Sulfhydryl Group Content of Chicken Progesterone Receptor: Effect of Oxidation on DNA Binding Activity†

Sara Peleg, William T. Schrader,* and Bert W. O'Malley

Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Received May 11, 1987; Revised Manuscript Received September 15, 1987

ABSTRACT: DNA binding activity of chicken progesterone receptor B form (PRB) and A form (PRA) has been examined. This activity is strongly dependent upon the presence of thiols in the buffer. Stability studies showed that PRB was more sensitive to oxidation than was PRA. Receptor preparations were fractionated by DNA–cellulose chromatography to DNA-positive and DNA-negative subpopulations, and sulfhydryl groups were quantified on immunopurified receptor by labeling with [^{3}H]-*N*-ethylmaleimide. Labeling of DNA-negative receptors with [^{3}H]-*N*-ethylmaleimide showed 21–23 sulfhydryl groups on either PRA or PRB form when the proteins were reduced and denatured. A similar number was seen without reduction if denatured DNA-positive receptor species were tested. In contrast, the DNA-negative PRB had only 10–12 sulfhydryl groups detectable without reduction. A similar number (12–13 sulfhydryl groups) was found for PRA species that lost DNA binding activity after exposure to a nonreducing environment in vitro. We conclude that the naturally occurring receptor forms unable to bind to DNA, as well as receptor forms that have lost DNA binding activity due to exposure to a nonreducing environment in vitro, contain 10–12 oxidized cysteine residues, likely present as disulfide bonds. Since we were unable to reduce the disulfide bonds when the native DNA-negative receptor proteins were treated with dithiothreitol (DTT), we speculate that irreversible loss of DNA binding activity of receptor in vitro is due to oxidation of cysteine residues that are not accessible to DTT in the native state.

The importance of sulfhydryl groups in steroid receptor–DNA interaction has been shown in several studies (Pike, 1981; MacDonald & Leavitt, 1982; Coty et al., 1983). Amino acid sequences of steroid receptor proteins share a consensus cysteine-rich region that is thought to be part of the DNA binding domain (Greene et al., 1986; Conneely et al., 1986; Miesfeld et al., 1986, 1987; Green & Chambon, 1987). Therefore, we considered the possibility that reduction/oxidation reactions of cysteines might take place on the receptor proteins which would be involved in maintenance of DNA binding activity.

Our earlier studies in vitro of the chicken progesterone receptor established the existence of two forms: receptor A (PRA)[1] with a molecular mass of ~78 kDa and receptor B (PRB)[1] with a molecular mass of ~108 kDa (Birnbaumer et al., 1983). We also found that PRA could bind tightly to DNA–cellulose (Schrader et al., 1972) and to soluble DNA (Compton et al., 1984). By contrast, PRB showed only weak binding to DNA–cellulose (Vedeckis et al., 1980) and no binding to DNA in solution (Schrader et al., 1972). Since we could not convert PRB into the DNA binding species (Vedeckis et al., 1980), we predicted that the two forms have different functions in the process of regulation of gene expression by progesterone in vivo (Schrader et al., 1981). A recent report by Gronemeyer et al. (1985) demonstrated that a significant proportion of PRB from hen oviduct could bind to DNA–cellulose. This improvement in PRB binding to DNA appeared to be the result of changes in the purification protocol, including stabilization of receptor aggregates by sodium molybdate. In this study we have reinvestigated the question of receptor affinity for DNA. DNA binding activity was determined by retention of receptor protein on columns containing calf thymus DNA–cellulose, and receptor-containing samples were analyzed by monoclonal or polyclonal antibodies specific to the chicken progesterone receptor (Sullivan et al., 1986; Birnbaumer et al., 1987).

We report here the effects of the reducing environment upon binding of PRA and PRB to DNA. We find that DNA binding activity of both receptor forms is dramatically decreased by elimination of thiols from the buffers. The effect is more pronounced for PRB. Dithiothreitol was the most effective stabilizing agent.

We also quantified the number of sulfhydryl groups on immunopurified receptor by labeling with [^{3}H]-*N*-ethylmaleimide. We used this method to define differences in oxidation state between DNA-positive and DNA-negative receptor forms.

Experimental Procedures

Chemicals and Buffers. [^{3}H]Progesterone (56 Ci/mmol), [^{3}H]R5020 ([*17α-methyl*-^{3}H]promegestone; 86 Ci/mmol), and *N*-([2-^{3}H]ethyl)maleimide (53.3 Ci/mmol) were obtained from New England Nuclear. Progesterone and triamcinolone acetonide (TAA)[1] were from Steraloids. Molecular mass

† This work was supported by National Institutes of Health Grant HD-07857. Some of the results in this paper were presented at the 16th Annual Meeting of the UCLA Symposia on Molecular and Cellular Biology, Jan 17–Feb 5, 1987.

* Author to whom correspondence should be addressed.

[1] Abbreviations: PRB, progesterone receptor B (108 kDa); PRA, progesterone receptor A (78 kDa); PR, progesterone receptor; [^{3}H]-R5020, [*17α-methyl*-^{3}H]-17,21-dimethyl-19-norpregna-4,9-diene-3,20-dione; TAA or triamcinolone acetonide, 9α-fluoro-11β,21-dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-dione; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; 1-TG, monothioglycerol; β-ME, β-mercaptoethanol; IgG, immunoglobulin G; Ab, antibody; NEM, *N*-ethylmaleimide; kDa, kilodalton(s).

0006-2960/88/0427-0358$01.50/0

standards for electrophoresis were phosphorylase B, 94 kDa; bovine serum albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 31 kDa; and soybean trypsin inhibitor, 21 kDa; all were from Bio-Rad. *N*-Ethylmaleimide was from Pierce, and dithiothreitol (DTT)[1] was from Sigma. Buffer TEG contained 10 mM Tris-HCl,[1] pH 7.4, 1 mM Na_2EDTA,[1] 10% (w/v) glycerol, and 50 mM NaCl. This buffer was supplemented with thiols when indicated in the text.

Preparation of Cytosol and Partial Purification of Progesterone Receptor. Oviducts were removed from diethylstilbestrol-treated 5-week-old chicks and rinsed in ice-cold 0.9% NaCl. Homogenization was done at 0 °C, in TEG buffer with or without thiols, as indicated in the text, at a ratio of 5 mL of buffer/g of wet tissue, by using a Polytron PT-10 at a setting of 5.5, with three 10-s bursts separated by 1-min intervals. The homogenate was then centrifuged at 27000*g* for 20 min. A floating lipid layer was removed, and the 27000*g* supernatant fraction was centrifuged for 1 h at 105000*g* to yield the high-speed cytosol.

High-speed cytosol prepared in TEG buffer with or without thiols was applied to a 30-mL phosphocellulose column, and the flow-through material was labeled for 2 h with [^{3}H]-progesterone. The receptor was precipitated at 40% saturation of ammonium sulfate. The pellet was collected after 30 min by centrifugation and redissolved in TEG buffer containing 100 mM NaCl with or without thiols.

To separate receptor able to bind to DNA from receptor unable to bind to DNA, we prepared cytosol and ammonium sulfate pellets as described above and applied the redissolved ammonium sulfate pellets onto a DNA–cellulose column. Receptor unable to bind to DNA was collected in the DNA–cellulose flow-through and in a 0.1 M NaCl wash of that column. This pool was precipitated by 50% saturation of ammonium sulfate. The DNA binding receptor fraction was eluted from the DNA–cellulose column by stepwise elution with 0.3 M NaCl in TEG buffer and precipitated by 50% saturation of ammonium sulfate.

Photoaffinity Labeling of Receptors. Photolabeling of receptor was performed by using receptor samples (see text) prepared from cytosol incubated with [^{3}H]R5020 instead of [^{3}H]progesterone (Birnbaumer et al., 1983). For UV irradiation, receptor solutions (10 mL) were placed in plastic Petri dishes (Fisher). The dishes were placed over a transillumination UV light box with a long-wavelength cutoff of 290 nm and irradiated for a period of 5 min. After irradiation the pools of receptor proteins were dialyzed at 4 °C against several changes of 50 mM NH_4HCO_3 (pH 8.5) and lyophilized. Electrophoretic separation, gel staining, and fluorography were carried out as described by Birnbaumer et al. (1983).

