Saier, M. H., Jr., Cox, D. F., & Moczydlowski, E. G. (1977b) *J. Biol. Chem. 252*, 8908–8916.
Saier, M. H., Jr., Cox, D. F., Feucht, B. U., & Novotry, M. J. (1982) *J. Cell. Biochem. 18*, 231–238.
Scholte, B. J., & Postma, P. W. (1981) *Eur. J. Biochem. 114*, 51–58.
Scholte, B. J., Schuitema, A. R. J., & Postma, P. W. (1982) *J. Bacteriol. 149*, 576–586.
Simoni, R. D., Hays, J. B., Nakazawa, T., & Roseman, S. (1973) *J. Biol. Chem. 248*, 957–965.
Waygood, E. B., Meadow, N. D., & Roseman, S. (1979) *Anal. Biochem. 95*, 293–304.

Purification and Characterization of the Androgen Receptor from Rat Ventral Prostate†

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ABSTRACT: The androgen receptor has been purified from rat ventral prostate cytosol by a combination of differential DNA–Sepharose 4B chromatography and testosterone 17β-hemisuccinyl-3,3′-diaminodipropylamine–Sepharose 4B affinity chromatography. Approximately 8 μg of protein was obtained from 38 g of rat ventral prostate, with a yield of 24%. The receptor was purified approximately 120 000-fold. Silver nitrate staining of a sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel revealed a major polypeptide band migrating at 86 000 daltons. Affinity labeling of a partially purified receptor preparation with either 17-hydroxy-17α-[^{3}H]methyl-4,9,11-estratrien-3-one or 17β-hydroxy-[1,2,4,5,6,7,16,17-$^3H_8$]-5α-androstan-3-one 17-(2-bromoacetate) produced a major band of radioactivity migrating at 86 000 daltons on a $NaDodSO_4$ gel. Under nondenaturing conditions, a M_r of 85 000 was determined by gel filtration (42 Å) and sucrose gradient sedimentation analysis (4.5 S). The purified receptor had an isoelectric point of 6.3. [^{3}H]-4,5α-Dihydrotestosterone, bound to the purified receptor, was displaced with 4,5α-dihydrotestosterone > testosterone ≫ progesterone > 5α-androstane-3α,17β-diol > 17β-estradiol > cortisol. A number of physicochemical properties of the purified receptor were similar to those of the receptor in crude cytosol.

Receptor proteins are essential for the biological function of androgens in male accessory sex organs [review articles by Liao et al. (1975), Mainwaring (1978), and Chan & Tindall (1981)]. By defining the molecular properties of these proteins, we will further our understanding of the mechanism by which receptors mediate biological events within target tissues. Recently, we developed a procedure to purify an androgen receptor from steer seminal vesicle to apparent homogeneity as determined by $NaDodSO_4$[1] gel electrophoresis (Chang et al., 1982). This procedure combined two techniques, differential DNA chromatography and steroid affinity chromatography, which had been used for purifying other steroid hormone receptors (Coty et al., 1979; Wrange et al., 1979; Westphal & Beato, 1980; Kuhn et al., 1975; Govindan & Sekeris, 1978; Sica & Bresciani, 1979). Because the rat prostate has been one of the most widely studied male accessory glands (Baulieu & Jung, 1970; Fang & Liao, 1971; Mainwaring & Irving, 1973; Tindall et al., 1975; Wilson & French, 1976, 1979), the purification of the prostate receptor would represent an important step in our efforts to understand the mechanism of androgen action in this target tissue. This paper describes the purification of the androgen receptor from rat ventral prostate cytosol and the characterization of a number of its physicochemical and steroid-binding properties.

Experimental Procedures

Materials. [1,2,4,5,6,7-$^3H_6$]Dihydrotestosterone, 143 Ci/mmol, was purchased from Amersham. 17-Hydroxy-17α-[^{3}H]methyl-4,9,11-estratrien-3-one ([^{3}H]R1881, 87 Ci/mmol), nonradioactive R1881, and Enhance were obtained from New England Nuclear (all other steroids were from Steraloids). Leupeptin was a gift from the United States–Japan Cooperative Cancer Research Program. 17β-Hydroxy-[1,2,4,5,6,7,16,17-$^3H_8$]-5α-androstan-3-one 17-(2-bromoacetate) (147 Ci/mmol) and 17β-hydroxy-5α-androstan-3-one 17-(2-bromoacetate) were synthesized and provided by Dr. Thomas J. Lobl of the Upjohn Co. All other reagents were of analytical grade.

Buffers. The following buffers were used: TED buffer, 50 mM Tris-HCl buffer containing 1.5 mM EDTA and 1.5 mM dithiothreitol (pH 7.4 at 22 °C); TEDG buffer, TED buffer containing 20% glycerol; TEDK buffer, TED buffer containing 0.5 M NaCl; sodium borate buffer, 25 mM sodium borate (pH 8.0 at 22 °C); imidazole buffer, 25 mM imidazole hydrochloride (pH 7.4 at 22 °C).

