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Rat androgen-binding protein: Evidence for identical subunits and amino acid sequence homology with human sex hormone-binding globulin

(cDNA nucleotide sequence/gene/precursor/*in vitro* translation)

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ABSTRACT **The cDNA for rat androgen-binding protein (ABP) was previously isolated from a bacteriophage λgt11 rat testis cDNA library and its identity was confirmed by epitope selection. Hybrid-arrested translation studies have now demonstrated the identity of the isolates. The nucleotide sequence of a near full-length cDNA encodes a 403-amino acid precursor (M_r = 44,539), which agrees in size with the cell-free translation product (M_r = 45,000) of ABP mRNA. Putative sites of N-glycosylation and signal peptide cleavage were identified. Comparison of the predicted amino acid sequence of rat ABP with the amino-terminal amino acid sequence of human sex hormone-binding globulin revealed that 17 of 25 residues are identical. On the basis of the predicted amino acid sequence the molecular weight of the primary translation product, lacking the signal peptide, was 41,183. Hybridization analyses indicated that the two subunits of ABP are coded for by a single gene and a single mRNA species. Our results suggest that ABP consists of two subunits with identical primary sequences and that differences in post-translational processing result in the production of 47,000 and 41,000 molecular weight monomers.**

Sertoli cells of the mammalian testis synthesize and secrete androgen-binding protein (ABP), which binds testosterone and 5α-dihydrotestosterone with high affinity (1, 2). After secretion into the lumen of the seminiferous tubule, ABP is transported to the epididymis. It is believed that ABP increases the concentration of androgens, thereby influencing the process of sperm maturation, spermatogenesis, or both, possibly acting as a carrier of androgens to the receptor (3–5). ABP may also play a role in protection of androgens from metabolism. In many species, including rabbit and human (6–8), the liver secretes a closely related protein, sex hormone-binding globulin (SHBG), which differs from testis ABP in its post-translational modification (6) and possibly in the primary amino acid sequence.

Rat ABP has been purified to homogeneity and polyclonal antibodies have been prepared (9, 10). Immunochemical studies and studies utilizing a steady-state polyacrylamide gel electrophoresis binding assay have demonstrated that cellular ABP levels and secretion of ABP are regulated by follicle-stimulating hormone and androgens (11–16). Studies with the purified epididymal protein have shown that the native protein has a molecular weight of 85,000–100,000 (9, 10). $NaDodSO_4$/polyacrylamide gel electrophoresis of the purified protein revealed two components with apparent molecular weights of 47,000 and 41,000, with approximately three times the amount of the heavy component (9, 10). The structure and significance of these two components have remained unknown (17).

To study the hormonal regulation of ABP mRNA, we have recently described the isolation of complementary DNA that encodes rat ABP (18). We now present the entire nucleotide sequence of the coding region for rat ABP mRNA and the deduced amino acid sequence. We also show that ABP shares homology with the amino-terminal amino acid sequence of human SHBG.

METHODS

Identification of Recombinants and Purification of cDNA Inserts. A rat testis cDNA library was prepared in λgt11 (18), an expression vector that was developed for the isolation of specific cDNAs by using antibody probes (19, 20). Recombinants containing ABP cDNA were identified by using rabbit ABP antiserum as previously described (18). Rabbit antiserum against highly purified rat epididymal ABP was judged to be monospecific by immunodiffusion, crossed immunoelectrophoresis, and immunocytochemical staining (9). The identities of the isolates were confirmed by epitope selection and immunoblot competition studies (18). After plaque purification, the recombinant phage were propagated in liquid culture and, banded thrice in cesium chloride density gradients, and the DNA was isolated by standard procedures (ref. 21, p. 80). The cDNA inserts were purified from agarose gels, with NA45 DEAE-paper (Schleicher & Schuell), after restriction endonuclease cleavage and electrophoresis.

