Protein-Protein Contacts in the Glucocorticoid Receptor Homodimer Influence Its DNA Binding Properties*

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We have investigated the influence of the N-terminal domain of the 94-kDa glucocorticoid receptor on the DNA:receptor interaction. An a-chymotrypsin-induced 39-kDa receptor fragment, containing the hormone and DNA binding domains, binds DNA with a reduced specificity compared to the intact 94-kDa receptor. Various footprinting assays did not reveal any qualitative differences when comparing the DNA contact points made by the two different receptor entities. Like the intact receptor, the 39-kDa receptor fragment binds as a dimer to DNA. Glutaraldehyde cross-linking demonstrated a difference in the protein:protein contacts of the two homodimers. Furthermore, the dimeric 94-kDa receptor did not recognize a half-DNA site, while the dissociated 94-kDa receptor dimer and the dimeric 39-kDa receptor fragment allowed binding to such a site. These results suggest that the loss of the N-terminal domain of the receptor affects the steric arrangement and/or rigidity of the two DNA binding domains of the receptor homodimer, resulting in a decreased DNA binding specificity of the 39-kDa receptor fragment.

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1The abbreviations used are: GR, glucocorticoid receptor; GRE, glucocorticoid response elements; bp, base pair(s); SDS, sodium dodecyl sulfate; DTT, dithiothreitol; MMTV, mouse mammary tumor virus.
strated that the cleavage site is located between amino acids 409 and 414 (Carlestedt-Duke et al., 1987); this leaves the entire ligand and DNA binding domains intact, and at least 26 amino acids of the N-terminal domain (see above). The fact that the 39-kDa a-chymotrypsin-induced receptor fragment shows discrete differences in DNA binding affinity implies that the N-terminal part either has one or more additional DNA contacts, or that this domain is indirectly involved in how GR recognizes and interacts with a specific DNA segment.

The 39-kDa GR fragment has a similar size, as well as identical DNA binding characteristics (i.e. increased affinity to nonspecific DNA) in vitro, as a group of GR mutants isolated from two glucocorticoid-resistant lymphoma cell lines (Yamamoto et al., 1976; Stevens and Stevens, 1981; Delweg et al., 1982). These receptor mutants show an increased nuclear transfer of the GR-hormone complex upon hormone treatment and are thus called nt'. Although cDNA for the nt' mutants have to our knowledge not been isolated, it has been shown that nt' cells contain a smaller transcript coding for GR than the wild type (Miesel et al., 1984) and that sequence information in the 5'-end is lacking (Northrop et al., 1986). Danielsen et al. (1987) have demonstrated, with a series of N-terminally deleted receptor mutants, an inverse correlation between loss of glucocorticoid-induced transcription and increased affinity for nonspecific DNA in vitro. This indicates that the nt' mutants in fact may be very similar to the a-chymotrypsin-induced 39-kDa GR fragment, and that their observed increase in affinity for nonspecific DNA may be of significance for the achievement of glucocorticoid resistance.

What then is the molecular basis of the increased affinity for nonspecific DNA binding exerted by the a-chymotrypsin-induced 39-kDa receptor fragment? In this report, we investigate the influence of the N-terminal domain on the DNA-GR interaction. Our results demonstrate that the N-terminal domain contains determinants which are involved in the protein-protein contacts made by the two receptor subunits in formation of the homodimer. The data illustrate how a domain, not directly involved in forming contacts with DNA, may exert significant effects on the DNA binding function. The decreased DNA binding specificity caused by the removal of the N-terminal domain is probably caused by an altered steric arrangement, and/or rigidity, of the two DNA binding domains in the GR homodimer.

MATERIALS AND METHODS

GR Preparation—GR-[3H]triamcinolone acetonide complex was purified from rat liver as described previously (Wrange et al., 1989), with the exception that the last DAE-DEAE column was replaced by chromatography on a 5-ml fast protein liquid chromatography Mono Q" column (Pharmacia LKB Biotechnology Inc.) (Perlmann and Wrange, 1988). GR was quantitated by analysis of bound [3H]triamcinolone acetonide by a gel retardation assay as described (Maniatis et al., 1982). The fragments were separated on a 5% (w/v) polyacrylamide gel, polynucleotide kinase, was done as described (Maniatis et al., 1982).

