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Purification and Characterization of Androgen Binding Protein from Rabbit Epididymis†

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ABSTRACT: A method for the purification of androgen binding protein (ABP) from the rabbit epididymis is presented. Epididymal extracts were submitted to sequential ammonium sulfate precipitation, androgen affinity chromatography, concanavalin A (Con A) affinity chromatography, and preparative polyacrylamide gel electrophoresis. Since the blood protein testosterone–estradiol binding globulin (TeBG) was a possible component of the epididymal extract, ABP was differentiated and separated from TeBG by affinity chromatography on Con A–Sepharose since the latter protein was shown to be completely adsorbed by the lectin while the former was not. The final product was shown to be pure by polyacrylamide gel electrophoresis. Electrophoresis in the presence of sodium dodecyl sulfate revealed that ABP is comprised of subunits.

Androgen binding protein (ABP)[1] is present in the male reproductive tract of rat, rabbit, and other mammalian species [for a review, see Hansson et al. (1975a) and Bardin et al. (1981)]. ABP is synthesized and secreted by the Sertoli cells under the influence of FSH and testosterone, and as a consequence, it has been used as a marker protein to study the physiology of the seminiferous tubule. The first highly purified ABP was isolated from rat epididymides (Musto et al., 1977, 1980); this was possible because the rat does not have testosterone–estradiol binding globulin (TeBG), a serum protein of hepatic origin which binds sex steroids (Khan et al., 1981; Corval & Bardin, 1973) and has properties very similar to ABP (Hansson et al., 1975b). The isolation of ABP from the rabbit and man has been complicated by the presence of TeBG in epididymal extracts. For example, Weddington et al. (1975b) partially purified a small quantity of ABP (rbABP) from rabbit epididymis, and antiserum generated against this preparation cross-reacted with both rbTeBG and rbABP (Weddington et al., 1975a). Since the fractionation procedures did not separate these two proteins, it is therefore possible that the antiserum used in these experiments was prepared against a mixture of both TeBG and ABP. In view of the interest in studying ABP production in species which also make TeBG, we thought it pertinent to develop a procedure for preparing highly purified rbABP which was free of rbTeBG. Such a technique was an essential prerequisite for determining whether rbABP and rbTeBG are similar or identical proteins. In this paper, we present the purification and characterization of rbABP which is free of rbTeBG.

Materials and Methods

Materials. Epididymides from mature rabbits were purchased from Pel-Freez Biological, Inc.; 3-oxo-17β-hydroxy-5α-androstane-17α-(6-hexanoic acid) (DHT-HA) was synthesized according to the procedure published previously (Musto et al., 1977). 1,2-5α-[^{3}H]Dihydrotestosterone ([^{3}H]DHT), 40 Ci/mmol, was obtained from New England Nuclear Corp. and purified on silica gel thin-layer plates developed in chloroform–methanol (25:2). Radioinert DHT, Sepharose 4B, concanavalin A–Sepharose, and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDAC) were obtained from Sigma Chemical Co. Epichlorohydrin was from Aldrich Chemical Co.; ammonium hydroxide was from Mallinckrodt, Inc. Ampholines pH 4–6 were from Bio-Rad Laboratories, and gelamide was from Polysciences, Inc. All the chemicals were reagent grade. Double-distilled water was utilized throughout.

Preparation of Affinity Column Matrix (DHT–Sepharose). Sepharose substituted with primary amino group was prepared by the method of Nishikawa & Bailon (1976) by using epichlorohydrin as follows: Sepharose 4B washed with water was suspended in an equal volume of 0.6 M NaOH. Epichlorohydrin was added to a final concentration of 0.24 M and the mixture incubated at 30 °C for 4 h with shaking. At the end of incubation, the gel was transferred to a glass filter and washed with water to pH 7 and then suspended in an equal volume of 2 M ammonium hydroxide solution. The suspension was incubated at room temperature overnight with shaking. The gel was washed with water on a glass funnel until the wash was neutral. It was further washed with 0.5 volume each of 0.02 M NaH_2PO_4 and 1 M NaCl. The gel was then washed

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[1] Abbreviations: ABP, androgen binding protein; DHT, 5α-dihydrotestosterone; TeBG, testosterone–estradiol binding globulin; rbABP, rabbit ABP; DHT-HA, 3-oxo-17β-hydroxy-5α-androstane-17α-(6-hexanoic acid); Con A, concanavalin A; EDAC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; TG buffer, 20 mM Tris, pH 7.4, and 10% glycerol; TDK buffer, 20 mM Tris, pH 7.4, 10% *N,N*-dimethylformamide, and 1 M KCl; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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extensively again with water until the pH of the filtrate was equal to that of distilled water.