Translation of Poly(A)$^+$ RNA, Immunoprecipitation, and Hybrid-Arrested Translation. Poly(A)$^+$ RNA (20 μg/ml) was translated by a wheat germ extract (22) essentially as described by Alexander and Miller (23), except that magnesium acetate was 2.0 mM and the synthesized protein was labeled with [^{3}H]leucine at 0.3 mCi/ml (1 Ci = 37 GBq). Mixtures (100 μl) were incubated for 90 min at 25°C.

For hybrid arrest, poly(A)$^+$ RNA was hybridized to ABP cDNA (clone 6) by the method of Paterson (24). Briefly, 125 ng of ABP cDNA and 4 μg of testis poly(A)$^+$ RNA from 26-day rats were heated at 85°C and cooled in ice water. RNA and DNA were hybridized in 80% (vol/vol) formamide at 48°C for 1.5 hr. The sample was diluted with water containing 10 μg of wheat germ tRNA and one half was not treated further. The other half was heated at 85°C to denature hybrids and cooled in a dry ice bath. Both samples were precipitated with ethanol, washed twice with 70% ethanol, dissolved in a minimal volume of water, and translated as described above.

Each translation mixture was processed as follows: Two aliquots (5 μl) were removed from each reaction mixture for trichloroacetic acid precipitation and liquid scintillation counting. The following solutions were added to the remaining 90 μl: 9 μl of 76 mM leucine, 270 μl of 50 mM Tris acetate,

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Abbreviations: ABP, androgen-binding protein; SHBG, sex hormone-binding globulin; bp, base pair(s); dATP[α-^{35}S], ^{35}S-labeled deoxyadenosine 5′-[α-thio]triphosphate.

pH 8.5/2.5% (wt/vol) Triton X-100, and 10 μl of Pansorbin (*Staphylococcus aureus*, Calbiochem). After a 15-min incubation at 25°C, the Pansorbin was removed by centrifugation and discarded. Finally, 5 μl of 200 mM phenylmethylsulfonyl fluoride and 1 μl of anti-ABP (9) were added and the mixture was incubated overnight at 4°C. In competition assays, the antiserum was incubated with the purified ABP for 15 min at 25°C before addition to the diluted translation mixture. Antibody–antigen complexes were removed by incubation with 40 μl of Pansorbin for 15 min at 25°C and centrifugation. Pellets were washed twice with 0.1 M potassium phosphate buffer, pH 7.4, containing 0.15 M NaCl, 5 mM EDTA, gelatin at 1 mg/ml, 0.05% Nonidet P-40, 0.001% Thimerosal, and 7.6 mM leucine. Pellets were heated at 85°C for 2 min in 20 μl of denaturing buffer (25) and centrifuged, and the products were fractionated by $NaDodSO_4$/polyacrylamide gel electrophoresis (25) and visualized by fluorography.

Other Analytical Procedures. Restriction endonuclease digestions of DNA and fractionation by agarose gel electrophoresis were done by standard techniques (ref. 21, pp. 98 and 151). Southern blotting and hybridization using nick-translated [^{32}P]DNA was as described by Wahl *et al.* (26). Blot hybridization analysis of ABP mRNA was done as described by Thomas (27), using nick-translated [^{32}P]DNA (28). For nucleotide sequencing, restriction endonuclease fragments were subcloned in bacteriophage M13 mp18 or mp19 (29). DNA sequences were determined by the dideoxy method of Sanger and co-workers (30, 31) with ^{35}S-labeled deoxyadenosine 5′-[α-thio]triphosphate (dATP[α-^{35}S]). Rat testis DNA was isolated as described by Blin and Stafford (32). DNA and protein sequences were analyzed with the MicroGenie computer program (Beckman).