Preparation of Cytosol. Male Sprague-Dawley rats (3–4 months old) were sacrificed 24 h after orchiectomy. Ventral prostate lobes were removed and frozen quickly in liquid ni-

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[1] Abbreviations: dihydrotestosterone, 4,5α-dihydrotestosterone; [^{3}H]R1881, 17-hydroxy-17α-[^{3}H]methyl-4,9,11-estratrien-3-one; R1881, 17-hydroxy-17α-methyl-4,9,11-estratrien-3-one; [^{3}H]dihydrotestosterone 17β-bromoacetate, 17β-hydroxy[1,2,4,5,6,7,16,17-$^3H_8$]-5α-androstan-3-one 17-(2-bromoacetate); leupeptin, a mixture of *N*-acetyl- and *N*-propionyl-L-leucyl-L-leucyl-DL-arginine aldehyde hydrochlorides; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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trogen prior to storage at −90 °C. Cytosol was prepared in TEDG buffer containing 10 μg/mL leupeptin as described previously (Chang et al., 1982).

Preparation of Affinity Resins. Two affinity resins containing either calf thymus DNA or testosterone 17β-hemisuccinate linked via 3,3′-diaminodipropylamine were used for the receptor purification. The procedures for synthesizing each affinity resin have been described previously (Chang et al., 1982).

Purification of Androgen Receptor. The protocol for purifying the prostate androgen receptor followed a procedure described previously for purifying the androgen receptor from steer seminal vesicle (Chang et al., 1982). The testosterone 17β-hemisuccinate affinity resin had a capacity of 10 pmol of androgen receptor/mL of packed resin as determined by saturation analysis. The dissociation constant of the receptor–resin complex obtained by Scatchard analysis was 2.5 nM, which was identical with the dissociation constant of the [^{3}H]testosterone–receptor complex (2.5 nM) obtained by Scatchard analysis (data no shown). These findings indicate that the receptor bound to the affinity resin via the hormone binding site. Moreover, the receptor could be eluted specifically from the resin with testosterone and 4,5α-dihydrotestosterone but not with progesterone, 17β-estradiol, or cortisol.

Polyacrylamide Gel Electrophoresis. Gel electrophoresis under denaturing conditions was performed in a slab gel by the procedure of Laemmli (1970) in which $NaDodSO_4$ was incorporated in the gel and buffer. Gel electrophoresis under nondenaturing conditions was performed in a slab gel with Laemmli's system except that $NaDodSO_4$ was excluded from all buffers. The acrylamide concentration in the running gel was 5% with a constant acrylamide/*N*,*N*′-methylenebis-(acrylamide) ratio of 30:1. The stacking gel contained 2.5% acrylamide. The electrophoresis was performed at 4 °C with 2 mA per lane in a Bio-Rad polyacrylamide gel electrophoresis apparatus. Heparin (0.01%) was added to the upper electrophoretic buffer to reduce aggregation and to enhance the penetration of receptor into the gel. After electrophoresis, one lane was sliced in 3-mm sections and incubated in 5 mL of tolune solution containing liquifluor (New England Nuclear, Inc.) for scintillation counting. A parallel lane was stained with silver nitrate as described previously by Wray et al. (1981).

Affinity Labeling. Affinity labeling the androgen receptor has been described previously (Chang et al., 1982). The receptor was precipitated with 45% ammonium sulfate, resuspended in 10 mM phosphate buffer (pH 7.4), and incubated with either 10 nM of the alkylating affinity label, [^{3}H]dihydrotestosterone 17β-bromoacetate, or 20 nM of the photoaffinity label, [^{3}H]R1881, in the presence or absence of 100-fold excess unlabeled ligand. Samples affinity labeled with [^{3}H]dihydrotestosterone 17β-bromoacetate were incubated at 0 °C for 2.5 h and further incubated at 23 °C for 30 min. Samples affinity labeled with [^{3}H]R1881 were first incubated at 30 °C for 30 min to exchange the [^{3}H]R1881 with unlabeled testosterone previously bound to the receptor. After being cooled for 10 min at 0 °C, the [^{3}H]R1881-labeled receptor was photolyzed for 30 min in an ice bath (0 °C) with an 85-W low-pressure mercury-vapor lamp (General Electric H85A3). All samples were treated with 10% trichloroacetate for 15 min and centrifuged at 1500*g* for 20 min. The precipitant was redissolved in sample buffer and applied to a $NaDodSO_4$ gel for electrophoresis as described in the previous section except that diallyltartardiimide was used as a cross-linker. Following electrophoresis, gels were washed extensively with 40% methanol–7% acetate to remove unbound steroid, treated with Enhance (New England Nuclear, Inc.), dried, and exposed to Kodak X-oMat AR film.