Immunoblotting (Western) Analysis. For immunoanalysis, receptor-containing aliquots (40 μL) were boiled in Laemmli sample buffer immediately after elution from the columns and kept frozen (−20 °C) for analysis. Gel electrophoresis of these samples was performed as described below. Proteins were electroeluted from the slab gels onto nitrocellulose paper in Tris–glycine buffer, as described previously (Towbin et al., 1979). After transfer, the protein binding sites on the paper were blocked by incubating the paper for 2 h at room temperature in Tris-HCl (pH 7.4) containing 0.2 M NaCl and 2% (w/v) nonfat dried milk (Carnation). Incubation with rabbit polyclonal anti-PRB IgG (Birnbaumer et al., 1987), at a final concentration of 1 μg of purified IgG/mL, was in the same buffer, at room temperature for 18 h. The excess was removed by four washes of the filter, 15 min each in the same buffer. The receptor–antibody complexes were detected by reaction with ^{125}I-protein A (Amersham) for 1 h at room temperature. Unbound protein A was removed by three washes in the same buffer. The nitrocellulose paper was dried and autoradiographed for 24–72 h at −70 °C by using Kodak X-OMAT XAR5 film and a Du Pont Cronex Quanta III intensifying screen.

Immunoprecipitation of Progesterone Receptor. Receptor-containing ammonium sulfate pellets were redissolved in TEG buffer in a final receptor concentration of 210–290 pmol/mL. Incubation mixtures for immunoprecipitation contained 21–29 pmol of receptor, 20 μg of plasmid DNA (pBR322), and 5 μg of anti-receptor mouse monoclonal antibody PR-22 (Sullivan et al., 1986), all in a final volume of 100 μL. Control samples contained all of the above except for antibody PR-22. After 2 h at 4 °C, 75 μg of anti-mouse IgG linked to agarose beads (Hyclone) was added and the mixtures were further incubated with gentle shaking for 2 h at 4 °C.

The receptor–IgG complexes were then washed 3 times with 1 mL of buffer [50 mM Tris-HCl, pH 7.5, containing 0.5 M NaCl and 0.2% (v/v) Triton X-100]. The pellet was either subjected directly to modification or heat denatured for 5 min at 90 °C in Laemmli buffer (Laemmli, 1970), before analysis by denaturing gel electrophoresis.

Modification of Immunoprecipitated Receptor. The washed receptor IgG–agarose beads were resuspended in 10 mM Tris-HCl, pH 7, containing 0.9% NaCl (Tris–saline buffer), with or without 2% (w/v) SDS. DTT was added at concentrations as indicated in the text. Samples containing SDS were boiled for 5 min and then quickly cooled in an ice bath. Then a mixture of [^{3}H]-*N*-ethylmaleimide and nonradioactive *N*-ethylmaleimide was added, such that the final concentration of *N*-ethylmaleimide was 0.4–3.2 mM. Specific radioactivity thus varied from 139 (when *N*-ethylmaleimide concentration was 0.4 mM) to 35 mCi/mmol (when *N*-ethylmaleimide concentration was 3.2 mM). Incubation with *N*-ethylmaleimide was carried out for 15–120 min as indicated in the text. The reaction was terminated by the addition of 4× Laemmli buffer followed by boiling for 5 min. After boiling, the samples were centrifuged for 3 min and the receptor-containing supernatant fraction was separated from the pellet of agarose beads. The samples were stored at −20 °C for analysis.

Analysis of [^{3}H]-N-Ethylmaleimide-Labeled Receptor Containing Samples. The electrophoretic separation of modified receptor-containing samples was carried out in slabs containing 1% SDS,[1] formed of 7.5% acrylamide/0.2% bis-(acrylamide). Gels were stained by Coomassie Blue (0.03%) dissolved in H_2O/isopropanol/acetic acid (35:10:4) for 1 h and then destained by several washes in 20% methanol. Each lane was cut into 2-mm slices. Each slice was incubated 16 h at room temperature with 0.4 mL of tissue solubilizer (NCS, New England Nuclear), and then 6 mL of scintillation fluid (ACS, Amersham) was added and the samples were counted in a liquid scintillation spectrometer. Counting efficiency for ^{3}H was 40%.

Results

Analysis of PRA and PRB Binding to DNA. Recently, others have shown that the progesterone receptor B form from hen oviducts binds to DNA–cellulose and elutes at a higher ionic strength than we had reported before (Gronemeyer et al., 1985). Since our previous studies had shown that this form does not bind to DNA at an ionic strength above 50 mM NaCl (Vedeckis et al., 1980), we decided to reexamine this question. We prepared receptor from chick oviducts as described under Experimental Procedures and applied samples to DNA–cel-

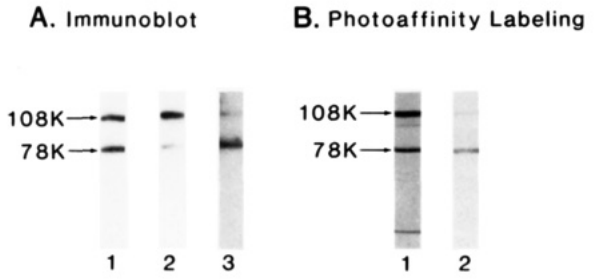

FIGURE 1: Analysis of receptor adsorption to DNA–cellulose. Panel A: Autoradiographic results obtained by Western immunoblotting with rabbit polyclonal anti-PRB. Receptors were prepared as described under Experimental Procedures, and the sample was analyzed for retention on a 2-mL DNA–cellulose column. Aliquots of the starting material (lane 1), material flowing through DNA–cellulose in 0.1 M NaCl (lane 2), and DNA-bound material eluting in 0.3 M NaCl (lane 3) were subjected to electrophoresis on 7.5% SDS–polyacrylamide slab gels. After electrotransfer from the gel to nitrocellulose filters, the filters were incubated with rabbit anti-PR IgG (1 μg/mL) and then with ^{125}I-protein A from *Staphylococcus aureus* as described under Experimental Procedures. Panel B: Fluorographic results of receptor retention by DNA–cellulose. The experiment was performed as described above except receptors were complexed with [^{3}H]R5020 instead of [^{3}H]progesterone. The DNA–cellulose flow-through fraction (lane 1) was obtained by washing the column with buffer containing 0.1 M NaCl. The receptor fraction eluting with 0.3 M NaCl (lane 2) was also collected. Both pools were UV-irradiated, dialyzed against NH_4HCO_3, and concentrated by lyophilization. The samples were then redissolved in Laemmli sample buffer and electrophoresed as in panel A. After staining by Coomassie Blue, the gel was processed for ^{3}H fluorography as described previously (Birnbaumer et al., 1983). Molecular weight determinations in both panels were made in comparison with stained protein standards run in companion lanes (not shown).

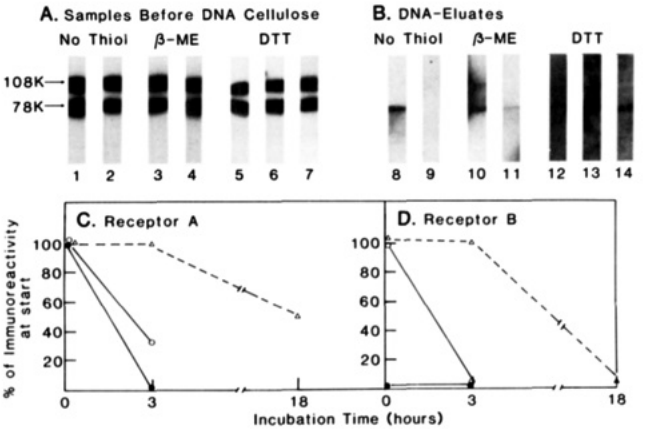

FIGURE 2: Effect of incubation time on DNA binding activity. [^{3}H]Progesterone receptor fractions were prepared as described in Figure 1 by using TEG buffer supplemented with either 5 mM β-mercaptoethanol or DTT. The pellets were resuspended in the same buffers and applied to DNA–cellulose columns immediately (lanes 1, 3, and 5 and 8, 10, and 12) or after 3-h incubation at 4 °C (lanes 2, 4, and 6 and 9, 11, and 13) or 18-h incubation at 4 °C (lanes 7 and 14). The unfractionated samples (panel A) or receptors retained by DNA–cellulose (panel B) were analyzed by Western immunoblot using rabbit anti-PRB antibodies to probe for PRA and PRB proteins as described in the legend to Figure 1. Panels A and B show the autoradiograms of the immunoblots. Panels C and D express quantitatively the results shown in panel B after densitometric scanning of the lanes in panel B. For each treatment, the data are normalized to the band intensity at $T = 0$, set at 100%. Panel C: Retention of PRA DNA binding activity. Panel D: Retention of PRB DNA binding activity. Intensities were determined for treatment without thiols (●—●), with β-mercaptoethanol (β-ME) (○—○), and with dithiothreitol (DTT) (△---△).

lulose columns. Receptor content was analyzed in the following fractions: (1) samples applied to the DNA–cellulose column; (2) samples washed through the column by 0.1 M NaCl; and (3) samples eluted from the column by a 0.3 M NaCl wash.