RESULTS

Hybrid-Arrested Translation of Poly(A)$^+$ RNA. The molecular weights of the two components of rat ABP are 47,000 and 41,000, at a ratio of approximately 3:1 (9, 10). It appeared likely that both forms originated from a single translation product but were modified differently (17, 33). To further investigate the origins of the different monomers, we analyzed the translation products by immunoprecipitation. Twenty six-day rat testis poly(A)$^+$ RNA was translated *in vitro* in a wheat germ system. The products were precipitated with ABP antiserum and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Fig. 1 (lane 2) shows that immunoprecipitation of the translation products with ABP antiserum yielded a single major protein band with a molecular weight of 45,000. The minor bands were due to nonspecific reactions, since they were precipitated with Pansorbin alone. Replacement of rat ABP antiserum with normal rabbit serum yielded no major protein band (Fig. 1, lane 1). The addition of purified rat ABP to the translation mixture prior to immunoprecipitation eliminated the ^{3}H-labeled band (Fig. 1, lanes 3–5).

Hybrid arrest of translation was used to provide further evidence that the cDNA clones code for ABP. Hybridization of insert DNA from clone 6 (18) with testis mRNA arrested the synthesis of the M_r 45,000 protein (Fig. 1, lane 7), whereas denaturation of the hybrid after hybridization permitted the mRNA to be translated (Fig. 1, lane 8). These data provide further evidence that the isolates contain ABP cDNA. Furthermore, these data provide the evidence that the primary translation product of ABP mRNA has a molecular weight of 45,000.

Nucleotide Sequence of Rat ABP cDNA. Restriction endonuclease fragments of three ABP cDNA isolates were subcloned in bacteriophage M13 DNA and sequenced by the dideoxy method (30, 31). The sequencing strategy is shown in Fig. 2. The entire coding region was sequenced on both strands and through all restriction sites that were used for subcloning. The entire nucleotide sequence of clone 6 (1395 bp) is shown in Fig. 3.

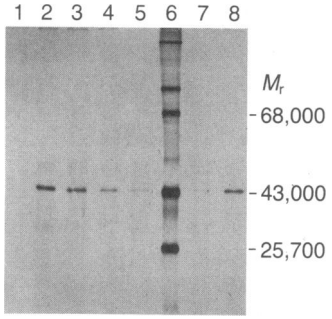

FIG. 1. *In vitro* translation and hybrid-arrested translation. Poly(A)$^+$ RNA isolated from testes of 26-day-old rats was translated *in vitro* in a wheat germ system with [^{3}H]leucine. The translation product was immunoprecipitated from the reaction mixture (0.1 ml) with 1.0 μl of antiserum and separated by $NaDodSO_4$/polyacrylamide gel electrophoresis, and bands were visualized by fluorography. ^{14}C-labeled molecular weight standards: myosin heavy chain, 200,000; phosphorylase *b*, 97,400; bovine serum albumin, 68,000; ovalbumin, 43,000; α-chymotrypsinogen, 25,700. Translation products were precipitated by using the following sera and conditions. Lane 1, normal rabbit serum; lane 2, anti-ABP; lane 3, anti-ABP plus 100 ng of purified ABP; lane 4, anti-ABP plus 320 ng of ABP; lane 5, anti-ABP plus 1000 ng of ABP; lane 6, molecular weight standards; lane 7, hybridized with ABP cDNA (clone 6), translated, and immunoprecipitated (hybrid-arrested); lane 8, hybridized, heated to denature, translated, and immunoprecipitated (unarrested).

None of the three cDNA isolates that were sequenced contained a poly(A) region at the 3′ end. These residues were probably removed by the DNA polymerase I during second-strand cDNA synthesis. Since the putative poly(A) addition signal (AATAAA) was detected 13 residues from the 3′-terminal nucleotide of the cDNA (Fig. 3), it is likely that only a few nucleotides are missing before the poly(A) addition site.

Predicted Amino Acid Sequence of ABP Monomer and Signal Peptide. Translation of the nucleotide sequence revealed an open reading frame from residue 34 (the first methionine codon from the 5′ end) to residue 1242, which is followed by a TAA termination codon (Fig. 3). The predicted sequence contains 403 amino acid residues and has a molecular weight of 44,539. This value is in good agreement with the size of the immunoprecipitated *in vitro* translation product of testis mRNA shown above (M_r = 45,000).