Molecular Weight Determinations. Gel-filtration chromatography was carried out in either an agarose A-1.5m (1.8 × 50 cm) or a Sephacryl S-200 (2.5 × 50 cm) column (Chang et al., 1982). Glycerol gradient sedimentation was preformed on gradients (5 mL) containing 5–20% glycerol in imidazole buffer containing 0.02% heparin. Stokes radii and sedimentation coefficients of standard proteins were used to determine molecular weights and frictional ratios as described by Siegel & Monty (1966). Gel-exclusion high-performance liquid chromatography (TSK-G3000SW, Beckman, Inc.) of the purified receptor was performed as described previously (Pavlik et al., 1982).

Binding Assays. Androgen receptor activity was determined with a charcoal binding assay as described by Korenman (1969) with minor modifications. Receptor preparations (250 μL) were incubated with 250 μL of TEDG buffer and 16 nM [^{3}H]dihydrotestosterone ± 1.6 μM unlabeled dihydrotestosterone for 4 h at 2 °C. After 10-min treatment with an equal volume of charcoal solution (1% charcoal, 0.1% dextran T-70, 0.1% gelatin, 1.5 mM EDTA, and 50 mM Tris-HCl, pH 7.4 at 22 °C), samples were centrifuged at 1500*g* for 15 min. The supernatant fluid was decanted for radioactivity determination. All receptor preparations following testosterone affinity chromatography were assayed for binding radioactivity by a steroid exchange assay. In order to reduce the loss of binding activity during charcoal treatment, ovalbumin (2 mg/mL) was routinely added to the sample. Samples were then incubated with equal volumes of charcoal solution for 10 min at 0 ~2 °C. After centrifugation at 1500*g* for 15 min, supernatant fluids were incubated with 16 nM [^{3}H]dihydrotestosterone plus or minus 1.6 μM unlabeled dihydrotestosterone for 30 min at 30 °C. The incubates were subjected to charcoal treatment as described previously. All samples were treated with ACS (Amersham) and counted in a Beckman LS300 liquid scintillation counter (40% counting efficiency).

Scatchard Analyses. Scatchard analysis was performed according to the method of Scatchard (1949). Cytosol was incubated with various concentrations of [^{3}H]dihydrotestosterone plus or minus 100-fold excess unlabeled dihydrotestosterone for 4 h at 2 °C. In order to reduce the loss of binding activity during charcoal treatment, the purified receptor preparation was mixed with boiled cytosol (2 mg/mL; proven to have no [^{3}H]dihydrotestosterone binding by Scatchard analysis) and precipitated with 45% ammonium sulfate. The pellet was resuspended in TEDG buffer for binding analysis. The binding activity was determined by the steroid exchange assay described previously.

Protein Determination. The quantity of protein in each sample was determined by the procedure of Lowry et al. (1951). Bovine serum albumin was used as the protein standard.

Results

Purification of Receptor. Cytosol prepared from 38 g of rat ventral prostate was passed through a DNA column (DNA I). This step resulted in a 0.8-fold purification and a 78% recovery of binding activity (Table I). The DNA I flow-through fractions were precipitated with ammonium sulfate at 40% saturation. This step resulted in an overall purification of 16.5-fold and a recovery of approximately 100%. Ammonium sulfate precipitation also served to transform the receptor to a DNA-binding state. Testosterone affinity chromatography

Table I: Purification of Cytoplasmic Androgen Receptor from Rat Prostate

purification step	total protein (mg)	total receptor sites (pmol)	sp act. (pmol/mg)[a]	yield (%)	purification (x-fold)
cytosol	4050[b]	370[c]	0.091	100	1.0
DNA-I flow-through	3978[b]	290[c]	0.073	78	0.8
40% pellet	248[b]	380[c]	1.5	100	16.5
affinity eluate	2.6[b]	110[d]	42	30	4.6×10^2
DNA-II pyridoxal 5′-phosphate eluate	0.008[e]	87[d]	10900	24	1.2×10^5

[a] For a protein of M_r 86 000, the maximum theoretical specific activity is 11 600 pmol/mg. [b] Protein was assayed by the procedure described under Experimental Procedures. [c] Receptor binding activity was assayed by the charcoal binding assay described under Experimental Procedures. [d] Receptor binding activity was assayed by the steroid exchange assay described under Experimental Procedures. [e] Protein was estimated from the silver nitrate staining (Wray et al., 1981) on the $NaDodSO_4$ gel as compared with bovine serum albumin at different concentrations.