Preparation of the 39-kDa Receptor Fragment—39-kDa receptor fragment was prepared as described by Wrange et al., 1984. The eluate from the Mono Q column (see above) was incubated with 0.5 μg of a-chymotrypsin (in 50 mM Tris-HCl, pH 7.8, 1 mM Na₂EDTA, and 10% (v/v) glycerol) containing 4.5 mM DTT, 0.1 mg/ml insulin, and 5 mM phenylmethylsulfonyl fluoride, and applied on a 5-ml DNA-cellulose column. The receptor was eluted by a linear 0.4-0.8 M NaCl gradient, thus separating the 39-kDa receptor fragment (which is eluted around 0.15-0.2 M NaCl) from undigested receptor, protease, and other degradation products. The proteins were analyzed by SDS-polyacrylamide gel electrophoresis on a 10% (w/v) acrylamide, 0.3% (w/v) N,N'-methylenebisacrylamide gel in a discontinuous buffer system (Laemmli, 1970) and stained with silver nitrate (Oakley et al., 1980). Analysis of purified receptor fragment by this technique shows no residual 94-kDa receptor (Fig. 1A).

Construction and Preparation of DNA Fragments—For the DNA constructs used in the footprinting studies, two oligonucleotides harboring the DNA sequence of the 200/-148 fragment in the MMTV long terminal repeat were synthesized on a Pharmacia CPG Assembler™. The nucleotide positions given are relative to the CAP site according to the nomenclature used by Scheidereit and Beato, 1984. This sequence includes a strong GR binding site as shown by a DNase I footprint covering a 24-bp segment at position -190/-167 (Wrange et al., 1983). The oligonucleotides form a double-stranded DNA fragment with EcoRI and BamHI ends. Insertion of one and four copies of this fragment, together with a PstI/BglII linker, into pGEM-1 (F. de Differ and Stillman, 1986). The plasmids are named PMTV(-200/-148)² and pMTV(-200/-148)², respectively. The construct used in glycerol gradient centrifugation and in gel shift analysis, named pMTV(-192/-164)² and harboring one GR site, was made as described by Wrange et al. (1989). Again, the oligonucleotides include a strong GRE derived from -190/-167 in the MMTV promoter, but in this case with flanking XhoI ends. pMTV(-192/-164)² (harboring a mutated binding site, see Fig. 5) was synthesized in the same way. This construct contained only the TGGTTCT half of the binding site; a BglII linker was inserted in the upstream part of the partial palindrome. This creates a dam (deoxyadenosine methylase) site (GATC). DNA propagated in either dam+ or dam- bacterial strains showed no differences in the experiments described in Fig. 5 (data not shown). Both constructs, harboring either a wild type or a mutated binding site, were inserted in the SalI site of pGEM-3. The orientation and the constructs were confirmed by DNA sequencing.

Labeling of the DNA fragments with [γ³²P]ATP, by use of T4 polynucleotide kinase, was done as described (Maniatis et al., 1982). The fragments were separated on a 5% (w/v) polyacrylamide gel, localized by autoradiography, and purified on a Schleicher & Schuell DEAE-membrane by electrophoresis, according to the procedure recommended by the manufacturer.

Exonuclease III Footprinting—Glucocorticoid receptor/39-kDa receptor fragment was bound to DNA in a volume of 110 μl of GR binding buffer (ETG buffer containing 5 mM DTT, 85 mM NaCl, 2% (v/v) polyvinyl alcohol, and 0.1 mg/ml pork insulin) for 15 min at 25°C. Then, MgCl₂ was added to a final concentration of 3 mM. The receptor-DNA complex was treated with 100 units of exonuclease III (Pharmacia) for 15 min at 37°C. The reaction was terminated by adding 17 μl of stop solution containing 1% SDS and 100 mM Na₂EDTA, extracted with phenol:CHCl₃ (2:1) followed by ethanol precipitation and analysis on a 6% (w/v) denaturing polyacrylamide gel. Sequence marker lanes were prepared as described by Maxam and Gilbert (1977).