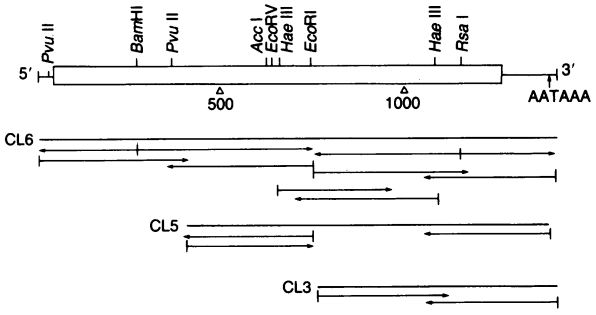

FIG. 2. Strategy for sequencing cloned cDNA that encodes rat ABP. The restriction endonuclease map of ABP cDNA displays the locations of cleavage sites as distances in base pairs (bp) from the 5′ terminus. The protein-encoding region is indicated by an open box. The direction and extent of sequencing determinations are shown by horizontal arrows under the lines representing each clone (CL3, CL5, CL6). Sequencing was done by the dideoxy method of Sanger and co-workers with dATP[α-^{35}S] (32, 33). The entire coding region was sequenced on both strands and through all restriction sites that were used for cloning.

```
                                                30                                                  60
ATA TTC TGA GCC ACT GGG TGA CAG CTG CTA ACT ATG GAG AAG GGA GAG GTG GCC TCC TTG
                                                    Met Glu Lys Gly Glu Val Ala Ser Leu              9

                                                90                                                 120
CGT TGC CGA CTG CTT CTG TTG TTG CTA CTA TTG ACG CTG CCT CCC ACC CAC CAG GGA CGG
Arg Cys Arg Leu Leu Leu Leu Leu Leu Leu Leu Thr Leu Pro Pro Thr His Gln Gly Arg             29

   *                                           150                                                 180
ACC CTG AGA CAC ATT GAC CCT ATC CAG AGT GCT CAG GAC TCT CCT GCT AAA TAC CTC AGC
Thr Leu Arg His Ile Asp Pro Ile Gln Ser Ala Gln Asp Ser Pro Ala Lys Tyr Leu Ser             49

                                               210                                                 240
AAT GGC CCA GGA CAA GAG CCC GTC ACT GTT CTG ACC ATT GAC CTC ACC AAA ATC AGC AAA
Asn Gly Pro Gly Gln Glu Pro Val Thr Val Leu Thr Ile Asp Leu Thr Lys Ile Ser Lys             69

                                               270                                                 300
CCC TCT TCC TCC TTT GAG TTT CGA ACC TGG GAT CCA GAG GGA GTG ATT TTT TAT GGG GAC
Pro Ser Ser Ser Phe Glu Phe Arg Thr Trp Asp Pro Glu Gly Val Ile Phe Tyr Gly Asp             89

                                               330                                                 360
ACC AAC ACT GAA GAT GAC TGG TTC ATG CTG GGA CTG CGG GAT GGC CAG CTT GAA ATC CAG
Thr Asn Thr Glu Asp Asp Trp Phe Met Leu Gly Leu Arg Asp Gly Gln Leu Glu Ile Gln            109

                                               390                                                 420
CTG CAC AAT CTC TGG GCT CGG CTT ACA GTA GGC TTT GGC CCT CGG CTG AAT GAT GGG AGA
Leu His Asn Leu Trp Ala Arg Leu Thr Val Gly Phe Gly Pro Arg Leu Asn Asp Gly Arg            129

                                               450                                                 480
TGG CAC CCG GTG GAG CTA AAG ATG AAC GGG GAT TCA CTG CTG CTA TGG GTG GAT GGA AAA
Trp His Pro Val Glu Leu Lys Met Asn Gly Asp Ser Leu Leu Leu Trp Val Asp Gly Lys            149

                                               510                                                 540
GAG ATG CTA TGC CTG AGA CAA GTT TCT GCA TCC CTG GCT GAC CAT CCC CAG CTC AGC ATG
Glu Met Leu Cys Leu Arg Gln Val Ser Ala Ser Leu Ala Asp His Pro Gln Leu Ser Met            169

                                               570                                                 600
AGG ATT GCA CTA GGG GGG CTC CTC CTC CCC ACT TCC AAA CTT CGG TTT CCG CTC GTT CCT
Arg Ile Ala Leu Gly Gly Leu Leu Leu Pro Thr Ser Lys Leu Arg Phe Pro Leu Val Pro            189

                                               630                                                 660
GCC CTG GAT GGC TGT ATA CGC CGA GAT ATC TGG CTG GGC CAC CAG GCC CAG CTC TCA ACC