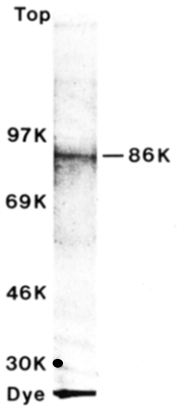

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of a purified receptor preparation from rat ventral prostate. The receptor was purified by the procedure described under Results. Peak fractions of androgen-binding activity from the second DNA column were precipitated with 10% trichloroacetate, and approximately 0.5 μg of protein was applied to a $NaDodSO_4$ gel containing 7.5% acrylamide (Laemmli, 1970). The following protein standards were used for molecular weight calibration: phosphorylase B, 97 000; bovine serum albumin, 69 000; ovalbumin, 46 000; carbonic anhydrase, 30 000. The gel was stained with silver nitrate as described under Experimental Procedures.

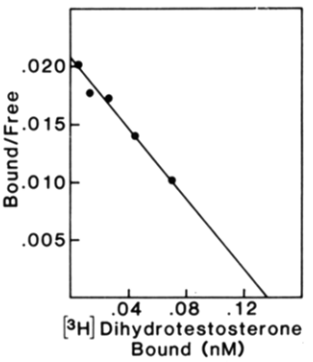

FIGURE 2: Scatchard analysis of purified receptor. The receptor was purified as described under Results and incubated with 0.4, 1, 2, 4, or 8 nM [^{3}H]dihydrotestosterone ± 100-fold excess unlabeled dihydrotestosterone for 4 h at 0 to ~2 °C. Specific binding was determined as described under Experimental Procedures.

Table II: Dissociation Constant, Isoelectric Point, and Molecular Size of the Androgen Receptor from Rat Prostate Cytosol

property	purified receptor[a]	cytosol
dissociation constant (nM)	6.5[b]	3.6[c]
isoelectric point (pI)[d]	6.3	6.5
Stokes radius (Å)[e]	42	42
sedimentation coefficient (S)[e]	4.5	4.5
molecular weight (M_r)[f]	85000	85000
frictional ratio (f/f_0)[f]	1.41	1.41

[a] The final product of the receptor purification, as described in Table I, was used in these experiments. [b] Purified receptor plus boiled cytosol, which had been proven to have no binding to [^{3}H]dihydrotestosterone by Scatchard analysis, was used for determining the dissociation constant by the method of Scatchard (1949). [c] Ventral prostate cytosol was incubated with various concentrations of [^{3}H]dihydrotestosterone ± 100-fold excess unlabeled dihydrotestosterone for 4 h. Bound activity was determined by a charcoal binding assay, and the dissociation constant was determined by the method of Scatchard (1949). [d] Chromatofocusing, as described previously (Chang et al., 1982), was used to determine the isoelectric point of the receptor. [e] These parameters were determined as described under Experimental Procedures. [f] Calculated from the combined values of Stokes radius and sedimentation coefficient.

was next performed. The overall purification at this point was 460-fold, and a 30% yield of binding activity was obtained. Binding activity of the purified receptor at this stage of purification may have been underestimated due to the presence of a large excess of testosterone. The testosterone affinity resin eluate was applied to a second DNA column (DNA II) and eluted with 10 mM pyridoxal 5′-phosphate in sodium borate buffer. This purification step resulted in an overall purification of 120 000-fold and a yield of 24%. This unusually large purification resulted from the selective elution of receptor by pyridoxal 5′-phosphate. In contrast, elution with sodium chloride resulted in only a 5000-fold purification. Approximately 8 μg of purified protein was obtained by using this protocol. The purified receptor was stable for more than 2 weeks when stored at 2 °C. When the purified material was electrophoresed through a $NaDodSO_4$ gel, a major polypeptide band corresponding to a molecular weight of 86 000 was observed by a silver nitrate staining procedure (Figure 1).

Characterization of Purified Receptor. We examined the binding properties of the purified receptor. Scatchard analysis of the purified receptor revealed a high-affinity binding component with a K_d of 6.5 nM (Figure 2), which was slightly higher than that found for the receptor in cytosol (K_d = 3.6 nM) (Table II). The higher K_d value may have resulted from the removal of some factor during the purification procedure since a similar change in K_d was noted following ammonium sulfate precipitation. On the basis of the receptor concentration (~0.14 nM) taken from the abscissa intercept of the Scatchard plot and a molecular weight of 86 000 determined from $NaDodSO_4$ gel electrophoresis, it was estimated that there is 0.8 mol of binding site/mol of protein. These data suggest that there is one hormone binding site per receptor molecule. The specificity of steroid binding to the purified receptor is shown in Table III. Testosterone exhibited less binding affinity (84%) than that of dihydrotestosterone (100%). Progesterone was not very effective in competing with [^{3}H]dihydrotestosterone for receptor binding (24%). Even less