Methylation Interference and Gel Retardation Assay—DNA fragments labeled at one of the 5'-ends were partially methylated in 200 μl of 50 mM Tris-HCl (pH 8.0) and 1 mM Na₂EDTA by addition of 1 μl of dimethyl sulfide, and incubation for 2 min at 20°C. The reaction was terminated by the addition of 50 μl of 1.5 M sodium acetate (pH 7.0), 1.0 M 2-mercaptoethanol, and 100 μg/ml tRNA. After two ethanol precipitations, the methylated DNA was incubated for 30 min at 25°C, with or without receptor/receptor fragment, in ETG buffer containing 5 mM DTT, 75 mM NaCl, 0.1 mg/ml pork insulin, and 50 μg of Sau 3A restricted pGEM-1 "wt", which was used as unspecific competitor. Bound receptor-DNA complex was separated from free non-specific DNA by a gel retardation assay as described above. If no unspecific competitor DNA was added, the 94-kDa GR formed a larger complex than seen in Fig. 1B, probably reflecting nonspecific interactions (cf. Fig. 5). The sample was applied on a 3.5% (w/v) polyacrylamide/bisacrylamide gel (55:1) in 67 mM Tris-HCl (pH 7.5), 1 mM sodium acetate, and 200 μg/ml T7 DNA polymerase. The sequence of one of the above DNA fragments was determined by a modified Sanger dideoxy sequencing method (Sanger et al., 1977) and analyzed on a 12.5% (w/v) denaturing polyacrylamide gel.

Ethylation Interference—The ethylation interference experiments were carried out essentially as described by Salonjali and Brown (1982). DNA labeled at either 5'-end was incubated in 20 μl of a 1:1
(v/v) mixture of 25 mM sodium cacodylate buffer (pH 8.0) and ethanol-saturated with ethyl nitrosourea (Sigma); incubation was for 30–80 min at 50 °C. The reaction was terminated by ethanol precipitation, using 4 μl of 3 M ammonium acetate and 40 μl of ethanol. The DNA fragments were reprecipitated three times. Binding reaction of receptor/receptor fragment to partially ethylated template, and separation of protein-bound from unbound DNA, were done as described under methylation interference.

Base cleavage was carried out in 200 μl of 10 mM Tris-HCl (pH 8.0) and 1 mM Na2EDTA, by the addition of 10 μl of 2 M NaOH and a subsequent incubation at 90 °C for 30 min. Samples were extracted with phenol:CHCl3 (2:1), followed by ethanol precipitation and analysis on a 12.5% (w/v) denaturing polyacrylamide gel.

**RESULTS**

**Mapping of Protein:DNA Contact Points**—To reveal potential differences in protein:DNA contact points that might account for the observed difference in DNA binding specificity, a careful comparison was performed, using purified intact 94-kDa receptor and α-chymotrypsin-induced 39-kDa receptor fragment (Fig. 1A).

The 3′-border of the protein:DNA complex was determined by exonuclease III footprinting (von der Ahe et al., 1985). In both cases, stops were observed between positions −165 and −164 on the top strand, and between positions −191 and −190 on the bottom strand (data not shown). Both receptor entities thus protect a 26-bp DNA segment symmetrically distributed over the partially palindromic sequence in the GR binding site (see Fig. 3 for summary of DNA:protein contact points). This is in good agreement with previously described exonuclease III (von der Ahe et al., 1985) and DNase I (Payvar et al., 1988; Scheiderer et al., 1988) footprinting data, which show a protected area covering the −190/−167 DNA segment.

Ten- to fifteen-fold more of the 39-kDa receptor fragment was required in place of intact 94-kDa receptor to obtain an exonuclease III footprint; this is probably due to the lower affinity and specificity of the receptor fragment for the specific DNA site.