Figure 1, panel A, shows analysis of receptor content by Western immunoblot. Receptor was probed with polyclonal antibodies prepared against a highly purified (95% pure) chicken PRB (Birnbaumer et al., 1987); these antibodies react equivalently with PRA. Due to the sensitivity of the antibodies (5 ng of receptor protein can be detected) no concentration step of receptor was required prior to analysis. The redissolved ammonium sulfate pellet (lane 1) contained equal amounts of PRA and PRB. Band intensities in the fraction not retained by DNA–cellulose (lane 2) showed that PRB was more abundant than PRA in this pool. Analysis of the material eluted from DNA–cellulose by 0.3 M NaCl (lane 3) showed preferential retention of PRA compared to PRB. Furthermore, comparison of the band intensities for PRB in the flow-through and retained pools showed only minimal adsorption of PRB to DNA–cellulose. These results were confirmed by using [^{3}H]R5020 photoaffinity labeling as shown in panel B.

Relative Stability of DNA Binding Activity of PRB and PRA. The experiments shown above indicate that PRA and PRB exist in both DNA binding and nonbinding states. We were unable to create additional DNA binding activity in either PRA or PRB pool by conditions such as incubation in high ionic strength or incubation at room temperature (data not shown). Furthermore, the DNA-negative pools were not aggregated or otherwise evidently different in their physical characteristics from the DNA-positive fractions. We speculated that perhaps local denaturation of the DNA binding sites might account for these results.

Maintenance of reduced sulfhydryl groups appears to be important for DNA binding activity of steroid receptors (Pike, 1981; Coty et al., 1983). One possible mechanism for receptor inactivation could be oxidation of these groups due to insufficient concentration of thiols in the buffers during isolation. To test this possibility, we prepared cytosol with or without different reducing agents and then assayed the extent of DNA–cellulose binding and the stability of this interaction after incubation at 4 °C for up to 18 h. Figure 2 shows the results of that experiment. Panel A shows immunoblot autoradiograms of samples before DNA–cellulose chromatography. Since all the samples had the same immunoreactivity, we conclude that proteolysis did not occur in the samples during 3 and 18 h of incubation. Panel B shows autoradiograms of the material eluted from the DNA–cellulose columns at various times. Panels C and D express these results quantitatively, after scanning densitometry of the films.

The experiment lacking thiols (lanes 8 and 9) showed the absence of PRB retention on DNA–cellulose under these conditions. It also showed that, within 3 h of incubation without thiols, PRA lost this activity as well.

A better stabilization of receptor DNA binding activity was obtained by using dithiothreitol (DTT).[1] During the first 3 h of incubation in buffer containing DTT, retention by the DNA–cellulose column did not decrease. After 18 h in DTT, PRA lost 50% of that activity while the PRB lost all its DNA binding activity. The results also indicate that DNA binding activity of PRB is more easily inactivated than that of PRA, even in the presence of DTT.

Since the experiments described above showed only partial maintenance of receptor binding to DNA at thiol concentrations of 5 mM, we tested higher concentrations of β-mercaptoethanol and DTT. We prepared labeled cytosol in TEG buffer containing 5 mM β-mercaptoethanol and precipitated receptor by ammonium sulfate. The pellets were redissolved in the same buffer supplemented with either DTT or β-mer-

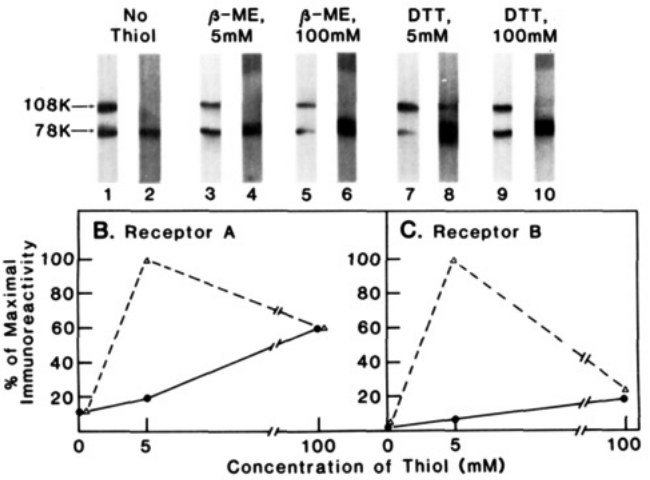

FIGURE 3: Effect of thiol concentration on progesterone receptor binding to DNA–cellulose. [^{3}H]Progesterone receptors were prepared in TEG buffer supplemented with 5 mM β-mercaptoethanol as described in Figure 2. The ammonium sulfate pellets were then resuspended in the same buffer containing 100 mM NaCl, and thiols were added. Then each sample was tested for DNA binding activity by using DNA–cellulose adsorption and elution. The upper panel shows the autoradiograms of samples analyzed after gel electrophoresis, transfer, and immunoblotting with rabbit anti-PRB antibodies as described in Figure 1. The odd-numbered lanes show the DNA flow-through material. The even-numbered lanes show samples eluted from the DNA–cellulose columns by 0.3 M NaCl. Panels B and C express quantitatively the results obtained by densitometric scanning of lanes showing the DNA-retained material. Immunoreactivity of PRA (panel B) or PRB (panel C) in samples eluted from the DNA–cellulose column in the presence of 5 mM DTT was considered as maximal immunoreactivity (100%). The plots show immunoreactivity in samples eluted from the DNA–cellulose column in the presence of β-mercaptoethanol (●—●) or immunoreactivity in samples eluted from the DNA–cellulose column in the presence of DTT (△---△).

captoethanol or without thiols and incubated for 3 h at 4 °C. Then the samples were applied to DNA–cellulose columns as described above, and receptor retention was measured. Figure 3 shows the results of this experiment. The upper panel shows autoradiograms of receptor samples not retained by DNA–cellulose and the material eluted from the columns. Panels B and C quantify the DNA-bound material after densitometric scanning of the autoradiograms. The experiment shows that β-mercaptoethanol was inferior to DTT in maintaining receptor binding to DNA, even at a concentration of 100 mM. However, when 100 mM DTT was used, the presence of this agent caused a significant decrease in DNA binding activity of both the PRA and the PRB (lane 10). Buffer containing dithiothreitol at a concentration of 5 mM was optimal in this experiment for DNA binding activity. Even under these conditions, however, only a small fraction of PRB was retained on DNA–cellulose.

We considered the possibility that DNA binding of receptor required an additional cofactor besides thiols: metal ions, such as zinc, have been suggested (Toft et al., 1979). It was possible that EDTA and DTT chelated the metal ions, thus causing progressive loss of DNA binding activity. In experiments not shown, we attempted to improve DNA binding of progesterone receptors by substitution of DTT by monothiols, which do not chelate zinc, and substitution of EDTA by EGTA, which chelates preferentially Ca^{2+}. The results of the experiments indicate that neither elimination of DTT nor its substitution by monothiols in the presence of EDTA or EGTA had any effect on DNA binding. Elimination of EDTA from the buffer decreased DNA binding activity significantly. However, this effect was the result of extensive proteolysis of receptor molecules, as indicated by Western immunoblotting analysis (not shown).

The data above support a role for reduced SH groups in receptor DNA binding activity. We quantified these groups as described in the next section.

Quantification of Receptor SH Groups Using N-Ethylmaleimide. Determination of sulfhydryl groups on a protein requires large quantities of highly purified protein. The method of choice to obtain sufficient amounts of highly purified receptor was immunopurification. Monoclonal antibody PR-22 can interact with both native receptor A and native receptor B without altering their DNA binding activity or steroid binding activity (Sullivan et al., 1986). Immunoprecipitation was performed in low salt, in the presence of soluble DNA and thiols, thus providing the optimal conditions for maintenance of DNA binding activity. The immunoprecipitated material contained IgG and some minor contaminants besides receptor protein. Therefore, receptor-containing samples were subjected to further purification by SDS gel electrophoresis. Since we wished to modify sulfhydryl groups on native as well as on denatured receptor protein, modification had to be performed prior to gel electrophoresis. The reagent *N*-ethylmaleimide, which modifies cysteines into *S*-(ethylsuccinimido)cysteine, is highly specific for sulfhydryl groups at pH 7 (Means & Feeney, 1971; Smyth et al., 1960). Thus radiolabeled *N*-ethylmaleimide was used to quantify sulfhydryl groups on receptor protein.