DHT-HA was coupled to the amino-Sepharose by using the water-soluble carbodiimide method as described by Parikh et al. (1974). The reaction was carried out in 70% dioxane by using 1 mg of DHT-HA/mL of packed resin and 5 mg of EDAC/mL of resin, added twice, 5 h apart. The suspension was shaken overnight. The finished product was washed with 4 volumes of 70% dioxane followed by 6 volumes of 80% methanol to ensure complete removal of noncovalently bound steroid. The gel was finally washed with 5 volumes of water and stored at 4 °C in the presence of 0.02% azide.

Purification of rbABP. (*A*) *Cytosol Preparation.* Mature rabbit epididymides were trimmed of fat, ground, and homogenized in a blender with 9 volumes of TG buffer. This homogenate was centrifuged at 10960*g* for 2.5 h, and the supernatant was filtered through glass wool. The supernatant was then treated with charcoal (1 mg/mL) for 1 h to remove endogenous steroid.

(*B*) *Ammonium Sulfate Precipitation.* Ammonium sulfate (361 g/L) was slowly added to the cytosol while the pH was maintained with the addition of NaOH. Following overnight incubation, the precipitate was recovered by centrifugation at 10960*g* for 2.5 h and the pellet dissolved in 100 mL of TG buffer by shaking overnight. This solution was centrifuged at 25000*g* for 1 h to remove insoluble material.

(*C*) *Affinity Chromatography.* The DHT–Sepharose was suspended in TDK buffer and packed in a 3-cm diameter column with a final bed volume of 200 mL. The ammonium sulfate fraction derived from 180 g of epididymides was applied to the column after preliminary studies showed that this sample size could be completely adsorbed by the affinity resin. The gel was then washed with 2 L of TDK buffer. For elution of the specifically bound rbABP, 400 mL of TDK buffer containing DHT (20 μg/mL) was added to the column. When this solution had completely replaced the bed volume, the flow was stopped for 16 h. This incubation allowed exchange of rbABP from the matrix to the free DHT. Following the incubation, rbABP was eluted by replacing the bed volume with 400 mL of the same buffer. The column was again stopped for 16 h and further eluted with 200 mL of buffer. The pooled eluates were diluted with an equal volume of TG buffer and concentrated to 50 mL in an Amicon TCF-10 concentrator with a PM-10 membrane. The volume was further reduced in a Model 52 Amicon concentrator with a UM-20 membrane. All the membranes were pretreated with TG buffer containing 0.2% gelatin and then washed sequentially with 0.1 M NaOH, water, 1 M NaCl, and water.

(*D*) *Concanavalin A–Sepharose Chromatography.* Con A–Sepharose was washed extensively with 50 mM Tris–0.5 M NaCl buffer, pH 7.4, containing 1 mM each of $CaCl_2$, $MnCl_2$, and $MgCl_2$. Partially purified rbABP was applied to the column (0.3 nmol/mL of gel). When the rbABP solution completely entered the column, the flow was stopped for 3 h to allow interaction between rbABP and Con A. The column was then eluted with the wash buffer at a rate of 15 mL/h until no more [^{3}H]DHT binding activity could be detected in the eluate. For dissociation of the Con A bound glycoproteins, the column was further eluted with the buffer containing 5% methyl α-D-glucopyranoside and 5% *N*,*N*-dimethylformamide.