We also performed DNase I footprinting (Galas and Schmitz, 1978, Wrange et al., 1986). These experiments were hampered by problems in obtaining enough concentrated 39-kDa receptor fragment preparations, required to reach near saturation of the specific DNA binding site, which is necessary in this assay. However, we did obtain DNase I footprints when using the bottom strand of a mouse mammary tumor virus (MMTV) −265/−110 promoter segment. These results did not reveal any qualitative difference between the DNA segments protected by either the intact 94-kDa receptor or the 39-kDa receptor fragment (data not shown).

The protein:DNA contact points of the two GR entities were further compared by methylation interference. Here the purified residues of a 58-bp DNA fragment, harboring the MMTV DNA segment −200/−148 (which contains a strong GRE located at −190/−167), was partially methylated prior to protein binding. Dimethyl sulfate methylates guanine at the N7 position (which is accessible in the major groove of the DNA double helix) and adenine residues at the N6 position (accessible in the minor groove). Following the binding reaction between the protein and the modified DNA fragment, the bound protein:DNA complex was separated from unbound DNA by use of the band shift assay (cf. Fig. 1B). Analysis of the retained DNA fragments on a sequencing gel displayed 2 guanosine residues on each DNA strand where methylation interfered with GR binding, thus creating gaps in the ladder, as compared with partially methylated DNA incubated without protein in the control lane (C in Fig. 2A). These were at positions −174 and −184 on the top strand, and at positions −171 and −180 on the bottom strand. Thus, the 39-kDa receptor fragment gave the same pattern of methylation interference as the intact 94-kDa receptor. Furthermore, there was no significant difference in the extent of methylation interference when comparing the two receptor entities, as revealed by two-dimensional densitometry (Fig. 2C). These results are in agreement with previously described methyla-

![Fig. 1](attachment:fig1.png)

Fig. 1. A, SDS-polyacrylamide gel electrophoresis of purified, intact 94-kDa glucocorticoid receptor and 39-kDa receptor fragment. Numbers refer to the relative molecular mass in kilodaltons of the standard proteins: phosphorylase b, bovine serum albumin, ovalbumin, and carbonic anhydrase, given in the order of the highest molecular mass first. B, gel shift assay of the 94-kDa receptor and the 39-kDa receptor fragment used for separation of bound from unbound DNA, e.g. in methylation and ethylation interference assay. C denotes free methylated or ethylated DNA. 94 kDa and 39 kDa represent intact receptor and receptor fragment, respectively, bound to a 58-bp DNA fragment harboring one strong GR binding site.

The 39-kDa GR fragment has an increased affinity for nonspecific DNA prompting us to investigate the contact points made with phosphate groups in the DNA backbone by use of ethylation interference (Sakonju and Brown, 1982). Ethylation by use of ethyl nitrosourea gives rise to a phosphotriester that interferes with the binding of the protein, either because of steric hindrance or the removal of the negative charge (Bushman et al., 1985). Ethyl nitrosourea also ethylates other residues of the DNA (especially the O6 position of guanine; Sun and Singer, 1975), although to a lower extent. The ethylated phosphates that interfere with the binding are detected on a sequencing gel after separation of receptor-bound DNA from unbound DNA, thus using a similar strategy as in the methylation interference assay.

Fig. 2B shows an ethylation interference footprint of the intact 94-kDa receptor and of the 39-kDa receptor fragment, from two different experiments using the 58-bp BglII/BamHI fragment of pMTV(−200/−148). There are two major segments on each strand where ethylated phosphates interfere with GR binding. These segments are located between residues −185/−181 and −174/−171 on the top strand and −182/−179 and −172/−169 on the bottom strand. Quantitation of