Ala Leu Asp Gly Cys Ile Arg Arg Asp Ile Trp Leu Gly His Gln Ala Gln Leu Ser Thr            209

                                               690                                                 720
TCT GCC CGA ACT AGC CTT GGG AAC TGT GAT GTG GAC CTG CAA CCT GGA CTG TTC TTC CCT
Ser Ala Arg Thr Ser Leu Gly Asn Cys Asp Val Asp Leu Gln Pro Gly Leu Phe Phe Pro            229

                                               750                                                 780
CCA GGG ACC CAT GCA GAA TTC AGT CTC CAA GAC ATT CCC CAG CCT CAC ACA GAC CCC TGG
Pro Gly Thr His Ala Glu Phe Ser Leu Gln Asp Ile Pro Gln Pro His Thr Asp Pro Trp            249

                                               810                                                 840
ACC TTT TCT CTG GAG CTG GGA TTT AAG CTG GTG GAT GGA GCA GGA CGA CTC CTT ACT CTT
Thr Phe Ser Leu Glu Leu Gly Phe Lys Leu Val Asp Gly Ala Gly Arg Leu Leu Thr Leu            269

                                               870                                                 900
GGG ACA GGG ACA AAT TCT TCT TGG CTC ACC CTT CAC CTC CAA GAC CAA ACG GTG GTT CTG
Gly Thr Gly Thr Asn Ser Ser Trp Leu Thr Leu His Leu Gln Asp Gln Thr Val Val Leu            289

                                               930                                                 960
TCT TCT GAA GCA GAA CCT AAA CTA GCT TTA CCC TTG GCG GTG GGA CTC CCT CTT CAA CTG
Ser Ser Glu Ala Glu Pro Lys Leu Ala Leu Pro Leu Ala Val Gly Leu Pro Leu Gln Leu            309

                                               990                                                1020
AAG CTG GAT GTA TTC AAA GTA GCC TTG AGC CAA GGA CCA AAG ATG GAG GTC CTT TCT ACA
Lys Leu Asp Val Phe Lys Val Ala Leu Ser Gln Gly Pro Lys Met Glu Val Leu Ser Thr            329

                                              1050                                                1080
TCT CTT TTA AGA CTT GCC TCC CTC TGG AGA CTC TGG TCC CAC CCT CAG GGC CAT CTC TCC
Ser Leu Leu Arg Leu Ala Ser Leu Trp Arg Leu Trp Ser His Pro Gln Gly His Leu Ser            349

                                              1110                                                1140
CTT GGG GCT TTA CCA GGA GAG GAC TCT TCT GCT TCC TTT TGC CTG AGT GAC CTT TGG GTA
Leu Gly Ala Leu Pro Gly Glu Asp Ser Ser Ala Ser Phe Cys Leu Ser Asp Leu Trp Val            369

                                              1170                                                1200
CAA GGA CAG AGA CTG GAC ATA GAC AAA GCC CTG AGC AGA AGC CAG GAC ATC TGG ACT CAT
Gln Gly Gln Arg Leu Asp Ile Asp Lys Ala Leu Ser Arg Ser Gln Asp Ile Trp Thr His            389

                                              1230                                                1260
AGT TGC CCT CAG AGC CCT AGC AAT GAT ACC CAC ACC TCC CAC TAA ACC CCT TTG AGA AGC
Ser Cys Pro Gln Ser Pro Ser Asn Asp Thr His Thr Ser His End

                                              1290                                                1320
AGG CAC GGC TGT GCA CAT CCT TAA ATC CCG GAG AGG TGA ATT GGG ACC TCA AGG TTA TCC

                                              1350                                                1380
TTG GCT ATA TGA CAT GTT TGA GGC CAG CCT GGG CTA CAT GAA ACC CTG CCT TCA AAA ATA

AAA AAG AAC TTT GGA