(*E*) *Preparative Gel Electrophoresis.* Preparative gel electrophoresis was performed with a Canalco "prep disc" apparatus. The gels were prepared according to the method described by Davis (1964) with the addition of 5% glycerol. The resolving gel was 10% T and 2.5% C using the cross-linker *N*,*N*-methylenebis(acrylamide). The gel area was 1.5 cm^2, and the length was 2.7 cm. The stacking gel was 5% T and 15% C using *N*,*N*′-diallyltartardiamide (DADTA) as cross-linker (Baumann & Chrambach, 1976). The volume of the stacking gel was slightly larger than the sample volume which was prepared by diluting the sample with one-third volume of upper gel buffer. In addition, 3 mg of bacitracin was added to the sample solution to enhance the recovery of rbABP (Kapadia & Chrambach, 1972). Electrophoresis was started at 5 mA (260–380 V) and eluted with 0.38 M Tris–5% glycerol, pH 8.9, at a rate of 30 mL/h. When the bromophenol blue dye marker reached the bottom of the resolving gel, conditions were changed to a constant voltage of 600 V. In addition, the elution buffer was changed to 0.83 M Tris–10% glycerol (pH 10.5), and the elution rate was increased to 60 mL/h. Fractions (4 mL/fraction) containing rbABP were pooled, concentrated in an Amicon concentrator with a UM-20 membrane, and dialyzed against TG buffer.

Assay for rbABP Activity. Androgen binding protein was quantified by a modification (Musto & Bardin, 1976) of the original dextran-coated charcoal assay for steroid binding proteins described by Korenman (1969). Samples containing rbABP were incubated with a constant concentration of [^{3}H]DHT (0.2 pmol) and increasing amounts of nonradioactive DHT (0–4 pmol) in a total volume of 0.2 mL. Tubes containing 1.9 nmol of nonradioactive DHT were used to correct for nonspecific binding. Gelatin (0.2%) was included in the incubation medium to prevent adsorption of protein by charcoal. After a 1-h incubation, the free DHT was separated from the protein-bound form by a 2-min exposure to dextran-coated charcoal (DCC) followed by centrifugation (8000*g* for 10 min). The protein-bound radioactivity in the charcoal-free supernate was quantified by liquid scintillation counting. In the presence of gelatin, no adsorption of rbABP by charcoal occurred (N. A. Musto, unpublished results). The concentration of binding sites and K_d were determined from Scatchard plots (Scatchard, 1949). rbABP preparations containing steroids were pretreated with DCC solution containing 0.2% gelatin before assay.

For the location of binding activity in column fractions, a two-point qualitative [^{3}H]DHT binding assay was performed. Aliquots were incubated with 1 nM [^{3}H]DHT and 0.1% gelatin at 4 °C for 1 h in a total volume of 0.2 mL. The unbound steroid was removed by adsorption with dextran-coated charcoal followed by centrifugation. Total bound steroid was determined from the radioactivity in the charcoal-free supernatant. Nonspecific binding was assessed in a second tube containing [^{3}H]DHT plus a 5000-fold excess of nonradioactive DHT.

Protein Measurement. Protein concentration in the solution was measured by the dye binding method of Bradford (1976). Bovine serum albumin was used as a standard.

Analytical Polyacrylamide Gel Electrophoresis. Electrophoresis in the presence of $NaDodSO_4$ was performed according to the method described by Laemmli (1970). rbABP preparations (approximately 10 μg of each) were denatured in 1% $NaDodSO_4$ and 1.6% β-mercaptoethanol at 95 °C for 5 min. Polyacrylamide gel electrophoresis under nondenaturing conditions was performed as described by Chrambach et al. (1976) using systems 1935 and 2860. System 1935 operates at pH 7.5, while system 2860 was at pH 10.25.

Isoelectric Focusing. Polyacrylamide gel containing 2% Ampholine, pH 4–6, and 5% glycerol was used to analyze the isoelectric point of rbABP. The gel tubes with an internal

Table I: Summary of the Purification of Mature Rabbit Epididymal ABP

	ABP[a] (nmol)	protein[b] (mg)	sp act.[c] (nmol/mg)	x-fold purification	ABP recovery (%)
cytosol	59.6	23750	0.0025	1	100
60% ammonium sulfate	45.6	10010	0.0046	1.8	77
affinity chromatography	19.8	2.6	7.17	3700	33
Con A chromatography peak I	12.2				21
G-50 chromatography	7.6	0.65	11.69	4700	13
preparative gel electrophoresis	7.3	0.34	21.47	8600	12

[a] The ABP concentration was measured by the [^{3}H]DHT binding activity by using the dextran-coated charcoal method. [b] The protein concentration was determined by the Coomassie blue dye binding assay. [c] Specific activity was expressed as nmol of DHT bound at saturation per mg of protein.

diameter of 5 mm were washed with methanol containing 10% KOH, rinsed with water, and finally coated with 1% gelamide (Nguyen et al., 1978). The gel concentration was 5% T and 15% C using *N,N'*-diallyltartardiamide as cross-linker. Each tube contained 2.5 mL of gel solution. The cathode solution was 0.05 M Tris with 0.112% calcium oxide. The anode was 0.05 M DL-glutamic acid (Nguyen & Chrambach, 1977). Isoelectric focusing was performed at 100 V for 1 h followed by 200 V for 18 h. The pH gradient was determined by slicing a parallel gel into 0.5-cm segments and soaking in 2 mL of deaerated water per slice, and the pH was measured with a standard pH electrode.

