyzed by chromatography on Sephadex G-150 as described in the legend to Fig. 1.

FIG. 4. Binding of [3H]dexamethasone to 61-Å (○ --- ○ and ○ — ○) and 36-Å (△ --- △ and △——△) receptor forms analyzed according to Scatchard. Liver homogenate was prepared in Buffer A and centrifuged for 15 min at 1000 × g. After this treatment the two samples contained 61- and 36-Å receptors, respectively (13). Each incubation mixture was centrifuged to obtain the cytosol fraction that was adjusted to a final concentration of 10% (w/v) glycerol. Eight samples were prepared with 0.4 ml of cytosol and 100 μl of a solution of [3H]dexamethasone in Buffer A. The samples contained a constant amount of [3H]dexamethasone and increasing amounts of unlabeled dexamethasone giving a final dexamethasone concentration ranging from 3.5 × 10^{-3} to 1.10^{-4} M in the different samples. After 2 h at 0°C each sample was treated with dextran-coated charcoal and assayed for radioactivity. The 61-Å receptor and 36-Å receptor preparations contained 12.5 A280-310 nm and 13.5 A280-310 nm/ml, respectively. The sizes of the complexes were checked by Sephadex G-150 chromatography. The data were plotted as described by Scatchard (18) and Chamness and McGuire (19).

RESULTS

Effects of Limited Proteolysis of Dexamethasone-Receptor Complex with Papain and α-Chymotrypsin — Since trypsin is a protease with a relatively narrow substrate specificity, we investigated whether by treatment with other proteases we could obtain fragments of the dexamethasone-receptor complex different from those by trypsin treatment. As seen in Fig. 2, small amounts of papain converted the 61-Å complex into a major complex with a Stokes radius of 36.5 Å. Larger concentrations of papain gave rise to a dexamethasone-receptor complex with a Stokes radius of 20.5 Å. Evidently treatment with papain induced proteolytic fragments of the dexamethasone-receptor complex of similar sizes and in a similar sequential manner as did treatment with trypsin (cf. Fig. 1).

Different results were obtained when α-chymotrypsin was used for limited proteolysis of the dexamethasone-receptor complex with a relatively narrow substrate specificity. We investigated whether by treatment with other proteases we could obtain fragments of the dexamethasone-receptor complex different from those by trypsin treatment. As seen in Fig. 2, small amounts of papain converted the 61-Å complex into a major complex with a Stokes radius of 36.5 Å. Larger concentrations of papain gave rise to a dexamethasone-receptor complex with a Stokes radius of 20.5 Å. Evidently treatment with papain induced proteolytic fragments of the dexamethasone-receptor complex of similar sizes and in a similar sequential manner as did treatment with trypsin (cf. Fig. 1).
complex. When added to cytosol in a concentration of 0.2 μg/A_{280-310} nm, α-chymotrypsin converted the 61-Å complex quantitatively to a dexamethasone-receptor complex with a Stokes radius of 35.0 ± 0.5 Å (mean ± S.D., n = 5) without any formation of a 19-Å complex. The size of the proteolytic fragment obtained by α-chymotrypsin treatment was not significantly different from the previously described 36-Å complex obtained by trypsin treatment (13) and will therefore also be referred to as 36-Å complex. When the concentration of α-chymotrypsin was further increased during incubation with the 61-Å complex, no smaller dexamethasone-receptor complex than the 36-Å complex was formed. One microgram of α-chymotrypsin/A_{280-310} nm caused an approximately 50% decrease in the amount of specifically bound dexamethasone but still no 19-Å complex was detected.

The 36-Å complex formed from the 61-Å dexamethasone-receptor complex following incubation with α-chymotrypsin was converted further to a 19-Å complex when digested with trypsin. When the 61-Å complex was treated with trypsin under identical conditions, considerably smaller amounts of 19-Å complex were formed (Fig. 3). The sequential digestion of the 61-Å complex to a 36-Å and a 19-Å complex demonstrated in this experiment indicates that the dexamethasone-receptor contains two regions ("61 → 36-Å complex digestion region" and "36 → 19-Å complex digestion region.")