Immunoprecipitated receptor was reacted with radiolabeled *N*-ethylmaleimide, and then the modified receptor was further purified by SDS slab gel electrophoresis. The gels were stained by Coomassie Blue, and then each lane was cut into a series of 2-mm slices. The slices were counted, and radioactivity in receptor bands served as a measure of cysteine residues on receptor. Figure 4A shows the profile of [^{3}H]-*N*-ethylmaleimide radioactivity seen when an immunopurified receptor sample was modified under nondenaturing conditions. Panel B shows the profile of radioactivity of a receptor-containing sample in which receptor was denatured before reaction with [^{3}H]-*N*-ethylmaleimide. Both panels show that there was a background radioactivity incorporated into nonreceptor proteins. Most of the radioactivity in the samples was in the IgG band, the main protein in the samples as shown in panel C. These panels also show two radioactive peaks present only when anti-receptor antibody was present, corresponding to PRB and PRA. The difference in labeling at these two positions due to immunoprecipitated receptors was an index of sulfhydryl groups on the two proteins. A comparison of the extent of [^{3}H]-*N*-ethylmaleimide incorporation in panel A with that in panel B shows the additional labeling due to denaturation of receptor proteins before modification. Panel C shows the Coomassie Blue stained gel of immunopurified unmodified receptors (lane 1). PRA and PRB appeared as distinct bands of 78 and 108 kDa, respectively. In addition, the gel showed some nonreceptor contaminants and the heavy band of immunoglobulins. The receptor bands were absent in a control immunoprecipitation of a receptor sample in the absence of Ab PR-22 (lane 3). After reaction with *N*-ethylmaleimide, PRB appeared as a sharper band, migrating slightly slower than the unmodified PRB (lane 2). The change in migration was more evident for modified receptor PRA; this species migrated at an apparent molecular mass of 81 kDa.

Figure 5 shows a concentration curve for labeling of denatured and reduced receptors by [^{3}H]-*N*-ethylmaleimide. The samples were reacted with 10 μCi of [^{3}H]-*N*-ethylmaleimide together with sufficient nonradioactive *N*-ethylmaleimide to

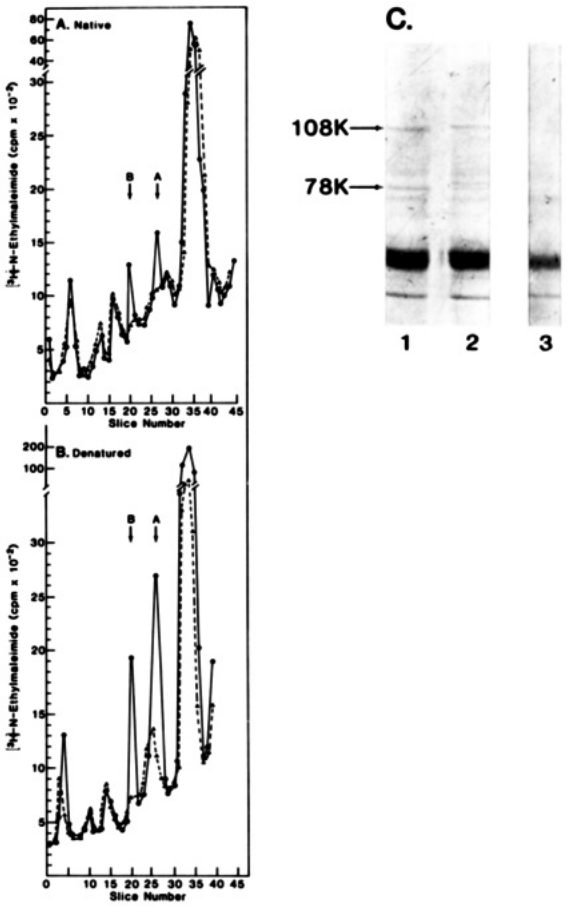

FIGURE 4: Modification of native denatured immunopurified progesterone receptor (PR) by [^{3}H]-*N*-ethylmaleimide. Receptor-containing ammonium sulfate pellets were prepared from chick oviduct cytosol. The receptor-containing pellets were resuspended in TEG buffer containing 5 mM DTT, and receptor proteins were immunoprecipitated as described under Experimental Procedures. Receptor–IgG complexes were either incubated for 2 h at room temperature in Tris–saline (pH 7) at a final concentration of 3.2 mM *N*-ethylmaleimide (panel A) or denatured in SDS (2%) for 5 min at 100 °C, cooled on ice, and then incubated with 3.2 mM *N*-ethylmaleimide for 2 h (panel B). Each incubation mixture also contained 10 μCi of [^{3}H]-*N*-ethylmaleimide. Samples were further purified by gel electrophoresis. The gels were stained, and each lane was sliced into a series of 2-mm pieces. The gel slices were dissolved in a tissue solubilizer (NCS) and counted. Receptor-containing samples were incubated only with goat anti-mouse IgG linked to agarose (▲---▲) or were incubated for 2 h with mouse anti-PR IgG (PR-22) and then with goat anti-mouse IgG (●—●). Panel C: Coomassie Blue stained gel containing immunopurified receptor samples. (Lane 1) Immunoprecipitated receptor, unmodified; (lane 2) immunoprecipitated receptor, modified by *N*-ethylmaleimide; (lane 3) mock immunoprecipitation of receptor-containing sample in the absence of PR-22.

bring the final *N*-ethylmaleimide molarity to the values shown. The DTT molar concentration in these samples was one-fourth of the *N*-ethylmaleimide concentration. The range of *N*-ethylmaleimide concentration (50–300-fold molar excess over total sulfhydryl groups in the sample) was calculated in relation to the IgG content (20–30 μg/sample) rather than to the concentration of receptor proteins (100–300 ng/sample). The labeling of receptor proteins was determined as described above, by subtraction of background radioactivity from radioactivity incorporated into receptor-containing bands. We calculated the number of reacted sulfhydryl groups per molecule, on the basis of the specific activity of [^{3}H]-*N*-ethylmaleimide and moles of receptor protein recovered after gel electrophoresis. Receptor concentration was determined from comparison of staining intensities of receptor-containing bands with staining intensities of protein standards, as shown in panel A. Panel B shows incorporation of [^{3}H]-*N*-ethylmaleimide into receptor proteins at several concentrations of nonradioactive *N*-ethylmaleimide. Panel C shows that, at 1.6–3.2 mM *N*-ethylmaleimide, a plateau was achieved for receptors A and B at about 20–21 sulfhydryl groups per molecule.

We performed a labeling time course experiment (data not shown) and observed a fast-labeling phase (0–30 min) and a slower increase in [^{3}H]-*N*-ethylmaleimide labeling up to 120 min. *N*-Ethylmaleimide also can modify lysine and histidine, although at a much slower rate than cysteine (Means & Feeney, 1971). Thus we adopted a 2-h labeling period as optimal for the reaction. This time point avoided increased background modification of amino acids other than cysteine.

Our experiments involved a reduction reaction prior to alkylation by *N*-ethylmaleimide. The reaction with *N*-ethylmaleimide was in the presence of DTT, which could inhibit *N*-ethylmaleimide by reacting with it. Therefore, we determined the optimal DTT concentration for reaction in the presence of a constant concentration of *N*-ethylmaleimide (data not shown). When receptors were labeled with 3.2 mM *N*-ethylmaleimide after reduction with increasing concentrations of DTT, we observed that DTT is inhibitory at concentrations higher than 0.8 mM. This concentration of DTT was chosen for subsequent experiments.

The experiment shown so far defined the conditions for optimal modification of sulfhydryl groups on progesterone receptor after immunoprecipitation. Assuming that all the labeling obtained was at cysteine residues, we detected a similar number of sulfhydryl groups on either receptor A form or receptor B form when the two proteins were reduced and denatured.