Table III: Steroid Specificity of Purified Androgen Receptor from Rat Prostate Cytosol

steroid	relative affinity (%)[a] purified receptor[b]	cytosol[c]
5α-dihydrotestosterone	100	100
testosterone	84	97
progesterone	24	16
5α-androstane-3α,17β-diol	17	23
17β-estradiol	7	<1
cortisol	1	<1

[a] Relative affinity was determined as the percentage of specific binding relative to dihydrotestosterone binding. [b] Receptor was purified as described in Table I. The purified receptor preparation was incubated with 16 nM [^{3}H]dihydrotestosterone in the absence or presence of 4 μM nonradioactive competitors under exchange conditions described under Experimental Procedures. [c] Cytosol was prepared as described under Experimental Procedures. The cytosol preparation was first incubated with 16 nM unlabeled testosterone. The cytosol was then treated with a charcoal solution and assayed for binding activity under exchange conditions as described above.

affinity was found for 5α-androstane-3α,17β-diol (17%), 17β-estradiol (7%), and cortisol (1%). The relative affinities of each of these steroids for the purified receptor were similar to those for receptor in crude cytosol (Table III).

The molecular charge of the purified androgen receptor was studied. Purified receptor was labeled wtih 16 nM [^{3}H]dihydrotestosterone by the steroid exchange assay procedure described under Experimental Procedures and applied to a chromatofocusing column. The column was eluted with a buffer gradient of pH 7.4–4. The purified receptor eluted at a pH of 6.3, which is similar to that found for the receptor in crude cytosol (pI = 6.5) (Table II).

The molecular weight of the receptor under denaturing conditions was examined by the use of affinity-labeling techniques. We have reported previously that [^{3}H]R1881 binds covalently to the androgen receptor from steer seminal vesicle (Chang et al., 1982). In order to demonstrate selective binding of this ligand to the prostate receptor, we used a partially purified receptor preparation (Figure 3, lane A). [^{3}H]R1881 was incubated with this receptor preparation and photoactivated with ultraviolet irradiation. Following $NaDodSO_4$ electrophoresis, fluorography demonstrated one band of radioactivity at 86 000 daltons (Figure 3, lane B). When excess unlabeled R1881 together with [^{3}H]R1881 was incubated with the same receptor preparation, binding activity was displaced (Figure 3, lane C). For confirmation of these results, a second affinity label with properties different from [^{3}H]R1881 was employed. [^{3}H]Dihydrotestosterone 17β-bromoacetate was incubated with a preparation of partially purified receptor. Following $NaDodSO_4$ gel electrophoresis, fluorography revealed a major band of radioactivity with a M_r of 86 000 (Figure 3, lane D). Labeling of the receptor in the presence of excess unlabeled dihydrotestosterone 17β-bromoacetate displaced the binding activity to the 86 000 component (Figure 3, lane E). Binding of R1881 to the receptor could be displaced effectively with both dihydrotestosterone and testosterone. Other steroids such as dehydroepiandrosterone, 5α-androstane-3α,17β-diol, progesterone, and cortisol demonstrated little or no competition for binding (data not shown). Although other minor bands or radioactivity were also observed with M_r of 65 000, 55 000, 40 000, and 35 000, these were not consistently displaced with excess unlabeled ligand.

The M_r of 86 000 was verified by the hydrodynamic properties of the nondenatured receptor (Table II). A major peak

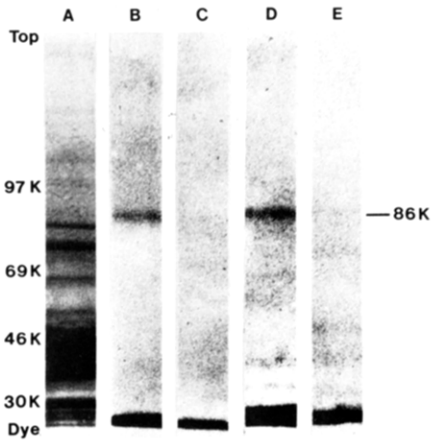

FIGURE 3: $NaDodSO_4$–polyacrylamide gel electrophoresis of the affinity-labeled androgen receptor. A partially purified receptor preparation (approximately 5000-fold purified) was prepared as described in Figure 1 except that the receptor was eluted from the second DNA column with TEDK buffer. Peak fractions of androgen-binding activity, determined by steroid exchange assay, were precipitated with 10% trichloroacetate. The pellet (approximately 10 μg of protein) was dissolved in sample buffer and applied to a $NaDodSO_4$ gel as described in the legend to Figure 1. The gel was stained with Coomassie blue (A). In order to affinity label the receptor, the sample eluted with TEDK buffer was incubated with either 20 nM[^{3}H]R1881 (B), 20 nM [^{3}H]R1881 plus 4 μM unlabeled R1881 (C), 10 nM [^{3}H]dihydrotestosterone 17β-bromoacetate (D), or 10 nM [^{3}H]dihydrotestosterone 17β-bromoacetate plus 2 μM unlabeled dihydrotestosterone 17β-bromoacetate (E) as described under Experimental Procedures. All samples were precipitated with 10% trichloroacetate. The precipitants were redissolved in $NaDodSO_4$ sample buffer, applied to a $NaDodSO_4$ gel, and electrophoresed. Fluorographic treatment of the gel was performed as described under Experimental Procedures.