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The initial, preferred formation of the 36-Å complex rather than the 19-Å complex following limited digestion of the 61-Å complex with trypsin or papain (cf. Figs. 1 and 2) may be due to (a) higher sensitivity of the 61 → 36-Å complex digestion region to proteolytic attack than the 36 → 19-Å complex digestion region or to (b) protection of the latter region from proteolytic attack as long as the former region is intact. The experiment documented in Fig. 3, the results of which were reproduced twice, seems to favor the latter alternative.

Following ligand binding and prior to intranuclear transfer, the dexamethasone-receptor has been claimed to undergo a conformational change ("activation") inducible by heat or high ionic strength (6, 10). Several experiments were carried out to investigate whether heat activation changed the sensitivity to proteolytic attack of the two protease-sensitive regions on the dexamethasone-receptor. In these experiments [3H]dexamethasone-labeled cytosol (containing 61-Å complex) was either activated at 20°C for 40 min or kept on ice (control).

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**Fig. 5.** Dissociation rate of [3H]dexamethasone from the 61-Å and 19-Å complexes in presence or absence of unlabeled dexamethasone. Cytosol was prepared in Buffer B and incubated for 1 h at 0°C with 50 μM [3H]dexamethasone. The labeled cytosol was divided into three pools of 5 ml each. The cytosol contained 49.7 μM A_{280-310} nm/ml. To prepare 19-Å complex, one pool was incubated with 1 μg of trypsin/A_{280-310} nm of cytosol for 30 min at 10°C and then 5 μg of soybean trypsin inhibitor/A_{280-310} nm of cytosol were added. The other two pools were incubated with 5 μg of soybean trypsin inhibitor/A_{280-310} nm or 1 μg of trypsin plus 5 μg of soybean trypsin inhibitor/A_{280-310} nm of cytosol for 30 min at 10°C. Then 2 × 2 ml from each pool were pipetted off into tubes and equilibrated for 30 min at 18°C, and then 10 μl of ethanol or 10 μl of ethanol containing 20 nmol of unlabeled dexamethasone were added to the respective 2-ml fractions of each pool. The 200-μl aliquots were taken off from each of the six incubation mixtures at indicated times and assayed for protein binding by charcoal analysis. Unspecific binding was measured as the protein-bound radioactivity remaining after incubation of an aliquot of each pool for 30 min at 40°C; all samples were cooled on ice prior to charcoal treatment. A shows Sephadex G-150 chromatography of pool I (△—△) and pool II (●—●) to check the sizes of the complexes after incubation at 10°C but prior to incubation at 18°C. Ferritin (Fe) and hemoglobin (Hb) were added as internal standards. V_s signifies the void volume. B shows the logarithm of the specifically bound radioactivity in disintegrations per min plotted as a function of time of incubation at 18°C. Pool I (19-Å complex), △—△ and △—△; pool II (61-Å complex incubated with trypsin inhibitor), ●—● and ●—●; pool III (61-Å complex incubated with trypsin and trypsin inhibitor), ■—■ and ■—■. Filled symbols signify the fractions containing [3H]dexamethasone. Open symbols signify the incubations containing a 200-fold excess of unlabeled dexamethasone (see also Table I).
Table I

<table>
<thead>
<tr>
<th>Type and treatment of dexamethasone-receptor complex</th>
<th>Apparent rate of degradation (half-life)</th>
<th>Apparent rate of dissociation (half-life)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-A complex + trypsin + trypsin inhibitor (Pool I; cf. Fig. 5)</td>
<td>2.9 *</td>
<td>*</td>
</tr>
<tr>
<td>61-A complex + trypsin inhibitor (Pool II; cf. Fig. 5)</td>
<td>160</td>
<td>3.9</td>
</tr>
<tr>
<td>61-A complex + trypsin + trypsin inhibitor (Pool II; cf. Fig. 5)</td>
<td>25 *</td>
<td>4.8 *</td>
</tr>
</tbody>
</table>

* Not possible to determine.