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FIG. 2. A, methylation interference experiment. C represents free methylated DNA isolated by band shift assay. 94 kDa and 39 kDa represent methylated DNA bound to intact receptor or 39-kDa receptor fragment, respectively, after separation by gel shift assay. Numbers denote the location of interfering guanosine residues. B, ethylation interference experiment showing interfering phosphate residues of the DNA backbone to the binding of intact 94-kDa receptor or 39-kDa fragment. GA and CT represent sequence ladders. C represents free ethylated DNA. Major sites where ethylated phosphates interfere with the binding of the receptor/receptor fragment are indicated with arrows. Numbers refer to positions (relative to the CAP site) of guanosines showing methylation interference. C and D, densitometric scanning of methylation (C) and ethylation (D) interference experiments. Percent interference refers to: ((band in control lane) - (band in either the 94-kDa lane or 39-kDa lane)) divided by (band in control lane). For all bands, the background density was first subtracted and normalized to nonprotected bands for variations in the amount of sample loaded. Fig. 2C shows only the guanine residues where methylation interfered with GR binding. Two-dimensional densitometry is described under "Materials and Methods.”

Stoichiometric Quantitation and Glutaraldehyde Cross-linking—The 94-kDa receptor forms a homodimer irrespective of binding to DNA (Wrang et al., 1989). This was demonstrated by use of glutaraldehyde cross-linking and by stoichiometric analysis of the receptor-DNA complex following a glycerol gradient centrifugation. To investigate whether the altered DNA binding properties of the 39-kDa fragment could be explained by an altered ability to form a homodimer, we performed similar experiments with this receptor fragment.

The intact 94-kDa GR sediments as a 4 S complex upon glycerol gradient centrifugation, but cross-linking with glutaraldehyde induces a shift to a 6 S complex (Fig. 4D). Glycerol gradient centrifugation of 94-kDa GR bound to DNA (a 35-bp DNA fragment harboring the MMTV DNA segment -192/-164 which contains one strong GRE at -190/-167) sediments as a 6 S complex. Stoichiometric analysis based on the quantitation of 3H-labeled hormone bound to GR and 32P-labeled DNA, both of known specific activity, shows that two GR molecules are bound per glucocorticoid-responsive element (Wrang et al., 1989).

Similar stoichiometric quantitation with the 39-kDa fragment bound to an identical DNA fragment shows that 2.1 ± 0.1 (mean ± S.D., n = 4) hormone ligands are bound per GR binding site (Fig. 4, A–C). The 39-kDa receptor-DNA complex sediments as a 5 S complex on glycerol gradients (Fig. 4C) and as a 4 S complex when not bound to the specific 35-bp DNA fragment (Fig. 4A). The quantitative data, together with the increased sedimentation rate of the protein-DNA complex, are compatible with the binding of two entities of the 39-kDa receptor fragment to one specific DNA site. This
The 39-kDa Glucocorticoid Receptor

FIG. 3. Summary of the footprinting data shown in a model of the DNA helix, drawn with 10.5 base pairs per helical turn. Symbols for interfering ethylated phosphate, interfering methylated guanine, and exonuclease III stop are indicated in the figure. Stars denote center of a 2-fold symmetry. The partially palindromic GRE consensus sequence is underlined.

finding is not unexpected, in light of the above described findings demonstrating a 2-fold symmetry of the 39-kDa GRE interaction, and the similarity with the homodimeric 94-kDa entity in contacting a GRE.

Glutaraldehyde cross-linking of the 39-kDa fragment, however, did not induce a shift similar to the one reproducibly seen after glutaraldehyde treatment of the 94-kDa GR (compare Fig. 4, D and E). Preforming the specific 39-kDa receptor-DNA complex at conditions where two 39-kDa entities are bound to each DNA fragment (see above), followed by cross-linking with glutaraldehyde again, did not cause any shift in the sedimentation rate of the receptor fragment. The same experiment concomitantly performed with the 94-kDa receptor resulted in a 4 S to 6 S shift, although glutaraldehyde treatment also results in total loss of DNA binding (Wrange et al., 1989; data not shown).

These glutaraldehyde cross-linking experiments demonstrate a qualitative difference between the 94-kDa GR and the 39-kDa receptor fragment, which probably reflects differences in the protein:protein contacts in the homodimer formed by these two receptor entities.