Determination of Oxidized Cysteine (Half-Cystine) Residues on Progesterone Receptor. To determine whether receptor in a form unable to bind to DNA was oxidized, we used the following strategy: cytosol was prepared in TEG buffer containing DTT and receptor was concentrated by ammonium sulfate precipitation. The resuspended ammonium sulfate pellet contained PRB, mostly in a state unable to bind to DNA (90%), and PRA, mostly in a state able to bind to DNA (60–70%). This unfractionated receptor mixture was immunoprecipitated in the presence of 5 mM DTT and soluble DNA (pBR322) to stabilize the DNA binding site. The washed receptor-containing pellet was briefly denatured by boiling in SDS in the presence or absence of DTT, cooled, and incubated with *N*-ethylmaleimide. Quantification of labeling by *N*-ethylmaleimide was done as described above.

Figure 6, panel A, shows the analysis of DNA binding activity in this preparation by Western immunoblot. Lane 1 shows the starting material containing equal amounts of PRA and PRB. This material was applied to a DNA–cellulose column. Lane 2 shows the material flowing through the DNA column, which contained the majority of the PRB. Lane 3 shows the material that was eluted from the DNA column; this pool contained mainly PRA. Lanes 4 and 5 show the results of immunoprecipitation of the unfractionated material after SDS gel electrophoresis and silver staining. Lane 5 shows PRA and PRB in equal amounts when immunoprecipitation was performed in the presence of Ab PR-22. Lane 4 shows the absence of receptor proteins when immunoprecipitation was performed without Ab PR-22. Panels B and C show the results of the [^{3}H]NEM reaction using this receptor preparation. Panel B shows a doubling of denatured PRB labeling by [^{3}H]-*N*-ethylmaleimide after reduction by DTT, indicating that this receptor subunit was oxidized. Only a slight increase

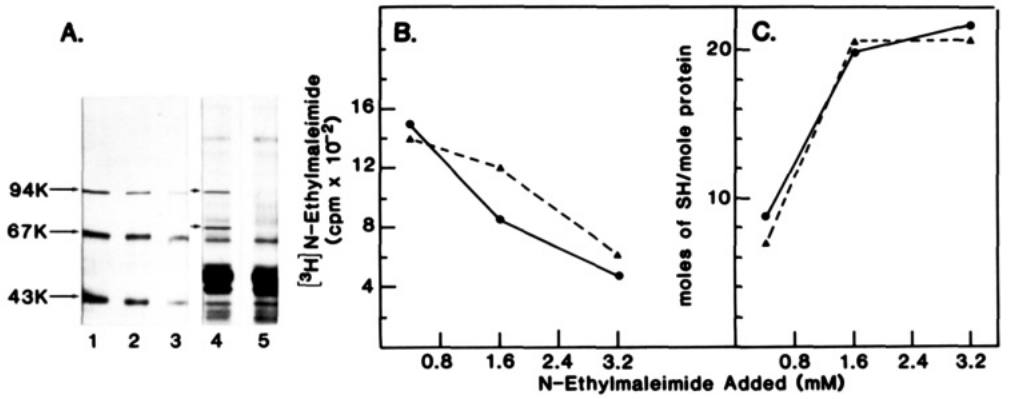

FIGURE 5: Concentration-dependent modification of immunopurified PR by *N*-ethylmaleimide. Receptor-containing ammonium sulfate pellets were prepared and immunoprecipitation was carried out as described under Experimental Procedures. The receptor–IgG complexes were heated in Tris–saline buffer (pH 7) containing SDS and increasing concentrations of DTT for 5 min, cooled, and incubated for 2 h at room temperature with increasing concentrations of *N*-ethylmaleimide. The ratio between DTT and *N*-ethylmaleimide was constant (1:4). All samples contained 10 μCi of [^{3}H]-*N*-ethylmaleimide. Modified receptor was further purified by SDS gel electrophoresis, the gels were stained, and receptor-containing bands were excised and counted. Panel A: Silver-stained SDS gel, demonstrating the estimation of receptor concentration by comparing staining intensity of known amounts of molecular mass standard proteins with staining intensity of receptor-containing bands. (Lanes 1, 2, and 3) Three standards (200, 100, and 50 ng, respectively): phosphorylase B (94 kDa), bovine serum albumin (67 kDa), and ovalbumin (43 kDa); (lane 4) immunoprecipitation of receptor-containing sample, in the presence of PR-22; (lane 5) immunoprecipitation of receptor-containing sample in the absence of PR-22. Panels B and C: *N*-Ethylmaleimide labeling data for PRA (▲---▲) and PRB (●—●). Panel B: [^{3}H]-*N*-Ethylmaleimide concentration dependent labeling of receptor bands. Panel C: Calculation of moles of SH per mole of receptor from the data of panels A and B.

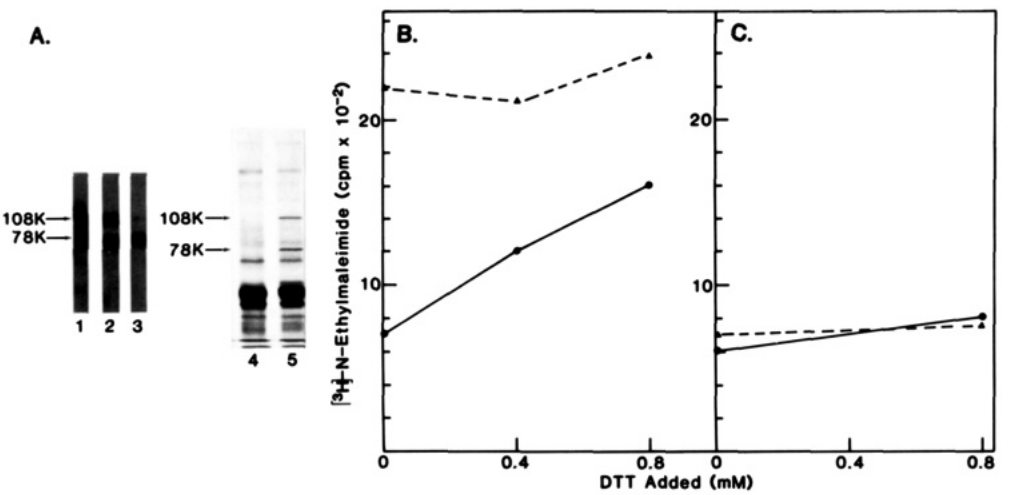

FIGURE 6: Detection of oxidized PRB in DTT-containing receptor preparations. Chick oviduct cytosol was prepared in TEG buffer containing 5 mM DTT. Receptor-enriched ammonium sulfate pellets were prepared and redissolved in the same buffer. A sample from this preparation was analyzed for DNA binding activity after chromatography on DNA–cellulose, by Western immunoblots. Immunoprecipitation of receptor from this preparation also was performed in the same buffer at a final NaCl concentration of 100 mM, and in the presence of 10 μg of plasmid DNA. Panel A shows autoradiograms of the Western immunoblot: receptor was analyzed in the starting material (lane 1), in the DNA–cellulose flow-through (lane 2), and in the material eluted from DNA–cellulose by 0.3 M NaCl (lane 3). Immunoprecipitation was performed as described under Experimental Procedures. Immunoprecipitated samples are shown in panel A: (lane 4) immunoprecipitation in the absence of antibody PR-22; (lane 5) immunoprecipitation in the presence of antibody PR-22. The receptor–IgG complexes were heat denatured in the presence of SDS, with or without DTT, cooled, and incubated 2 h at room temperature with a mixture of 3.2 mM *N*-ethylmaleimide and 10 μCi of [^{3}H]-*N*-ethylmaleimide (panel B) or directly subjected to incubation with the *N*-ethylmaleimide mixture (panel C). The reaction was terminated by heating the samples in Laemmli buffer. Samples were further purified by SDS gel electrophoresis and analyzed as described in Figure 2 for PRB (●—●) and PRA (▲---▲).

in labeling of PRA occurred after reduction, suggesting that this receptor form is oxidized to a lesser extent. Panel C shows lower labeling values of receptor proteins under nondenaturing conditions and no evident increase in labeling due to reduction by DTT.

We wished to determine if the in vitro oxidation treatment, which abolished DNA binding activity of PRA, would indeed produce oxidized residues on this subunit. Cytosol was prepared in TEG buffer in the absence of thiols, and receptor was concentrated by ammonium sulfate precipitation. The ammonium sulfate pellet was resuspended in buffer TEG in the absence of thiols and incubated at 4 °C in an uncovered container for 2 h. Figure 7, panel A, shows the analysis of DNA binding activity in the preparation by Western immunoblot: lane 1 shows that the starting material contained equal amounts of PRA and PRB. This material was applied to a DNA–cellulose column. Lane 2 shows the material flowing through the DNA–cellulose column, which contained equal amounts of PRA and PRB. Lane 3 shows that there was no detectable immunoreactivity in the material that was eluted from the DNA–cellulose by 0.3 M NaCl. The results indicate that PRA and PRB lost their DNA binding activity completely by the treatment described above.