Cross-Linking of rbABP Protomers. Dimethyl suberimidate (Davies & Stark, 1970) was used as a cross-linking reagent as follows: A highly purified preparation of rbABP (65 μg) was dialyzed overnight against 0.2 M triethanolamine, pH 8.5. Dimethyl suberimidate (80 μg) in the same buffer was added, and the mixture was incubated at room temperature for 3 h. At the end of incubation, 2 mL of 0.1 M ammonium bicarbonate was added to quench the reaction and the solution lyophilized.

Peptide Mapping of the H and L Components. Purified rbABP was electrophoresed into $NaDodSO_4$-containing polyacrylamide gels and stained with Coomassie blue. The H and L components were cut out of these gels and the peptide maps produced as described by Cleveland et al. (1977).

Results

Purification of Androgen Binding Protein. The isolation procedure for rbABP is shown in Table I. rbABP was first precipitated from cytosol at 2.1 M ammonium sulfate. This step increased the specific activity 2-fold with a recovery of 80% and reduced the volume by a factor of 10. The affinity chromatography of rbABP on DHT–Sepharose produced a cumulative 3700-fold purification over the starting material. The rbABP eluted from the androgen affinity column was then applied to a Con A–Sepharose affinity column, and three peaks of binding activity were eluted (Figure 1). Peak I appeared in the void volume, peak II was retarded by the column, and peak III was eluted by methyl α-glucoside. Each peak was pooled, concentrated, and assayed for [^{3}H]DHT binding activity by the dextran-coated charcoal assay and Scatchard analysis. Of the recoverable binding activity, 60% was found in peak I and 20% each in peaks II and III. Peak I was selected for further purification of rbABP. This selection was

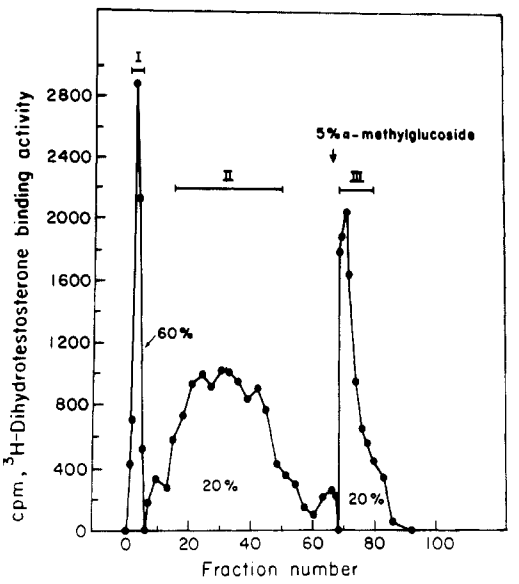

FIGURE 1: Con A–Sepharose chromatography of rabbit ABP. The affinity-purified ABP (5.2 nmol), specific activity 7 nmol/mg of protein, was applied to a Con A–Sepharose column (1 × 19 cm). The column was eluted with 50 mM Tris–0.5 M NaCl buffer, pH 7.4, containing 1 mM each of $CaCl_2$, $MgCl_2$, and $MnCl_2$. At the arrow, the column was eluted with the same buffer containing 5% methyl α-glucoside and 5% *N,N*-dimethylformamide. Each fraction was 5.8 mL. [^{3}H]DHT binding activity was monitored as described under Materials and Methods. The number under each peak gives the percentage of total recovered DHT binding activity as determined on the pooled peaks by the dextran-coated charcoal assay and Scatchard analysis.

made on the basis that rbTeBG is quantitatively adsorbed by Con A–Sepharose (Lea, 1979; Kotite & Musto, 1982). As a consequence, peaks I and II are not observed when unfractionated serum or purified TeBG are run under identical conditions (Lea, 1979; Kotite & Musto, 1981). Thus, peaks I and II are unique to the epididymis, with no comparable peaks in female rabbit serum, while peak III is similar to rbTeBG. The binding proteins in each of the peaks eluted in the same positions when fractionated a second time on the Con A column. The final steps of purification required to produce homogeneous rbABP were Sephadex G-50 chromatography (to remove concanavalin A) and preparative polyacrylamide gel electrophoresis. This purification scheme increased the specific activity of rbABP 8600-fold with an overall recovery of 12% (Table I).