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FIG. 3. Nucleotide sequence of cloned cDNA encoding rat ABP and the deduced amino acid sequence of the ABP precursor. Nucleotide residues are numbered from the 5′ terminus relative to the mRNA. The poly(dG)·poly(dC) tract at the 5′ end and linker residues are not shown. The number of the amino acid residue beginning with the first methionine residue is indicated at the right of each line. The putative signal peptide is underlined and the * indicates the putative cleavage site. Potential sites of N-glycosylation are in bold print and underlined. The poly(A) addition signal sequence (AATAAA) beginning at nucleotide residue 1377 is also underlined.

The predicted amino-terminal sequence contains a hydrophobic region with eight consecutive leucine residues. Although the amino-terminal sequence of rat ABP has not been directly determined, the signal peptide cleavage site can be tentatively assigned. On the basis of the homology with human SHBG (see below) the site is likely between amino acid residues 30 (Thr) and 31 (Leu).

The amino acid composition of rat ABP that was determined by direct analysis (9) is compared with the composition predicted from the nucleotide sequence in Table 1. Good agreement is observed. There is unusually high leucine and low tyrosine content relative to the average amino acid distribution in proteins (34). Discrepancy was noted only in the Asp plus Asn content (15% measured vs. 9.3% predicted). On the basis of the amino acid composition the molecular weight of the ABP precursor without the signal peptide is 41,183 Da. Table 1 also shows that the protein contains 33% hydrophobic amino acid residues. The distribution of hydrophilic and hydrophobic domains was analyzed by hydropathy graphing as described by Kyte and Doolittle (35) (data not shown). ABP was found to be very hydrophobic, with 55% of the amino acid residues on the hydrophobic side of the midpoint line.

Since ABP is known to be glycosylated, we searched the amino acid sequence for potential N-glycosylation sites (Asn-Xaa-Ser or Asn-Xaa-Thr). Potential glycosylation sites are present at amino acid residues 274 and 397 (Fig. 3).

Amino Acid Sequence Homology with Human SHBG. In several mammalian species, including human but not adult rat, the liver produces an SHBG that is closely related to ABP. Differences between the proteins include glycosylation and possibly other post-translational modifications (6). Whether there are minor differences in primary sequence remains to be determined. Recently, Hammond *et al.* (7) have

Table 1. Amino acid composition of rat ABP

Amino acid residue	Predicted: No. of residues	Predicted: mol %	Direct analysis mol %
Alanine	19	5.1	5.3
Arginine	17	4.6	4.2
Asparagine	8	2.1	15.0
Aspartic acid	27	7.2	
Cysteine	5	1.3	1.3
Glutamine	21	5.6	8.7
Glutamic acid	13	3.5	
Glycine	29	7.8	8.3
Histidine	13	3.5	2.8
Isoleucine	12	3.2	2.0
Leucine	63	16.9	18.1
Lysine	12	3.2	3.3
Methionine	5	1.3	1.2
Phenylalanine	13	3.5	3.2
Proline	26	7.0	6.3
Serine	37	9.9	9.8
Threonine	22	5.9	5.7
Tryptophan	12	3.2	—
Tyrosine	2	0.5	0.3
Valine	17	4.6	4.8

The predicted amino acid residues were calculated after removal of the putative signal peptide of 30 residues. The direct analysis was from Feldman *et al.* (9) and data were converted to molar percent.

obtained by direct analysis the amino-terminal amino acid sequence of human SHBG. Fig. 4 shows a comparison of the amino-terminal amino acid sequence of SHBG with the predicted sequence of rat ABP. Seventeen of 25 amino acid residues are identical, including a stretch of 8 identical residues beginning at residue 48 of the ABP precursor and residue 18 of SHBG. Analysis of the similarity of ABP and SHBG by the algorithm of Lipman and Pearson (36) showed the relationship to be significant, with an initial Z value of 15 standard deviations above the mean of randomized sequences. Hammond *et al.* (7) have proposed that residue seven of SHBG may be a glycosylated residue. This aligned residue of rat ABP is not glycosylated.