of binding activity with Stokes radius of 42 Å was observed on a Sephacryl S-200 column, and a major binding peak with a sedimentation coefficient of 4.5 S was observed on a 5–20% glycerol gradient. These data were used to calculate a M_r of 85 000 and a fractional ratio of 1.41 for the nondenatured protein. Although 0.02% heparin was added to prevent extensive receptor aggregation, two small peaks of binding activity with higher Stokes radii and sedimentation coefficients were also observed. Similar peaks of binding activity were observed by gel-exclusion high-performance liquid chromatography (data not shown). The receptor protein detected at A_{210} corresponded with the peaks of binding activity, suggesting that both the 85 000-dalton component and its aggregate forms were present under these experimental conditions. When the purified receptor was applied to a nondenaturing 5% polyacrylamide gel, we observed one band of silver-stained protein that corresponded to one peak of radioactivity at the very top of a parallel lane (data not shown). Addition of 0.01% heparin in the upper electrophoretic buffer did not facilitate the migration of the receptor into the gel. It therefore appeared that the purified receptor was aggregated under these conditions and could not enter the gel.

Discussion

These results demonstrate that the androgen receptor from rat ventral prostate was purified to near homogeneity. The purification of the prostate receptor was based on three unique properties of the receptor protein (Liao et al., 1975; Wilson & French, 1976; Mainwaring & Mangan, 1973; Gustafsson & Pousette, 1975), i.e., high affinity for androgens, altered affinity for DNA before and after receptor transformation by ammonium sulfate, and selective elution from DNA–Sepharose with low concentrations of pyridoxal 5′-phosphate (10 mM).

The purified receptor was characterized with respect to its steroid binding and physicochemical properties. Two affinity labels were used to determine the molecular weight of the receptor under denaturing conditions.

Earlier studies by Bruchovsky et al. (1975) demonstrated that the rat prostate androgen receptor in crude cytosol had a Stokes radius of 48 Å and a sedimentation coefficient of 4.4 S, which was calculated to be a molecular weight of 87 000. This would suggest that the 86 000-dalton protein band observed by $NaDodSO_4$ gel electrophoresis (Figure 1) is the receptor protein. Since the 86 000-dalton component was the largest molecular form found by two different androgen affinity labels under denaturing conditions, we feel that we have isolated the receptor protein as an intact polypeptide. Gel filtration and sucrose gradient analyses of the purified receptor demonstrated peaks of binding activity at 42 Å and 4.5 S, respectively, which were in agreement with values for the receptor in crude cytosol (Table II). Although leupeptin was added in the cytosol preparation to prevent the receptor protein from proteolytic degradation (Sherman et al., 1978; Chang & Tindall, 1983), we occasionally observed smaller molecular weight species, suggesting that there may have been other proteolytic enzymes present that were resistant to this inhibitor.

A molecular weight of 85 000–90 000 has been reported for the glucocorticoid receptor (Govindan & Sekeris, 1978; Yamamoto et al., 1976; Simons & Thompson, 1981; Nordeen et al., 1981), suggesting that both the androgen receptor and the glucocorticoid receptor have a similar molecular size. In contrast, it has been reported that M_r of the estrogen receptor from calf uterus is 70 000 (Sica & Bresciani, 1979), which is identical with that reported for the progesterone receptor from rabbit uterus (Lamb et al., 1982). The progesterone receptor from chick oviduct has been found to consist of subunits of M_r of 78 000 and 106 000 (Coty et al., 1979; Kuhn et al., 1975). The nontransformed chick progesterone receptor has been reported to have a M_r of 90 000 (Puri et al., 1982). These findings suggest that all steroid receptors do not have the same molecular weight.

We reported recently (Chang et al., 1982) the purification of the androgen receptor from steer seminal vesicle by the same purification protocol described in this paper. It had a molecular weight of 60 000–70 000. The differences in molecular weights of receptors from these two tissues could be due to several possibilities. One reason for this difference could be due to proteolysis. The tissue obtained from the slaughter house took longer to process than that obtained in the laboratory. Moreover, the steers had been castrated for up to 1 year, which markedly altered the tissue morphology. Indeed, hsitology of the tissue revealed a reduced epithelial cell population and mostly stromal elements present. Finally, an obvious possible reason is species differences. Nevertheless, it is interesting that these receptors did have different molecular weights. We are currently trying to determine the source of these differences.