Table II

<table>
<thead>
<tr>
<th>Type of steroid-receptor complex</th>
<th>Radioactivity in 0.4 M KCl extract of nuclei following incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonactivated complex</td>
<td>Activated complex</td>
</tr>
<tr>
<td>61-A complex</td>
<td>14,700</td>
</tr>
<tr>
<td>36-A complex</td>
<td>28,800</td>
</tr>
<tr>
<td>19-A complex</td>
<td>13,800</td>
</tr>
</tbody>
</table>

And then subjected to limited proteolysis with trypsin, α-chymotrypsin, papain, or extract from the 1,000 x g to 10,000 x g pellet of liver homogenate. The degree of receptor activation was controlled by chromatography on DNA-cellulose (cf. below). In these experiments, activated and nonactivated (control) 61-A complex were digested in an identical manner, both qualitatively and quantitatively.

Kinetics of Binding of Dexamethasone to 61-, 36-, and 19-A Receptor Forms—Cytosol containing 36-A receptor form was prepared by hypotonic dilution of liver homogenate (see "Materials and Methods" and legend to Fig. 4). The binding of [3H]dexamethasone to the 61- and 36-A receptor forms was investigated by analysis according to Scatchard (18). The 61- and 36-A complexes had dissociation constants of 6.8 x 10^-9 M and 8.9 x 10^-9 M, respectively (means of two determinations). In these experiments, the 61-A receptor preparation also contained some 36-A receptor (10 to 30% of total receptor contents). Similar experiments were also performed when cytosol was prepared in Buffer B and when the 36-A receptor was obtained by limited α-chymotrypsin digestion. These procedures gave preparations of the 36-A receptor containing very little 61-A receptor and vice versa (cf. Fig. 3). The dissociation constants calculated for the 61- and 36-A dexamethasone-receptor complexes in these experiments were 7.8 x 10^-9 and 6.1 x 10^-9 M, respectively (means of two determinations). In all experiments, 12 to 15% of the specific binding sites for dexamethasone were lost during preparation of the 36-A receptor.

The kinetics of dexamethasone binding to the 19-A receptor could not be analyzed according to Scatchard since the ligand-binding site on the receptor has to be occupied by steroid prior to preparation of the 19-A complex by limited proteolysis. Unoccupied 19-A receptor seems to be destroyed during proteolytic digestion (13). Therefore, we compared the dissociation rates of [3H]dexamethasone from the binding sites on the 19-A and the 61-A receptors, respectively, in the presence and absence of unlabeled dexamethasone. Rat liver cytosol was incubated with [3H]dexamethasone and divided into three pools. One pool was trypsinized to produce 19-A complex, the other two served as controls. Each pool was divided into two halves and a 200-fold excess of unlabeled dexamethasone was added to one of the halves. Each sample was kept at 18 ± 1°C and aliquots were taken for charcoal treatment after varying periods of time. By chromatography on Sephadex G-150 the sizes of the two complexes were found to be 19.8-A (calibrated column) and approximately 61-A (Fig. 5A), respectively. The results given in Fig. 5B and Table I indicate that the 61-A complex is considerably more stable (about 30-fold) than the 19-A complex under the conditions used. Insufficient inactivation of trypsin by a 5-fold excess of soybean trypsin inhibitor could not alone explain this difference in stability, since the rate of degradation of the 61-A complex only increased about 6-fold in the presence of trypsin plus trypsin inhibitor (cf. Fig. 5B). The rate of dissociation of the 61-A [3H]dexamethasone-receptor complex was similar in the presence of trypsin inhibitor (t1/2 = 4.8 h) or trypsin + trypsin inhibitor (t1/2 = 3.9 h) (Fig. 5B and Table I). The rate of disappearance of the 19-A [3H]dexamethasone-receptor complex did not increase significantly in the presence of an excess of unlabeled ligand indicating either that the unoccupied 19-A receptor-fragment degraded rapidly and before reassociation with [3H]dexamethasone or that it could not reassociate with its ligand for other reasons. If the latter alternative is the correct explanation, the high apparent rate of degradation of the 19-A complex (t1/2 = 2.9 h) may mainly reflect the dissociation of [3H]dexamethasone from the receptor and may not necessarily indicate a rapid proteolysis of the receptor protein.