Panel B shows the results of labeling by [^{3}H]-*N*-ethylmaleimide of this receptor preparation after immunoprecipitation. Labeling of SDS-denatured receptor with and

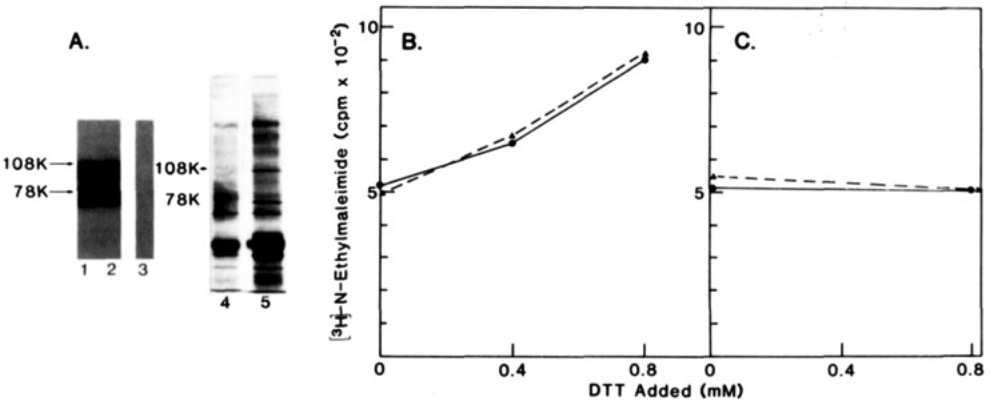

FIGURE 7: Detection of oxidized progesterone receptor A in receptor preparations lacking DTT. Chick oviduct cytosol was prepared in TEG without thiols. The ammonium sulfate pellets were resuspended in the same buffer and incubated for 2 h at 4 °C in open containers. A sample from this material was then analyzed for DNA binding activity, as described in Figure 6. Autoradiograms of the Western immunoblots of samples fractionated by the DNA–cellulose column are shown in panel A: (lane 1) starting material; (lane 2) DNA–cellulose flow-through; (lane 3) material eluted from DNA–cellulose by 0.3 M NaCl. Immunoprecipitation was carried out in the same buffer. Silver-stained samples are shown in panel A after SDS gel electrophoresis: (lane 4) immunoprecipitation without antibody PR-22; (lane 5) immunoprecipitation with antibody PR-22. Immunoprecipitated samples were subjected to modification by a mixture of 3.2 mM *N*-ethylmaleimide and 10 μCi of [^{3}H]-*N*-ethylmaleimide exactly as described in legend to Figure 5. Panel B: Modification of SDS and heat-denatured receptor. Panel C: Modification of nondenatured receptor. Data are shown for PRB (●—●) and PRA (▲---▲).

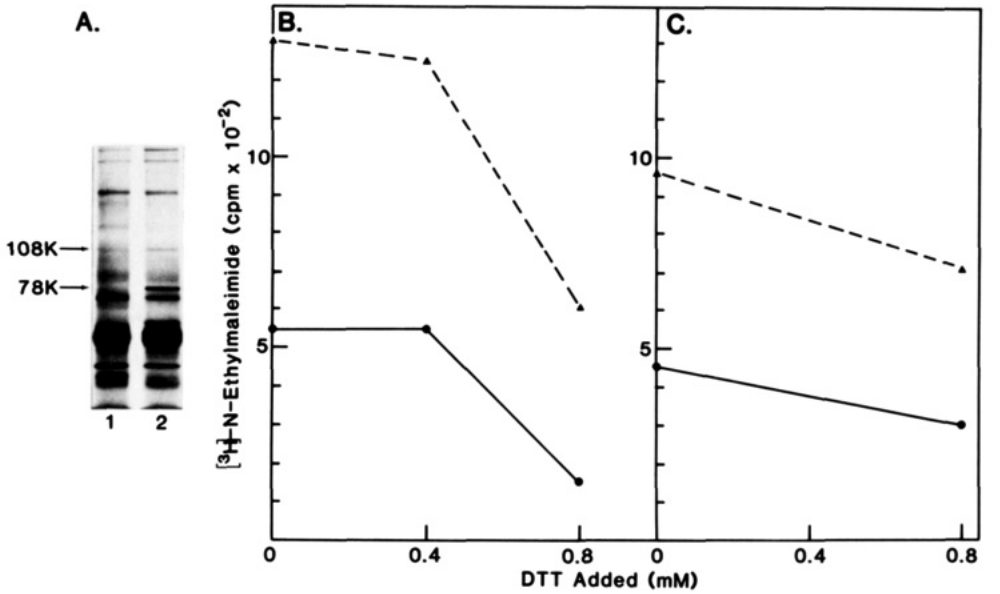

FIGURE 8: Modification by *N*-ethylmaleimide of progesterone receptor eluted from a DNA–cellulose column. Chick oviduct cytosol was prepared in TEG containing 5 mM DTT. Ammonium sulfate pellets prepared from that cytosol were redissolved in the same buffer and immediately applied to a DNA–cellulose column. The DNA-bound material was eluted by 0.3 M NaCl and concentrated by precipitation in ammonium sulfate at a final saturation of 50%. The pellets were resuspended in the same buffer and incubated in the presence of 10 μg of plasmid DNA (pBR322) during the immunoprecipitation. Analysis of the immunoprecipitated material after SDS gel electrophoresis and silver staining is shown in panel A: (lane 1) immunoprecipitation in the absence of antibody PR-22; (lane 2) immunoprecipitation in the presence of antibody PR-22. The immunoprecipitated receptor was subjected to modification by a mixture of 3.2 mM *N*-ethylmaleimide and 10 μCi of [^{3}H]-*N*-ethylmaleimide, exactly as described in legend to Figure 6. Panel B: Modification of SDS and heat-denatured samples. Panel C: Modification of nondenatured samples. Data are shown for PRB (●—●) and PRA (▲---▲).

without DTT was done as described above. In the experiment both PRA and PRB had a similar pattern: nearly a doubling in labeling of denatured receptor in the presence of DTT was seen for both forms. We therefore conclude that both receptor forms were oxidized in this preparation and oxidation was associated with a loss of DNA binding activity of PRA. Panel C shows that oxidized cysteine residues were not reduced by DTT in the native receptor molecules. Thus the oxidized residues were not accessible either to DTT or to maleimide or to both.

Most of the PRB (85–90%) and some of the PRA (30–40%) are found in a form that does not bind to DNA even when cytosol is prepared in the presence of thiols (see Figure 1). We wished to determine if these inactive receptor PRA and PRB molecules are indeed oxidized whereas the DNA binders are reduced.

For that purpose we prepared cytosol in TEG buffer containing DTT. Receptor was concentrated by ammonium sulfate precipitation. The pellet was resuspended in the same buffer and applied to a DNA–cellulose column to separate DNA binders from receptors unable to bind to DNA. Receptor able to bind to DNA was step-eluted from the DNA–cellulose column, concentrated again by ammonium sulfate precipitation, and resuspended in TEG buffer containing 5 mM DTT. Receptor in the DNA column flow-through was treated similarly.