Assessment of Purity. The purity of the final rbABP preparation was assessed by polyacrylamide gel electrophoresis. Electrophoresis of native gels at pH 7.5 (system 1935) and at pH 10.25 (system 2860) was performed, and the results are shown in Figure 2. A single band was found at gel concentrations of 5% T and 10% T in system 1935 (lanes 1 and 2). In system 2860, one band was observed at 5% T (lane 3). At 10% T, two minor bands which based on their mobilities may represent dissociated subunits are seen (<1%) running in front of rbABP (lane 4). For demonstration that the final product actually bound androgens, purified protein was electrophoresed in the presence of [^{3}H]DHT. A single peak of binding activity was observed which corresponded to the protein band stained by Coomassie blue (data not shown).

When this rbABP preparation was fractionated on $NaDodSO_4$-containing gels, two bands were seen. In addition, there appeared to be an unequal amount of these components with more of the light component than the heavy (Figure 3, lane 2). A comparison of the distribution of these species in the other binding proteins resolved by Con A chromatography

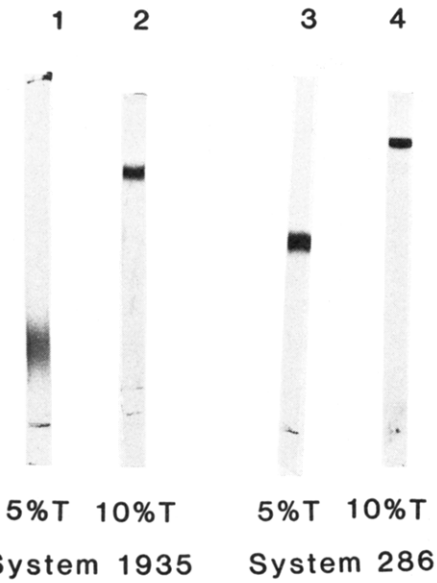

FIGURE 2: Polyacrylamide gel electrophoresis of purified rbABP in system 1935 (pH 7.5) and system 2860 (pH 10.25) at two gel concentrations (5% T and 10% T). Gels were stained with Coomassie blue R-250.

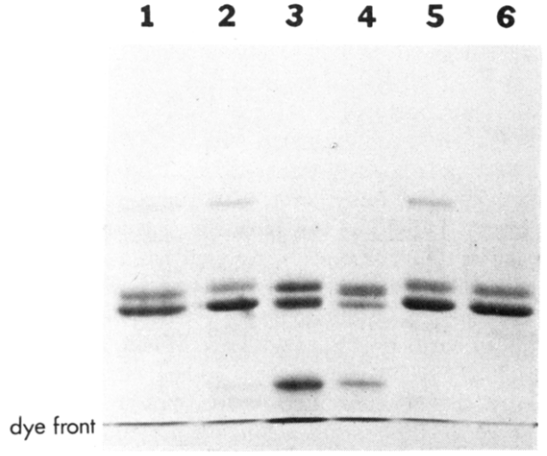

FIGURE 3: $NaDodSO_4$–polyacrylamide gel electrophoresis of ABP during fractionation. Lane 1, DHT eluate from affinity chromatography; lane 2, Con A chromatography peak I (see Figure 1); lane 3, Con A chromatography peak II; lane 4, Con A chromatography peak III; lane 5, peak I after Sephadex G-50; lane 6, postpreparative gel electrophoresis.

reveals a different pattern. Peak II has approximately equal amounts of the heavy and light components (Figure 3, lane 3) while peak III shows more heavy than light (Figure 3, lane 4).