Affinity of Dexamethasone-Receptor Complexes with Different Stokes Radii to Purified Nuclei—As reported by Hig-
Following incubation of [3H]dexamethasone-labeled cytosol with purified nuclei, the nuclear pellet was trypsinized and extractable by limited trypsin digestion in low ionic strength. Extracted stepwise in low ionic strength and in 0.4 M KCl (O-O). Cytosol was prepared in Buffer A, labeled with [3H]dexamethasone, and heat-activated (40 min at 20°). Cytosol, 14 ml, was incubated for 1 h at 0° with 9.4 x 10⁸ freshly purified rat liver nuclei. After incubation the nuclei were sedimented by centrifugation at 10,000 x g for 15 min and suspended in 0.88 M sucrose, 1.5 mM CaCl₂, 1 mM MgSO₄, 10 mM Tris/HCl, pH 7.4, and washed by sedimentation through 10 ml of 2.2 M sucrose at 56,000 x g for 30 min (13). The nuclei were resuspended in 4 ml of 0.88 M sucrose and an aliquot was taken off and extracted with ethanol for measurement of radioactivity. The total nuclear pellet contained 1.74 x 10⁹ dpm. The suspended nuclei were divided into two fractions. Following addition of 10 μg of trypsin to one fraction, both samples were incubated for 30 min at 10°. The incubation mixtures were cooled on ice and 40 μg of soybean trypsin inhibitor were added to both samples that were subsequently centrifuged at 8,000 x g for 5 min. The supernatant of the trypsinized nuclei contained 2.99 x 10⁸ dpm (x- - x) and the supernatant of the control nuclei 4.3 x 10⁸ dpm. Both nuclear pellets were then extracted with 3 ml of Buffer A containing 0.4 M KCl for 40 min at 0° and centrifuged at 10,000 x g for 15 min. The supernatants of the trypsin-treated nuclei (O-O) and the control nuclei (O-O) contained 2.79 x 10⁹ dpm and 3.56 x 10⁹ dpm, respectively. Ferritin (Fe) and hemoglobin (Hb) were added as internal standards and the samples were analyzed on Sephadex G-150 columns. V₀ indicates the void volume of the columns. The chromatograms have been superimposed upon each other with the help of the internal standards.
the dexamethasone-receptor complex becomes activated during the gel filtration step and therefore no accurate comparison between Stokes radius of activated and nonactivated receptor could be done in these experiments.

To investigate the affinity of the 61-, 36-, and 19-A dexamethasone-receptor complexes to DNA, a [3H]dexamethasone-labeled cytosol preparation was subjected to limited trypsin digestion and applied on a DNA-cellulose column containing double-stranded DNA. When the column was eluted with a linear KCl gradient, a double peak appeared at 0.13 and 0.18 M KCl, respectively (Fig. 7). Gel filtration showed that the Stokes radii of these dexamethasone-receptor complexes were 57 and 30 Å, respectively (Fig. 8). The 61- and 36-A complexes were also prepared separately and chromatographed on DNA-cellulose separately. The 61-A complex was eluted at 0.11 to 0.13 M KCl as a single peak; subsequent gel filtration indicated a Stokes radius of 50 to 60 Å (mean value 56 Å). The 36-A complex gave a single peak at 0.15 to 0.20 M KCl, the Stokes radius of which was determined to approximately 30 to 32 Å (Table III). Similar results were obtained when the 36-A complex was prepared by limited digestion with trypsin or α-chymotrypsin or by dilution of the 1000 × g liver homogenate supernatant with hypotonic buffer. When the 19-A complex was chromatographed on DNA-cellulose, no [3H]dexamethasone was retained in the column. Subsequent gel filtration on Sephadex G-150 showed that the radioactivity eluted from the DNA-cellulose column still chromatographed like a 19-A complex. This experiment indicates that the 19-A complex does not bind to DNA.