Southern Blot Analysis of Rat Genomic DNA. We have previously shown that ABP cDNA hybridizes to a single 1600-base mRNA species in rat testis or Sertoli cell poly(A)$^+$ RNA (18). To characterize the gene(s) that codes for ABP mRNA, we analyzed rat DNA by Southern blot hybridization with ABP cDNA (clone 6) as probe. Fig. 5 shows the results that were obtained with the DNA from testes of three individual rats. Identical results were obtained with all three DNA preparations. *Eco*RI, which cleaves ABP cDNA at one site, yielded two bands of hybridization (17 and 5 kbp). *Hin*dIII and *Sst* I, which do not cleave the cDNA, yielded two bands (>20 and 3.3 kbp) and one band (5.5 kbp), respectively. Thus, the entire gene appears to be located within a single 5.5-kbp *Sst* I fragment.

DISCUSSION

We have identified the cDNA that encodes rat ABP. Characterization of the cDNA has revealed the predicted amino acid sequence of the ABP precursor, the putative signal peptide, and potential sites of glycosylation. On the basis of the predicted amino acid sequence, ABP was found to share homology with human SHBG.

Previous studies have shown that purified rat ABP from epididymis is a dimer with a native molecular weight of 85,000–100,000 (9, 10). When fractionated under denaturing conditions, components with molecular weights of 47,000 and 41,000 were identified, in a ratio of approximately 3:1. It was also shown that the components are very closely related and have apparently identical steroid-binding sites (17, 33). The evidence suggested that the components differ in carbohydrate composition and possibly in the length of the primary structure. On the basis of the deduced amino acid sequence, we have established that the unglycosylated form of the ABP precursor has a molecular weight of 45,000. Our *in vitro* translation studies have confirmed that the primary translation product has a molecular weight of 45,000. Although antibody to ABP reacts with both components of ABP, it precipitates a single translation product. Without the putative signal peptide, the molecular weight deduced from the nucleotide sequence is 41,183. It is consistent with previous findings that the small component (M_r = 41,000) of ABP that was seen on NaDodSO$_4$ gels is this component or is minimally glycosylated. The large component (M_r = 47,000) is derived from the M_r 45,000 precursor after cleavage of the signal peptide and post-translational glycosylation. Thus, rat ABP appears to consist of two identical subunits, some of which are modified differently. In support of this idea we have shown recently that rabbit anti-ABP, affinity purified with the recombinant fusion protein (epitope selection), reacts with both the heavy and light components of ABP (18). Thus, the two components share epitopes with the recombinant fusion protein, which is coded for by the rat ABP cDNA recombinant. The related protein, SHBG (see below), also is a dimer composed of two identical subunits (7, 37).

Our studies indicate that the two components of ABP are coded for by a single gene and a single mRNA species. Blot hybridization analysis of testis and Sertoli cell poly(A)$^+$ RNA, with ABP cDNA as probe, revealed a single 1600-base mRNA species (18). Furthermore, the *in vitro* translation studies described above indicate there is only one mRNA species. In view of the relatedness of the two components of ABP, it is likely that they are transcribed from a single mRNA species.