Steroid Binding Properties of Purified rbABP. Purified rbABP was incubated with increasing concentrations of DHT; bound and free steroids were separated by DCC, and the results were plotted according to Scatchard (Figure 4). The apparent equilibrium association constant of the rbABP–DHT complex at 4 °C is 1.6×10^8 M^{-1} as determined by the dextran-coated charcoal method. The specific binding activity of this preparation is 21.5 nmol/mg of protein.

Apparent Molecular Weights of the H and L Components of rbABP. The molecular weight of the rbABP components was determined by $NaDodSO_4$–polyacrylamide gel electrophoresis. This method was validated by Ferguson analysis as described by Frank & Rodbard (1975). In this procedure, $NaDodSO_4$ disc gel electrophoresis of rbABP and molecular weight standards using gel concentrations from 6% T to 10% T was performed, and the log R_f vs. percent T was plotted

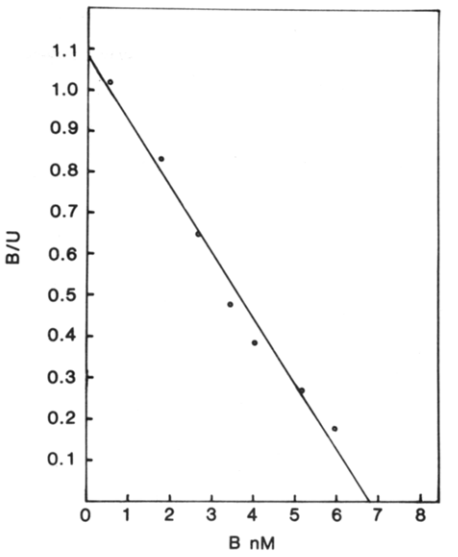

FIGURE 4: Scatchard analysis of DHT binding by purified rbABP. Saturation analysis was done by the dextran-coated charcoal assay described under Materials and Methods.

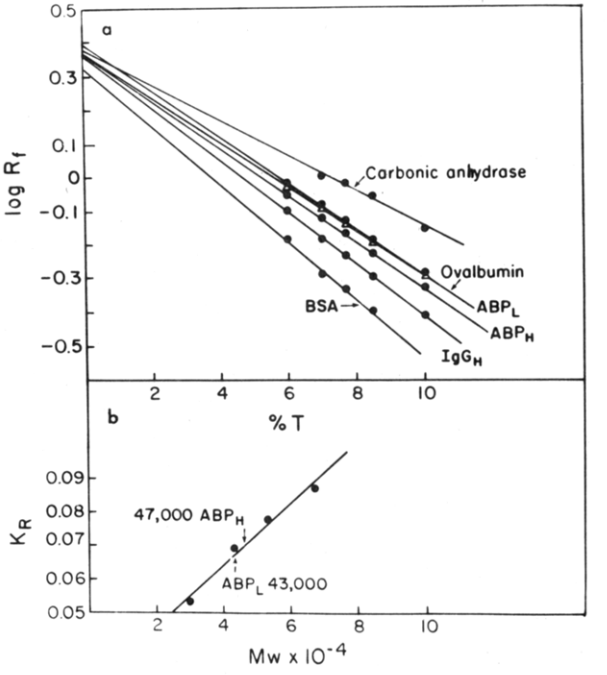

FIGURE 5: Molecular weight determination of ABP components in $NaDodSO_4$–polyacrylamide gel electrophoresis. ABP and standard proteins were electrophoresed into $NaDodSO_4$ gels with concentrations from 6% T to 10% T. (a) Ferguson analysis was performed by plotting log R_f vs. % T for the heavy (ABP_H) and light (ABP_L) components of ABP and molecular weight standards. (b) Molecular weights of ABP_H and ABP_L were determined by interpolation of the K_R (negative of slopes from log R_f vs. % T) vs. M_r standards.

(Figure 5a). A common y intercept of the two rbABP components and the standards (indicative of identical free mobilities) validated the use of $NaDodSO_4$–polyacrylamide gel electrophoresis for molecular weight estimates by demonstrating that each has a similar charge density (in the presence of $NaDodSO_4$) and their mobilities are a function of their molecular weight. The molecular weights of rbABP components were determined by plotting the slope (K_R) obtained in the Ferguson analysis vs. the molecular weights of standard proteins (Figure 5b). This analysis indicated that the molecular weights of the $NaDodSO_4$-resolved species are 43 000 for the light ($rbABP_L$) component and 47 000 for the heavy ($rbABP_H$) component. Cross-linking of the rbABP promoters