Experiments were then performed where dexamethasone-receptor complex was first adsorbed to a DNA-cellulose column. Subsequently the column was equilibrated with a buffer of low ionic strength containing trypsin. After incubation for 30 min at 10° the DNA-cellulose column was eluted stepwise with Buffer A and Buffer A containing 0.3 M KCl. An aliquot of each eluate was chromatographed on Sephadex columns for determination of Stokes radius (Fig. 9). The low salt eluate contained a dexamethasone-receptor complex with a Stokes radius of 19 to 22 Å (range of three experiments). The high salt eluate showed a more complex chromatogram with some radioactivity in the void volume and two radioactivity peaks with Stokes radii of 55 and 32 Å, respectively (Fig. 9). In control experiments where trypsin was not added prior to incubation of the DNA-cellulose column at 10° for 30 min insignificant amounts of radioactivity were eluted with the buffer of low ionic strength, whereas practically all radioactivity was recovered in the 0.3 M KCl eluate; Sephadex G-150 chromatography of the eluted complex indicated a Stokes radius of 50 to 60 Å.

Heat activation could occur before or after proteolytic conversion of 61-A to 36-A complex with identical increase in degree of DNA retention. Furthermore, as shown previously by Kalimi et al. (20), the glucocorticoid receptor may be activated and bound to DNA-cellulose only in the presence of...
Table III

Binding of dexamethasone-receptor complexes of different Stokes radius to DNA-cellulose

Dexamethasone-receptor complexes of different Stokes radius were prepared either by hypotonic dilution of the 1000 × g supernatant of liver homogenate (H.D.) or by trypsin (T) or α-chymotrypsin (α-CT) digestion (see "Materials and Methods"). A typical experiment (No. 8) is described and documented in Figs. 7 and 8. The Stokes radius of complexes after DNA-cellulose chromatography was determined by Sephadex gel chromatography. The Stokes radius is given as a range if the Sephadex column was not calibrated; in these cases the approximate Stokes radius determined by use of ferritin and hemoglobin (see "Materials and Methods").

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Size(s) of complex(es) applied on DNA-cellulose; method of receptor proteolysis is given within parentheses</th>
<th>Nature of DNA bound to cellulosea</th>
<th>Concentration of KCl at the radioactive peak(s) eluted from DNA-cellulose</th>
<th>Stokes radius of complexes after DNA-cellulose chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>S.S.</td>
<td>0.11</td>
<td>0.11-0.13 M KCl 0.15-0.18 M KCl 30-36</td>
</tr>
<tr>
<td>2</td>
<td>61; 36; 19 (T)</td>
<td>S.S.</td>
<td>0.12; 0.16</td>
<td>50-61 31</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>S.S.</td>
<td>0.11</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>61; 36; 19 (H.D.)</td>
<td>S.S.</td>
<td>0.12; 0.15</td>
<td>ndb</td>
</tr>
<tr>
<td>5</td>
<td>61; 36; 19 (H.D.)</td>
<td>S.S.</td>
<td>0.11; 0.15</td>
<td>nd</td>
</tr>
<tr>
<td>6</td>
<td>36; 19 (T)</td>
<td>D.S.</td>
<td>; 0.15</td>
<td>nd</td>
</tr>
<tr>
<td>7</td>
<td>nd (H.D.)</td>
<td>D.S.</td>
<td>; 0.17</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>61; 36; 19 (T)</td>
<td>D.S.</td>
<td>0.13; 0.18</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td>36 (α-CT)</td>
<td>D.S.</td>
<td>; 0.19</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>36 (α-CT)</td>
<td>D.S.</td>
<td>; 0.20</td>
<td>nd</td>
</tr>
</tbody>
</table>

a S.S., denatured; D.S., native.
b nd, not determined.

Figure 9. Sephadex G-150 (A) and G-200 (B) chromatography of dexamethasone-receptor complex bound to DNA-cellulose and trypsinized and eluted stepwise in low ionic strength (A) and 0.3 M KCl (B). Ten milliliters of cytosol were incubated with 3H-dexamethasone, heat-activated by incubation at 25°C for 30 min, and subjected to gel filtration on a 100-ml Sephadex G-25 column equilibrated with Buffer A. The protein-bound fraction containing 2.9 × 10^6 dpm was applied on a 10-ml DNA-cellulose column (native DNA) that was washed with Buffer A to remove unbound radioactivity (1.01 × 10^6 dpm). Then 10 ml of Buffer A containing 4 µg of trypsin were applied to the DNA-cellulose column, the flow was stopped and the column incubated in a water bath at 30°C for 30 min at 106 ligand. As shown in Table IV, this is true both for the 61- and the 36-Å receptor forms.