Binding of GR to a Half-GRE—To further investigate potential differences in the dimerization ability, we constructed a DNA fragment harboring only half of the previously used partially palindromic sequence (which constitutes the -190/-167 MMTV GRE). Fig. 5 shows a gel retardation assay using both the wild type and the half-binding site. The 94-kDa GR

FIG. 4. Glycerol gradient centrifugation analysis. A–C represents stoichiometric analysis of 39-kDa receptor fragment bound to DNA. A, 0.71 pmol of 39-kDa receptor fragment. B, 0.4 pmol of a 35-bp 32P-end-labeled DNA fragment, derived from pMTV(-192/-164) by XhoI cleavage harboring one strong GR binding site. C, 0.71 pmol of 39-kDa receptor fragment with a specific radioactivity of 1 cpm/0.772 fmol, incubated with 0.5 pmol of the 35-bp specific DNA fragment with a specific radioactivity of 1 cpm/0.045 fmol. D and E represent glutaraldehyde cross-linking of 94-kDa receptor and 39-kDa receptor fragment respectively, performed in parallel. The receptor and receptor fragment were preincubated at 25 °C for 5 min, then incubated for an additional 5 min at the same temperature, in the presence or absence of 0.01% glutaraldehyde. For further experimental details, see Wrange et al. (1989).
The 39-kDa Glucocorticoid Receptor

HindIII-restricted pMTV (-192/-164) and pMTV (-192/-164), respectively. The triangle denotes the mobility of a 94-kDa homodimer, and the star denotes the mobility of a 39-kDa homodimer, as revealed by methylation and ethylation interference. Amounts of unlabeled pGEM-1* used as competitor DNA are indicated in ng. MMTV sequences of DNA constructs used are shown below. Boxed base pairs in the sequence refer to mutated bases compared to the wild type. Imperfect palindromic sequences are underlined.

incubated with the wild type DNA binding site resulted in a retarded complex of characteristic mobility which is relatively resistant to competition with unspecific DNA (cf. Fig. 1B and the 1st lane in Fig. 5) and which is caused by the binding of one GR dimer (according to data presented above). If no DNA competitor is added, a complex of slower mobility was usually formed, probably reflecting nonspecific protein:DNA contacts (not shown). When the intact 94-kDa receptor was incubated with the half-GRE, the nonspecific complex was obtained (Fig. 5, 2nd lane). However, increasing amounts of nonspecific competitor DNA did not result in the appearance of a complex with the expected mobility of the dimer; instead, low amounts of a smaller complex was occasionally seen (Fig. 5, lanes 3 and 4). The smaller complex may represent a 94-kDa monomer, since its mobility is close to the 39-kDa dimer, but this assumption has not been experimentally confirmed. A corresponding analysis of the 39-kDa receptor fragment with the half-GRE resulted in a band with identical mobility as the dimer of the 39-kDa fragment bound to the wild type GRE (compare lanes 5 and 6–8 in Fig. 5).

We conclude that the 94-kDa receptor was unable to form a complex corresponding to the size of a dimer when incubated with the half-DNA binding site. This was in clear contrast to the 39-kDa receptor fragment, which formed a complex of identical size as the dimer formed with a wild type DNA site. Repeated experiments, involving the titration with various amounts of competing nonspecific DNA, showed that there was a clear-cut decrease in the affinity of the 39-kDa receptor to a half-site as compared to a wild type DNA site (not shown). This was not unexpected but we want to stress the fact that we could never obtain a 94-kDa receptor-half-GRE complex with the expected mobility of a dimer, which was repeatedly seen with the 39-kDa receptor fragment.