The purified receptor from rat prostate cytosol was found to have an isoelectric point of 6.3 (Table II), which was similar to that found for the purified receptor (6.6) from steer seminal vesicle cytosol (Chang et al., 1982). This is also in agreement with the findings of Mainwaring & Irving (1973) that the heat-activated receptor or nuclear receptor complex has an isoelectric point of 6.5. In contrast, the unactivated androgen receptor has an isoelectric point of 5.8 as determined by sucrose gradient focusing (Mainwaring & Irving, 1973) and polyacrylamide gel electrofocusing (Tindall et al., 1975). However, chromatofocusing of the activated or unactivated androgen receptors revealed no differences in pI values.[2]

Results from these studies demonstrate that we have purified a protein from rat prostate cytosol, which is similar to the β-protein (complex II) but different from the α-protein (complex I) reported by Liao et al. (1975). The purified receptor was different from androgen-binding protein (ABP) in that ABP has a faster dissociation rate (6 min), has a lower pI value (4.6), and requires higher concentrations of ammonium sulfate for precipitation (40 to ~50%) than the prostatic androgen receptor (Tindall et al., 1975, 1977, 1978). It is not likely that we have purified a serum sex steroid binding protein since no such protein is found in rat serum (Chan & Tindall, 1981).

This paper presents a rapid and efficient procedure for the purification of androgen receptor from rat ventral prostate. However, the present procedure only allowed us to obtain a limited quantity of purified receptor from each preparation. It is obvious that we need to scale up the purification of the receptor in order to study in detail its physiochemical properties and to produce monospecific antibodies against the protein. This work is in progress.

Acknowledgments

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Registry No. Dihydrotestosterone, 521-18-6; testosterone, 58-22-0; progesterone, 57-83-0; 5α-androstane-3α,17β-diol, 1852-53-5; 17β-estradiol, 50-28-2; cortisol, 50-23-7.

References

Baulieu, E.-E., & Jung, I. (1970) *Biochem. Biophys. Res. Commun. 38*, 599–606.

Bruchovsky, N., Rennie, P. S., & Vanson A. (1975) *Biochim. Biophys. Acta 394*, 248–266.

Chan, L., & Tindall, D. J. (1981) in *Pediatric Endocrinology* (Collu, R., Ducharme, J. R., & Guyda, H., Eds.) pp 63–97, Raven Press, New York.

Chang, C. H., & Tindall, D. J. (1983) *Endocrinology 113*, 1486–1493.

Chang, C. H., Rowley, D. R., Lobl, T. J., & Tindall, D. J. (1982) *Biochemistry 21*, 4102–4109.

Coty, W. A., Schrader, W. T., & O'Malley, B. W. (1979) *J. Steroid Biochem. 10*, 1–12.

Fang, S., & Liao, S. (1971) *J. Biol. Chem. 246*, 16–24.

Govindan, M. V., & Sekeris, C. E. (1978) *Eur. J. Biochem. 89*, 95–104.

Gustafsson, J.-Å., & Pousette, Å. (1975) *Biochemistry 14*, 3094–3101.

Korenman, S. G. (1969) *Steroids 13*, 163–177.

Kuhn, R. W., Schrader, W. T., Smith, R. G., & O'Malley, B. W. (1975) *J. Biol. Chem. 250*, 4220–4228.

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

Lamb, D. J., Holmes, S. D., Smith, R. G., & Bullock, D. W. (1982) *Biochem. Biophys. Res. Commun. 108*, 1131–1135.

Liao, S., Tymoczko, J. L., Castaneda, E., & Liang, T. (1975) *Vitam. Horm. (N.Y.) 33*, 297–317.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Mainwaring, W. I. P. (1978) in *Receptors and Hormone Action II* (O'Malley, B. W., & Birnbaumer, L., Eds.) pp

[2] C. H. Chang, D. R. Rowley, D. J. Tindall, unpublished observations.