Discussion

As shown in this and a previous (13) study, the hepatic dexamethasone-receptor complex with a Stokes radius of approximately 61 Å may be proteolytically digested with trypsin, α-chymotrypsin, papain, and rat liver protease to form two steroid-binding fragments with Stokes radii of approximately 36 and 19 Å, respectively. Similar fragments were formed irrespective of the protease used, except following α-chymotrypsin digestion, where only 36-Å dexamethasone-receptor complex was formed. Since the different proteases used in this study have different substrate specificities they will most probably not give rise to completely identical receptor fragments. On the other hand, the two regions sensitive to proteolytic attack may well consist of several amino acid residues; hydrolysis of any of the peptide bonds in one region would thus create a receptor fragment of similar size. The 36-Å dexamethasone-receptor complex has an increased affinity to DNA-cellulose and the same range of dissociation constant for dexamethasone when compared to the 61-Å dexamethasone-receptor complex. The former characteristic of the 36-Å complex may be explained by the reduced size of the protein and/or a sterical change of the receptor molecule, allowing a closer contact between DNA and the DNA-binding
Liver cytosol was prepared in Buffer B and incubated for 30 min at 10° in the presence or absence of 0.2 μg of α-chymotrypsin/ A 19-A complex of cytosol. Following incubation, 4 μg of lima bean trypsin inhibitor/ A 19-A complex was added to both incubation mixtures. The proteolyzed and nonproteolyzed preparations were then divided into two parts, which were incubated for 20 min at 20° (activation) or 0°, respectively. Aliquots were removed from each sample for determination of specific dexamethasone-binding sites. [3H]Dexamethasone was then added to all four preparations to yield a final concentration of 10 nM. Following incubation on ice for 30 min, the samples previously not exposed to heat were kept at 20° for 20 min; aliquots of these two samples were then chromatographed on Sephadex G-150 for determination of Stokes radius. Of each of the four samples, 2 ml were diluted with 2 ml of Buffer A and applied on 5 ml of DNA-cellulose columns. These were first eluted with Buffer A and then the retained dexamethasone-receptor complex was eluted with Buffer A containing 0.4 M KCl.

<table>
<thead>
<tr>
<th>Complex and treatment</th>
<th>[3H]Dexamethasone-receptor complex applied on DNA-cellulose</th>
<th>[3H]Dexamethasone-receptor complex retained on DNA-cellulose</th>
<th>[3H]Dexamethasone-receptor complex applied on DNA-cellulose</th>
<th>[3H]Dexamethasone-receptor complex retained on DNA-cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>61-A complex</td>
<td>dpm</td>
<td>dpm</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Heated before labeling</td>
<td>1,220,000</td>
<td>70,000</td>
<td>5.7</td>
<td>5.7</td>
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<tr>
<td>Labeled before heating</td>
<td>1,280,000</td>
<td>602,000</td>
<td>47.0</td>
<td>47.0</td>
</tr>
<tr>
<td>36-A complex</td>
<td></td>
<td></td>
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<tr>
<td>Heated before labeling</td>
<td>1,180,000</td>
<td>64,700</td>
<td>5.5</td>
<td>5.5</td>
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<tr>
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<td>1,270,000</td>
<td>592,000</td>
<td>46.6</td>
<td>46.6</td>
</tr>
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</table>

In conclusion, the present investigation has demonstrated that the hepatic dexamethasone-receptor molecule contains three structurally distinct parts separated by regions sensitive to limited proteolytic digestion. One of these parts contains the DNA-binding site, another part contains the hormone-binding site and a third part has yet unknown functions.

Acknowledgment - The precise technical assistance of Miss Ingalill Ramberg is gratefully acknowledged.

REFERENCES
Functional Regions of Glucocorticoid Receptor

Separation of the hormone- and DNA-binding sites of the hepatic glucocorticoid receptor by means of proteolysis.
O Wrange and J A Gustafsson