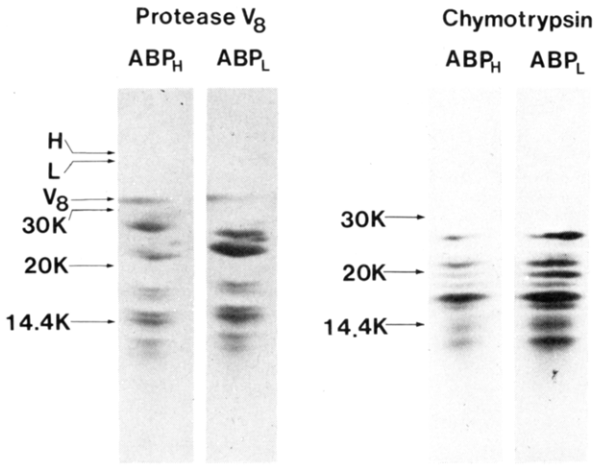

FIGURE 6: Peptide maps of rbABP H and L components. H and L were isolated by electrophoresing purified rbABP on $NaDodSO_4$–polyacrylamide gel electrophoresis. The protein bands were then visualized by staining with Coomassie blue R-250, cut out, and reelectrophoresed in the presence of protease V_8 (1 μg) or chymotrypsin (1 μg) as described by Cleveland et al. (1977). H, position of unhydrolyzed $rbABP_H$; L, position of unhydrolyzed $rbABP_L$; V_8, position of protease V_8.

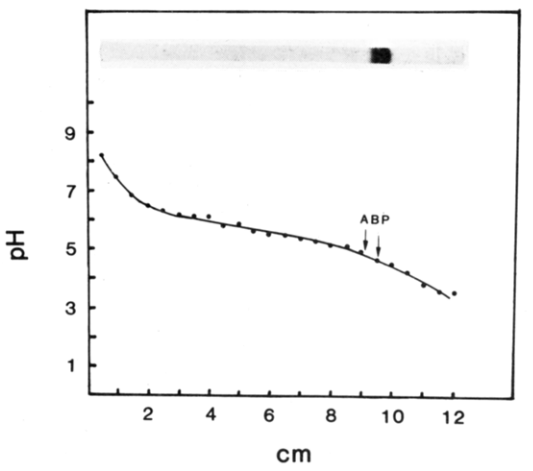

FIGURE 7: Isoelectric point determination of native ABP. Focusing was performed in 5% T–15% C DATDA gels for 19 h. The catholyte was 0.05 M Tris–0.112% CaO, and the anolyte 0.05 M DL-glutamic acid. The pH gradient was determined by slicing the gel, soaking in degassed deionized water, and measuring the pH with a standard electrode.

in their native association and subsequent analysis by $NaDodSO_4$–polyacrylamide gel electrophoresis as described by Davies & Stark (1970) revealed a single higher order species with a molecular weight of 94000. This result is in agreement with the dimeric structure of rbABP by confirming the stoichiometry of association for native rbABP and produces an estimate of the native molecular weight for rbABP independent of molecular shape. These data taken together suggest that the rbABP protomers are heterogeneous with regard to size and exist as two types, $rbABP_H$ and $rbABP_L$.

Structural Comparison of $rbABP_H$ and $rbABP_L$ Components. The H and L components of highly purified rbABP were separated by $NaDodSO_4$–polyacrylamide gel electrophoresis and analyzed by the peptide mapping procedure described by Cleveland et al. (1977). The peptide fragments generated by two enzymes (Figure 6) were very similar, suggesting that $rbABP_H$ and $rbABP_L$ were closely related with regard to primary structure. The appearance of at least one unique peptide in the L component with protease V_8 digestion suggests the possibility that they may not be absolutely identical with regard to their primary structure. Alternatively, the presence of a different carbohydrate moiety may alter the size of a peptide, thereby producing a dissimilar pattern with proteins of similar primary sequence.

Charge Heterogeneity of rbABP. An analysis of the isoelectric point of native rbABP is seen in Figure 7. The data show that this material possesses some microheterogeneity with the appearance of at least two variants with p*I* values of 4.9 and 4.6.