105–120, Academic Press, New York.
Mainwaring, W. I. P., & Irving, R. (1973) *Biochem. J. 134*, 113–127.
Mainwaring, W. I. P., & Mangan, F. R. (1973) *J. Endocrinol. 59*, 121–139.
Nordeen, S. K., Lan, N. C., Showers, M. O., & Baxter, J. D. (1981) *J. Biol. Chem. 256*, 10053–10508.
Pavlik, E. J., Nagell, J. R. V., Jr., Muncey, M., Donaldson, E. S., Hanson, M., Kenady, D., Rees, E. D., & Talwalkar, V. R. (1982) *Biochemistry 21*, 139–145.
Puri, R. K., Grandics, P., Dougherty, J. J., & Toft, D. O. (1982) *J. Biol. Chem. 257*, 10831–10837.
Scatchard, G. (1949) *Ann. N.Y. Acad. Sci. 51*, 660–672.
Sherman, M. R., Pickering, L. A., Rollwagen, F. M., & Miller, L. K. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 167–173.
Sica, V., & Bresciani, F. (1979) *Biochemistry 18*, 2369–2378.
Siegel, L. M., & Monty, K. J. (1966) *Biochim. Biophys. Acta 112*, 346–362.
Simons, S. S., Jr., & Thompson, E. B. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3541–3545.
Tindall, D. J., Hanson, V., McLean, W. W., Ritzen, E. M., Nayfeh, S. N., & French, F. S. (1975) *Mol. Cell. Endocrinol. 3*, 83–101.
Tindall, D. J., Miller, D. A., & Means, A. R. (1977) *Endocrinology (Philadelphia) 101*, 13–23.
Tindall, D. J., Cunningham, G. R., & Means, A. R. (1978) *J. Biol. Chem. 253*, 166–169.
Westphal, H. M., & Beato, M. (1980) *Eur. J. Biochem. 106*, 395–403.
Wilson, E. M., & French, F. S. (1976) *J. Biol. Chem. 251*, 5620–5629.
Wilson, E. M., & French, F. S. (1979) *J. Biol. Chem. 254*, 6310–6319.
Wrange, Ö., Carlstedt-Duke, J., & Gustafsson, J.-Å. (1979) *J. Biol. Chem. 254*, 9284–9290.
Wray, W., Boulikas, T., Wray, V. P., & Hancock, R. (1981) *Anal. Biochem. 118*, 197–203.
Yamamoto, K. R., Gehring, U., Stampfer, M. R., & Sibley, C. H. (1976) *Recent Prog. Horm. Res. 32*, 3–32.

Histone Deacetylase from HeLa Cells: Properties of the High Molecular Weight Complex†

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ABSTRACT: In previous work [Hay, C. W., & Candido, E. P. M. (1983) *J. Biol. Chem. 258*, 3726–3734], we have shown that the histone deacetylase from HeLa cell nuclei is associated with a high molecular weight, nuclease-resistant complex. This complex was found to contain a variety of non-histone proteins, and indirect evidence for the importance of protein–protein interactions in the maintenance of its structure was obtained. In the present report, we examine the effects of β-mercaptoethanol and neocuproine on the deacetylase complex and present data on the level of histone acetylation and the presence of satellite DNA sequences in this material. HeLa cell histone deacetylase complex partially dissociates in 10 mM β-mercaptoethanol, resulting in a loss of non-histone proteins. The presence of 10 mM β-mercaptoethanol during the micrococcal nuclease digestion of HeLa cell nuclei results in a greatly reduced yield of histone deacetylase complex, with a correspondingly large increase in the production of small oligonucleosomes and mononucleosomes. Histone deacetylase activity on endogenous labeled histone within the complex is strongly inhibited by either 1 or 10 mM β-mercaptoethanol or 3 mM neocuproine. This loss of histone deacetylase activity does not seem to be due to an inactivation of the enzyme but appears to be a consequence of the disruption of the structure of the deacetylase complex itself. Histone H4 in the deacetylase complex prepared from HeLa cell nuclei by micrococcal nuclease digestion was more highly acetylated than H4 in bulk nucleosomes. Restriction enzyme analysis of the DNA associated with the histone deacetylase complex revealed neither an enrichment nor a depletion of major satellite sequences in this material.

Nucleosomes, the basic unit of chromatin organization, have been well characterized structurally (McGhee & Felsenfeld, 1980). Although the amino acid sequence of the nucleosome core histones H2A, H2B, H3, and H4 are highly conserved, the histones exhibit considerable heterogeneity which is created by a variety of posttranslational modifications including acetylation, methylation, phosphorylation, and ADP-ribosylation (Elgin & Weintraub, 1975; Ogata et al., 1980). As these modifications can cause considerable alterations in the charge distributions of histones and are incomplete in nature, they introduce heterogeneity into the chromatin structure. One of the best-studied modifications is the reversible acetylation at ε-amino groups of specific lysyl residues located near the amino terminal of each core histone (Dixon et al., 1975).

While the exact function of histone acetylation remains unclear, it has been proposed that the reduction in the positive charge in the acetylated core histones may alter the conformation of chromatin and result in a concomitant increase in the accessibility of the DNA to specific nuclear enzymes or regulatory proteins. This suggestion is based upon correlations between high levels of gene activity and high levels of histone acetylation (Vavra et al., 1982; Davie et al., 1981) and on the observation that the nucleosomes of transcriptionally competent chromatin have high levels of histone acetylation (Davie & Candido, 1980; Levy-Wilson et al., 1979). Recently, it has also been suggested that all of the chromatin in a cell may experience cycles of acetylation and deacetylation which may

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