```
                                                  10                                  20
ABP   Met Glu Lys Gly Glu Val Ala Ser Leu Arg Cys Arg Leu Leu Leu Leu Leu Leu Leu Leu

                                                  30                                  40
ABP   Thr Leu Pro Pro Thr His Gln Gly Arg Thr Leu Arg His Ile Asp Pro Ile Gln Ser Ala
SHBG                                 1-Leu Arg Pro Val Leu Pro  X  Gln Ser Ala

                                                  50                                  60
ABP   Gln Asp Ser Pro Ala Lys Tyr Leu Ser Asn Gly Pro Gly Gln Glu Pro Val Thr Val Leu
SHBG  His Asp Pro Pro Ala Val His Leu Ser Asn Gly Pro Gly Gln Glu-25
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FIG. 4. Comparison of the ABP precursor amino acid sequence with the amino acid sequence of human SHBG. The amino acid residues for ABP are numbered from the initiation methionine residue. The amino-terminal amino acid sequence of SHGB (7) is shown below the ABP sequence. X represents an unknown amino acid residue (7). Residue numbers are shown at the termini of the SHBG sequence. Identical amino acid residues are underlined.

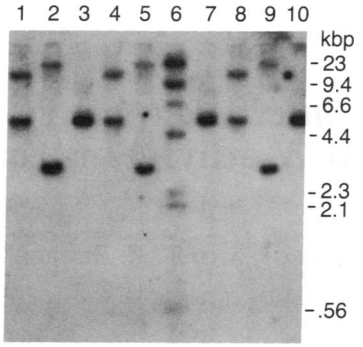

FIG. 5. Southern blot analysis of rat ABP gene(s). Testis DNA (0.02 mg) from each of three rats was cleaved with each restriction endonuclease and fractionated by agarose gel electrophoresis. After transfer to a nylon membrane and hybridization with ABP [^{32}P]cDNA (clone 6), the bands were visualized by autoradiography. A ^{32}P-labeled *Hin*dIII digest of λ phage DNA was electrophoresed in an adjacent lane to provide size markers. Lane 1, *Eco*RI; lane 2, *Hin*dIII; lane 3, *Sst* I; lane 4, *Eco*RI; lane 5, *Hin*dIII; lane 6, marker [^{32}P]DNA; lane 7, *Sst* I; lane 8, *Eco*RI; lane 9, *Hin*dIII; lane 10, *Sst* I. DNA from rat 1, lanes 1–3; rat 2 DNA, lanes 4, 5, and 7; rat 3 DNA, lanes 8–10.

Southern blot analysis of rat genomic DNA with various restriction endonucleases yielded DNA fragments that are consistent with the presence of one gene. Moreover, we have recently cloned the gene for rat ABP (unpublished results). A preliminary restriction endonuclease map of this isolate is consistent with the ABP DNA-containing restriction fragments observed by Southern blot analysis of rat DNA.

Testis ABP and liver SHBG within a species have been shown to be closely related and share immunological, physical, chemical, and steroid-binding properties (6–8). However differences in glycosylation were noted. Our results show that there is considerable homology between rat ABP and human SHBG at the amino terminus. Recently the entire sequence of human SHBG has been determined by protein sequence analysis (Phillip Petra, personal communication). Comparison of human SHBG and rat ABP sequences showed that both monomers contain 373 amino acid residues and 68% of the corresponding residues are identical. This similarity of rat ABP and human SHBG lends support to the idea that ABP and SHBG are either identical or very similar in primary structure, but glycosylated differently. Because these proteins are similar in their steroid-binding properties it is likely that their active sites reside in a conserved region(s). Comparison of their amino acid sequences will therefore facilitate studies to further reveal steroid–protein interactions.

Note Added in Proof. We and Michael Baker (University of California at San Diego) have found that ABP is related to the carboxyl-terminal domain of protein S, a vitamin K-dependent blood clotting factor.

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