Glycerol gradient centrifugation was also used for studies of the GR-DNA interaction with a half-GRE binding site. As mentioned above, the GR sedimented as a monomer on glycerol gradients unless the homodimer was stabilized by glutaraldehyde cross-linking or specific DNA binding (Wrange et al., 1989). Fig. 6A illustrates the result when the 94-kDa GR was incubated with an 80-bp DNA fragment containing one wild type GRE. The GR-DNA complex had a sedimentation rate of 6 S, while the free GR sedimented at 4 S (filled arrow). The free DNA sedimented slightly faster than free GR (empty arrow). Stoichiometric analysis showed a 2:1 relationship for GR and DNA fragment. Fig. 6B demonstrates the same experiment, but instead conducted with a half-DNA binding site in a 80-bp DNA fragment. In this case, the 94-kDa GR-DNA complex had a sedimentation rate of 5 S, and the stoichiometric analysis showed a 1:1 GR/DNA relationship; thus, one single monomer of 94 kDa interacts with the half-GRE. This is in accordance with previous findings, suggesting that the GR homodimer dissociates into monomeric form during glycerol gradient centrifugation.

DISCUSSION

The aim of this study was to investigate the molecular basis of the altered DNA binding properties of the α-chymotrypsin-induced 39-kDa receptor fragment, i.e. its decreased sequence specificity and its higher affinity for nonspecific DNA (Payvar and Wrange, 1983; Wrange and Gustafsson, 1978). A detailed comparison of the protein:DNA contact points, made by the intact 94-kDa receptor and by the 39-kDa receptor fragment, did not reveal any qualitative differences. There was, however, a quantitative difference in the dependence of the complex on phosphate contacts, as revealed by a significantly higher sensitivity of the 39-kDa receptor in the ethylation interference assay (Fig. 2D). This result implicates that the 39-kDa receptor-DNA complex relies to a higher degree on the nonspecific phosphate contacts than the 94-kDa receptor-DNA complex does, a finding which may be compatible with the decreased DNA sequence specificity of the 39-kDa receptor fragment.
As displayed in Fig. 3, there is an almost perfect 2-fold symmetry in the DNA contacts located by exonuclease III, methylation interference, and ethylation interference, for both receptor entities. This is in agreement with our previous report, which showed that GR bound DNA as a homodimer (Wrang et al., 1989). The glycerol gradient centrifugation experiments (Fig. 4) also fit with a dimer model (see "Results"). It is of interest to note that in the cross-linking experiment using glutaraldehyde, an effect was seen on the sedimentation rate of the 39-kDa fragment (Fig. 4, D and E). This indicates an alteration in the protein:protein contacts of the GR homodimer as a consequence of the removal of the N-terminal domain. We have not been able to determine potential differences in the homodimer stability of the two receptor entities in the absence of DNA.

However, two lines of indirect evidence support the idea that the 39-kDa receptor fragment exists as a homodimer in the absence as well as in the presence of DNA. First, no DNA-protein complex migrating faster than dimeric complex was observed, even after incubation of the 39-kDa protein fragment with a large excess of DNA. Second, the same 39-kDa receptor-DNA complex was present also when the DNA component consisted of half of a GRE palindrome. These results are in agreement with observations made by Kumar and Chambon (1988), indicating that the C-terminal ligand binding domain of the structurally related estrogen receptor was important in maintaining the dimeric state.