Figure 8, panel A, shows the results of immunoprecipitation of DNA binding receptor molecules. As expected, the prep-

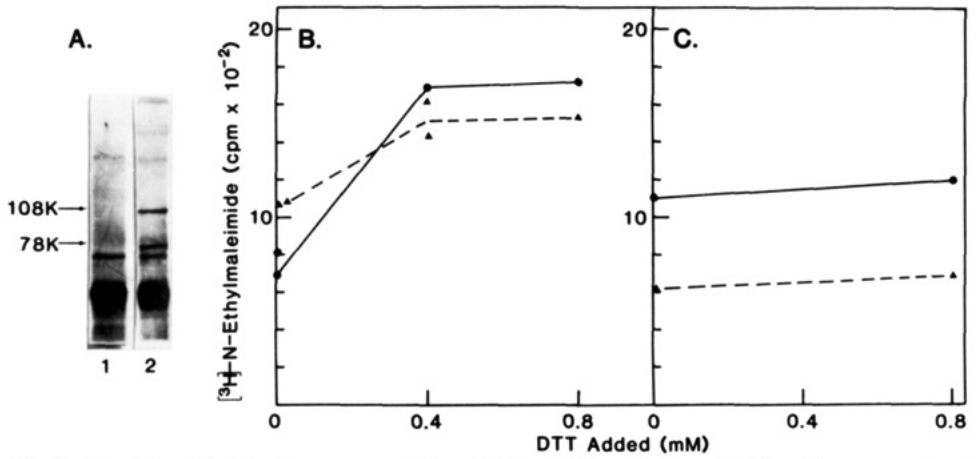

FIGURE 9: Modification by *N*-ethylmaleimide of receptor unable to bind to DNA–cellulose. Chick oviduct cytosol and ammonium sulfate pellets were prepared and DNA cellulose chromatography was performed as described in the legend to Figure 8. The DNA flow-through material was concentrated by ammonium sulfate precipitation, and immunoprecipitation was carried out after dissolving the pellets in TEG buffer containing DTT and DNA as described above. Analysis of the immunoprecipitated material after SDS gel electrophoresis and silver staining is shown in panel A: (lane 1) immunoprecipitation in the absence of antibody PR-22; (lane 2) immunoprecipitation in the presence of antibody PR-22. Immunoprecipitated receptor was subjected to modification by a mixture of 3.2 mM *N*-ethylmaleimide and 10 μCi of [^{3}H]-*N*-ethylmaleimide, exactly as described in the legend to Figure 4. Panel B: Modification of SDS and heat-denatured receptor. Panel C: Modification of nondenatured receptor. Data are shown for PRB (●—●) and PRA (▲---▲).

arations contained mostly PRA and a small proportion of PRB (lane 2).

This receptor preparation was denatured by SDS in the presence or absence of DTT and modified by [^{3}H]-*N*-ethylmaleimide. The results of this experiment are presented in panel B. These data showed that no increase in labeling of PRB or PRA DNA binders occurred in the presence of DTT, thus indicating that there are no oxidized residues on these receptor forms. The inhibition of *N*-ethylmaleimide labeling obtained by 0.8 mM DTT could be due to interaction of DTT with this reagent as discussed above. Panel C shows that labeling of nondenatured PRA and PRB was lower than that of the denatured receptor protein. Since it appeared that receptor proteins able to bind to DNA were fully reduced (panel B), we must conclude that some of the sulfhydryl groups were not accessible to *N*-ethylmaleimide in the nondenatured receptor species of that preparation.

Receptor unable to bind to DNA–cellulose (DNA flow-through material) was also immunopurified. Figure 9, panel A, shows the result of immunoprecipitation of receptor unable to bind to DNA–cellulose. Lane 2 shows, as expected, that this preparation contained both PRB and PRA.

Modification of the immunopurified, denatured receptor with and without DTT revealed, as shown in panel B, that both PRA and PRB were oxidized, since an increase of 90% in labeling of PRB by *N*-ethylmaleimide occurred in the presence of DTT, as well as an increase of 60% in labeling of PRA. Again, the oxidized residues were not available to DTT in the native molecules (panel C), since no increase in labeling of these molecules occurred in the presence of DTT.

The results of these experiments are summarized in Table I. To simplify the calculations, we assumed that only cysteine residues were modified and calculated moles of cysteine per mole of receptor protein. The amount of receptor protein recovered after gel electrophoresis was estimated by comparison with the staining intensity of standard protein as has been demonstrated in Figure 5. From Table I it is evident that the maximal number of cysteine residues recovered on denatured receptor was 21–23 per molecule. Oxidation was not detectable on PRA or PRB forms able to bind to DNA, but 10–12 of the cysteine residues were oxidized on PRA or PRB unable to bind to DNA. Similarly, 10 sulfhydryl groups were oxidized on PRA which lost in vitro DNA binding activity after exposure to an environment lacking reducing agents.

Table I: Determination of Sulfhydryl Groups on Different Forms of Chicken PR

receptor form	mol of SH/mol of receptor protein[a]			
	nonreduced			
	DNA binders[b]	non-DNA binders[c]	oxidized in vitro[d]	reduced in vitro
PRA	20.8 ± 2.2[e]	13.1 ± 1.7[f]	10.1 ± 1.5[e]	21.0 ± 2.6[g]
PRB	25 ± 1.1[e]	11.6 ± 1.7[f]	10.2 ± 1.7[e]	22.6 ± 3.1[g]

[a] Immunoprecipitated receptor, heat denatured with SDS, in the absence of DTT (nonreduced) or in the presence of DTT (reduced in vitro), was modified by a mixture of 3.2 mM *N*-ethylmaleimide and 10 μCi of [^{3}H]-*N*-ethylmaleimide. The samples were further purified by SDS gel electrophoresis, and receptor-containing gel slices were processed as described under Experimental Procedures. The values shown are the mean of *N* determinations ± SD. [b] PRA and PRB were prepared as described in Figure 8. [c] PRA and PRB were prepared as described in Figure 9. [d] PRA and PRB were prepared as described in Figure 7. [e] $N = 4$. [f] $N = 3$. [g] $N = 11$.

DISCUSSION

The objective of this study was to define the molecular basis for the difference between chicken PR forms that have binding activity to DNA and PR forms unable to bind to DNA. Originally, it was thought that the DNA binding activity was unique to PRA (78 kDa) while PRB (108 kDa) lacked this activity. However, a recent study showed that a significant population of PRB did have DNA binding activity that was at least comparable to that of the PRA (Gronemeyer et al., 1985).

We confirm in this paper that a fraction of PRB molecules possess DNA binding activity. However, this fraction does not exceed about 15%, regardless of the incubation conditions used.

In addition, the analytical methods being employed in this study revealed that although most of the PRA molecules do bind to DNA–cellulose, a fraction of this form did not possess this activity. It thus appears that the two forms exist in the chicken oviduct cytosol both in a state in which they bind to DNA and in a state in which they do not. The fraction of either PRA or PRB that does not bind to DNA did not acquire this activity upon "activation" by either heat or salt treatment. In addition, filtration of receptor through Sephadex G-25 or

Sephadex G-100, which removed an inhibitor of DNA binding in the case of the glucocorticoid receptor (Cake et al., 1976; Bailly et al., 1977), failed to increase DNA binding activity of chicken progesterone receptor (not shown).

We considered the possibility that the DNA-negative receptors are formed due to irreversible inactivation during in vitro manipulation. In search for an explanation for this event, we considered oxidation as a possible mechanism. It has been shown before that activation of hamster progesterone receptor into a DNA binding state required thiols (MacDonald & Leavitt, 1982). Another study showed an inhibition of DNA binding of activated chicken progesterone receptor by sulfhydryl-reactive reagents (Coty et al., 1983). Here, we show that DNA binding of the activated chicken progesterone receptor is extremely sensitive to oxidation, thus confirming the importance of sulfhydryl groups for DNA binding activity of progesterone receptor. The DNA binding of PRB appeared to be more labile than that of PRA; a higher concentration of thiols was required to maintain any PRB in a DNA binding form. But all concentrations of thiols tested failed to increase the proportion of receptor B able to bind to DNA over about 15%. An explanation for these results could be that active and inactive forms of receptor exist already in vivo and thiols cannot alter the inactive DNA binding site in vitro. Another explanation could be that DNA binding of receptor requires an additional cofactor that is being lost during receptor preparation.

To test for alterations in free SH group content, we immunopurified and modified receptor protein by *N*-ethylmaleimide, which is highly specific for cysteines under the conditions employed (Means & Feeney, 1971). We found that modification of receptor in its native form was incomplete, as had been reported for several other proteins (Fernandez-Diez et al., 1962). Therefore, the determination of sulfhydryl groups needed to be performed on the denatured receptor protein. We determined 21–22 sulfhydryl groups on the denatured and reduced PRA or PRB. This number is very close to the number of cysteines predicted from the cDNA sequence of the chicken oviduct progesterone receptor gene (Conneely et al., 1987), thus confirming the reliability of the method employed in this study for determination of cysteine residues.

We found that denatured and nonreduced receptor forms unable to bind to DNA had only 10–12 sulfhydryl groups. However, a final number of 21–22 sulfhydryl groups was found on these same receptor molecules in the presence of thiols. The number of sulfhydryl groups on nonreduced receptor forms that had DNA binding activity was 21–25, and no additional sulfhydryl groups were detected on these receptor species by the addition of thiols. We concluded that receptor in a form unable to bind to DNA contained 10–12 oxidized cysteines while oxidation of cysteines in the DNA binders was undetectable.