Discussion

In the male rabbit, there are two extracellular high-affinity androgen binding proteins found in different anatomical compartments. TeBG, which is thought to be of hepatic origin (Bordin & Petra, 1980; Khan et al., 1981), is secreted into the vascular space of both sexes while ABP, which is produced by the Sertoli cell (Hansson et al., 1975a), is found primarily in the tubules of the testis and the epididymis. It is this compartment that contains the spermatozoa and many other secretory products of the testis. Many investigators have questioned whether TeBG and ABP are in reality the same protein made in two different sites or totally different proteins with similar biological and immunological properties (Hansson et al., 1975b; Weddington et al., 1975a,b; Hsu & Troen, 1978; Bordin & Petra, 1980). In order to distinguish between these possibilities, it was essential to isolate these two proteins and compare them. The earlier efforts of Weddington et al. (1975a) were successful in producing small quantities of rbABP from rabbit epididymides; however, their final product was undoubtedly contaminated with TeBG inasmuch as no effort was made to remove this protein from epididymal extracts. A method was, therefore, required to isolate rabbit ABP free of TeBG. In this paper, we have shown that the androgen binding properties, isolated from an epididymal extract, exhibit marked heterogeneity with regard to concanavalin A affinity chromatography. This contrasts with the behavior of purified TeBG (Kotite & Musto, 1982) or crude TeBG (Lea, 1979) which appears homogeneous with regard to affinity for this lectin. We have used this property to isolate one species of the androgen binding proteins found in the epididymis. We suggest that this binding protein, which is unique to the epididymis and not found in the serum of female rabbits, represents at least part of the ABP produced by the Sertoli cell. As such, we have characterized it and found its physical properties indistinguishable from those reported for crude rbABP (Hansson et al., 1975b).

The marked heterogeneity observed during Con A–Sepharose chromatography is not limited to the purified epididymal steroid binding proteins of the rabbit. Hsu & Troen (1978) have shown that the androgen binding protein in extracts of human testis could be fractionated into two peaks by Con A affinity chromatography, one peak passing through in the void volume while the other bound to the lectin matrix, and could be eluted with the appropriate sugar. In addition, they and others (Lea, 1979) showed that human TeBG, like that of rabbit, chromatographed on Con A–Sepharose as a single peak elutable by sugar. These results suggest that TeBGs differ from ABP in at least two species by the amount of terminal mannose residues associated with the polysaccharide moieties that are added to the polypeptide chains. This difference in posttranslational modification from tissue to tissue is similar to that seen for conalbumin and transferrin in the fowl (Williams, 1962) or serum transferrin and transferrin made by Sertoli cells (Skinner & Griswold, 1980).

While we have chosen to study only the rbABP in the void volume (peak I, Figure 1) of the Con A column, we cannot

exclude the possibility that all of the binding protein specifically retained by the column (peak III) is rbTeBG. In fact, the appearance of the second peak (see Figure 1, peak II) in epididymal extracts suggests that ABP does possess some degree of heterogeneity with regard to Con A affinity, an observation also made for rat ABP (N. A. Musto, unpublished experiments). In addition, heterogeneity with regard to Con A affinity has also been demonstrated for other steroid binding proteins, e.g., rat CBG (Lea, 1979) and α-fetoprotein (Kerckaert et al., 1979).

It is of interest to note that rbABP, like all extracellular androgen binding proteins thus far isolated [i.e., rat ABP (Musto et al., 1980), rbTeBG (Mickelson & Petra, 1978; Kotite & Musto, 1982), human TeBG (Mickelson et al., 1978), bovine TeBG (Suzuki et al., 1977), and canine TeBG (Suzuki et al., 1979)], is comprised of subunits. The significance of this is presently unknown. In addition to the observation of subunit structure common to all binding proteins, another universal observation is the presence of size hybrids in the protomers. This idea is derived from the observation that in $NaDodSO_4$–polyacrylamide gel electrophoresis there are two size species which appear in unequal proportion and which show great similarity with regard to primary structure. Thus, all of the androgen binding proteins isolated by this laboratory to date appear to have multiple size species in their protomers. These include rat ABP (Musto et al., 1980), rabbit TeBG (Kotite & Musto, 1982), and rabbit ABP. The function or the source of this size heterogeneity in the protomers is not known.

In summary, a method is described for isolating rbABP from epididymal extracts which is free of TeBG. The protein prepared by this procedure can now be compared with rbTeBG from the plasma of female rabbits.

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