The qualitatively different protein:protein contacts in the 39-kDa receptor suggested by the glutaraldehyde cross-linking experiments may result in an altered steric arrangement and/or an altered rigidity of the homodimer. In this context, it is interesting to note the distinctly different results of the gel shift experiment that were obtained when a half-GR was used, as compared with those obtained with the 94-kDa GR. The 94-kDa GR does not show any specific dimeric complex formation (similar to the one observed with an intact GRE), while the 39-kDa receptor forms a qualitatively indistinguishable complex with the half-GRE or an intact GRE (Fig. 5). When the 94-kDa receptor is bound to a half-GR and then analyzed by glycerol gradient centrifugation, one receptor monomer will bind to each half-GR, as shown both by stoichiometric analysis and by the partial shift in sedimentation rate (Fig. 6). We previously demonstrated that the receptor homodimer is dissociated during glycerol gradient centrifugation, unless stabilized by a specific DNA binding site or by prior glutaraldehyde treatment (Fig. 4 and Wrang et al., 1989). This is not the case during the gel shift assay. In this method, only trace amounts of a more rapidly moving complex, perhaps representing monomeric GR, were sometimes observed when using a half-GR (Fig. 5, lane 3). Thus, our results imply that the integrity of the receptor homodimer prevents the 94-kDa GR from binding to a half-GR. On the other hand, a 39-kDa homodimer is able to bind to a half-palindrome. Taken together, these results suggest that the structural coordination of the two receptor monomers in forming the DNA binding homodimer is affected in the 39-kDa receptor fragment. In this context, crystallographic data of the phage 434 repressor-operator complex suggest that a tight fit between 434 repressor and DNA could not be achieved with nonspecific DNA since such a DNA segment would displace the repressor 2–3 Å outward from the DNA axis (Anderson et al., 1987). This model may be applied to our results, showing that the 39-kDa, but not the 94-kDa entity, binds as a dimer to a half-GR. The observed difference could be explained by a less rigid 39-kDa homodimer. An increased flexibility of the 39-kDa homodimer may allow the formation of a specific interaction with a half-DNA site, although the other half of the 39 kDa homodimer is partly displaced from the DNA axis by the adjacent major groove harboring a nonspecific segment. This requires a kink to be formed within the homodimer. This model would suggest that the more rigid 94-kDa GR homodimer prevents the kinked structure required for the formation of a complex with a half-GRE.

Our analysis of the protein:DNA contacts does not rigorously exclude the possibility that there may still exist qualitative differences in contacts formed by the two receptor entities. However, the clear-cut difference seen in the glutaraldehyde cross-linking experiment, as well as in the gel shift analysis involving a half-GRE, strongly favors the above-mentioned explanation. It is probably of importance to keep the two protein domains in a stiff and precise steric arrangement in relation to each other in order to gain optimal DNA binding specificity. Furthermore, our data suggest that each receptor monomer contacts one of the two major grooves on the DNA that constitutes the GRE; the finding of one receptor monomer interacting with a half-GRE during glycerol gradient centrifugation lends particular support to this hypothesis.

The N-terminal part of GR has probably different functional domains that may be structurally overlapping, such as transcriptional activation (Giguère et al., 1986; Godowski et al., 1988; Hollenberg and Evans, 1988) and the maintenance of optimal specific DNA binding (Danielsen et al., 1987). The latter is probably achieved via protein:protein contacts required for optimal steric arrangement and/or rigidity of the two DNA binding domains in the receptor homodimer (above results). An alternative explanation proposed by Danielsen et al. (1987), is that the more positive net charge of a N-terminally deleted GR, similar to the α-chymotrypsin-induced 39-kDa fragment, could account for the increased affinity for nonspecific DNA. An understanding of the molecular basis of the decreased hormone induction seen in transfection experiments using different N-terminally deleted receptor mutants requires that the different functional activities are individually evaluated. Our results illustrate how a deletion outside of the domain which forms direct contacts with DNA may affect DNA binding. The physiological significance of these effects on DNA binding is presently not known in quantitative terms. The reported inverse relationship between functional hormone induction in vivo and an increased affinity for nonspecific DNA binding in vitro in various N-terminal deletions (Danielsen et al., 1987) does suggest that the altered DNA binding reported above for the 39-kDa entity has functional significance. The functional importance of the putative transcription activation domain(s) within the deleted N-terminal region remains unknown, until the effects caused by altered DNA binding properties (e.g. by an altered steric arrangement of the two monomers forming the DNA dimer) can be quantitated and subtracted from the overall effect of the mutant in question.

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REFERENCES

Anderson, J. E., Ptashne, M., and Harrison, S. C. (1987) Nature 326, 846–853.

Bushman, F. D. (1989) Cell 56, 335–344.

Bushman, F. D., Anderson, J. E., Harrison, S. C., and Ptashne, M. (1988) Nature 316, 651–653.

Carlstedt-Duke, J., Strömstedt, P.-E., Wrang, Ö., Bergman, T.,
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P Eriksson and O Wrange

*J. Biol. Chem.* 1990, 265:3535-3542.

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