We were unable to reduce the oxidized receptor in its native form, which suggests that the oxidized residues (likely engaged in disulfide bridges) are not accessible to the thiols. These results can explain why loss of DNA binding activity of receptor is irreversible by thiols in vitro.

The study cannot explain why PRB is more sensitive to oxidation than PRA. Originally, we thought that PRB might contain some additional cysteines which make it more susceptible to oxidation. Indeed, the amino acid sequence of PRB as predicted from the nucleic acid sequence of the cloned cDNA of chicken progesterone receptor does show two additional cysteines in the N-terminal portion of the molecule; these are not included in the shorter sequence representing PRA protein (Conneely et al., 1987). A difference in cysteine content between PRA and PRB was detected in our determinations (see Table I). The average number (from 10 determinations) of cysteines determined on PRA was 21. The average number of cysteines on PRB (11 determinations) was 22.6. However, due to variation between experiments in the determination of sulfhydryl groups and receptor concentration, this difference was not statistically significant.

Another explanation for the higher sensitivity of PRB to oxidation could be that this receptor form has a different conformation from that of PRA. Evidence for such a difference comes from a recent study by Puri and Toft (1986). That study showed that PRA and PRB had very similar peptide maps but were phosphorylated in vivo in different sites. This finding indicates that phosphorylation sites on the two molecules are not similarly exposed to kinases.

Oxidation of receptor molecules apparently leads to an inactivation of the DNA binding site, an event that is irreversible in vitro. Since we had an efficient maintenance of DNA binding activity of receptor proteins in the presence of DTT, we were unable to explain how receptor forms unable to bind to DNA were formed even when purification was carried out in the continuous presence of this thiol. One possible explanation could be that the oxidized and nonoxidized receptor species exist already in the unbroken cells, and the transition from one form to another is important for steroid receptor function in vivo. Grippo et al. (1983, 1985) have reported that an NADPH-dependent thioredoxin thioreductase system is essential to maintain the glucocorticoid receptor in a steroid binding state (in the absence of thiols). This enzyme or the reduced thioredoxin is associated with receptor, and its removal caused receptor inactivation. It is possible that a similar system might be important for the chicken progesterone receptor to maintain its biological function. If this were the case, any factor in cells that affects the levels of thioredoxin itself or thioreductase activity might cause a transition of receptor from oxidized to reduced state and vice versa, thereby affecting the functional state of this regulatory molecule.

Finally, these studies are consistent with—but do not prove—a role for the receptor cysteine-rich region as a functional DNA binding site in vivo. The chicken progesterone receptor, for example, also binds strongly to a non-histone chromosomal protein in chick oviduct chromatin. This "acceptor protein" (Thrall & Spelsberg, 1980) is required for high-affinity binding of chicken PR to chromatin in vitro. Such an interaction may be required for effective alteration of target gene promoter function as we have proposed previously (Schrader et al., 1981). Similarly, the glucocorticoid receptor copurifies with an associated protein whose removal dramatically reduces DNA binding activity (Wrange et al., 1986). Thus, it remains a possibility that receptor SH oxidation affects DNA binding in part by changing the interaction of the protein with accessory proteins. This hypothesis is under active investigation at this time.

ACKNOWLEDGMENTS

We thank Dr. D. O. Toft for the generous gift of antibody PR-22. We are grateful to Greg Caldwell and Ernest Charles for expert technical assistance and to Cheryl McCarthy for typing the manuscript.

Registry No. Cys, 52-90-4; progesterone, 57-83-0.

REFERENCES

Bailly, A., Sallas, N., & Milgrom, E. (1977) *J. Biol. Chem.* *252*, 858–863.

Birnbaumer, M., Schrader, W. T., & O'Malley, B. W. (1983) *J. Biol. Chem. 258*, 1637–1644.

Birnbaumer, M., Hinrichs-Bosello, M. V., Cook, R. G., Schrader, W. T., & O'Malley, B. W. (1987) *Mol. Endocrinol. 1*, 249–259.

Cake, M. H., Goidl, J. A., Parchman, L. G., & Litwack, G. (1976) *Biochem. Biophys. Res. Commun. 71*, 45–52.

Compton, J. G., Schrader, W. T., & O'Malley, B. W. (1984) *J. Steroid Biochem. 20*, 89–94.

Conneely, O. M., Sullivan, W. P., Toft, D. O., Birnbaumer, M., Cook, R. G., Maxwell, B. L., Zarucki-Schulz, T., Greene, G. L., Schrader, W. T., & O'Malley, B. W. (1986) *Science (Washington, D.C.) 233*, 767–770.

Conneely, O. M., Dobson, A. D. W., Tsai, M.-J., Beattie, W. G., Toft, D. O., Huckaby, C. S., Zarucki, T., Schrader, W. T., & O'Malley, B. W. (1987) *Mol. Endocrinol. 1*, 517–525.

Coty, W. A., Klooster, T. A., Griest, R. E., & Profita, J. A. (1983) *Arch. Biochem. Biophys. 225*, 748–757.

Fernandez-Diez, M. J., Osuga, D. T., & Feeney, R. E. (1964) *Arch. Biochem. Biophys. 107*, 449–456.

Green, S., & Chambon, P. (1987) *Nature (London) 325*, 75–78.

Greene, G. L., Gilna, P., Waterfield, M., Baker, A., Hort, Y., & Shine, J. (1986) *Science (Washington, D.C.) 231*, 1150–1153.

Grippo, J. F., Tienrungroj, W., Dalmer, M. K., Housley, P. R., & Pratt, W. B. (1983) *J. Biol. Chem. 258*, 13658–13664.

Grippo, J. F., Holmgren, A., & Pratt, W. B. (1985) *J. Biol. Chem. 260*, 93–97.

Gronemeyer, H., Govindan, M. V., & Chambon, P. (1985) *J. Biol. Chem. 260*, 6916–6925.

Hollenberg, S. H., Weinberger, C., Ong, E. S., Cerelli, G., Oro, A., Lebo, R., Thompson, E. B., Rosenfeld, M. G., & Evans, R. M. (1985) *Nature (London) 318*, 635–641.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

MacDonald, R. G., & Leavitt, W. W. (1982) *J. Biol. Chem. 257*, 311–315.

Means, G. E., & Feeney, R. E. (1971) in *Chemical Modification of Proteins*, pp 110–114, Holden-Day, San Francisco, CA.

Miesfeld, R., Rusconi, S., Godowski, P. J., Maler, B. A., Okret, S., Wikstrom, A. C., Gustafsson, J. A., & Yamamoto, K. R. (1986) *Cell (Cambridge, Mass.) 46*, 389–399.

Miesfeld, R., Goodowski, P. J., Maler, B. A., & Yamamoto, K. R. (1987) *Science (Washington, D.C.) 236*, 423–427.

Pike, T. W. (1981) *Biochem. Biophys. Res. Commun. 100*, 1713–1719.

Puri, R. K., & Toft, D. O. (1986) *J. Biol. Chem. 261*, 5651–5657.

Schrader, W. T., Toft, D. O., & O'Malley, B. W. (1972) *J. Biol. Chem. 247*, 2401–2407.

Schrader, W. T., Birnbaumer, M. W., Hughes, M. R., Weigel, N. L., Grody, W. W., & O'Malley, B. W. (1981) *Recent Prog. Horm. Res. 37*, 583–633.

Smyth, D. G., Nagamatsu, A., & Fruton, J. S. (1960) *J. Am. Chem. Soc. 82*, 4600–4610.

Sullivan, W. P., Beito, T. G., Proper, J., Krco, D., & Toft, D. O. (1986) *Endocrinology (Baltimore) 119*, 1549–1557.

Thrall, G. T., & Spelsberg, T. C. (1980) *Biochemistry 19*, 4130–4138.

Toft, D. O., Roberts, P. E., Nishigori, H., & Mougil, V. K. (1979) *Adv. Exp. Med. Biol. 117*, 329–336.

Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.

Vedeckis, W. V., Schrader, W. T., & O'Malley, B. W. (1980) *Biochemistry 19*, 343–349.

Von der Ahe, D., Janich, S., Scheidereit, C., Renkawitz, R., Schutz, G., & Beato, M. (1985) *Nature (London) 313*, 706–709.

Wrange, O., Carlstedt-Duke, T., & Gustafsson, J. A. (1986) *J. Biol. Chem. 261*, 11770–11778.