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ANALYSIS OF THE OLIGOSACCHARIDES ON ANDROGEN-BINDING PROTEINS: IMPLICATIONS CONCERNING THEIR ROLE IN STRUCTURE/FUNCTION RELATIONSHIPS

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Summary—Rat androgen-binding protein (rABP), human testosterone-binding globulin (hTeBG) and rabbit (rb) TeBG are heterodimeric proteins. The source of the heterogeneity arises from the differential glycosylation of a common protein core. This glycosylation results in a heavy subunit (more glycosylation) and a light subunit (less glycosylation). Glycosylation is one factor responsible for multiple charged species seen when rABP, hTeBG, and rbTeBG are analyzed by two-dimensional gel electrophoresis. Enzymatic digestion with the endoglycosidase, peptide: *N*-glycosidase F indicated that all three proteins have asparagine (Asn)-linked oligosaccharides as their major glycan substituent. Treatment with exoglycosidases provided evidence for terminal sialic acid, galactose and mannose and *N*-acetylglucosamine residues. About 16–22% of the mass of the heavy subunit and about 8–14% of the mass of the light subunit is contributed by carbohydrate.

Serial lectin chromatography indicated that rABP is glycosylated differently from hTeBG and rbTeBG. About 40% of the rABP contains tri and tetraantennary complex oligosaccharides, while only about 20% of the hTeBG and TeBG from pregnant rabbits contains these types of glycans. About 9% of the TeBG from male rabbits bears these types of oligosaccharides. All of the biantennary complex oligosaccharides on rABP are fucosylated on the chitobiose core, but only 8% of those on hTeBG and none of those on rbTeBG are fucosylated in this manner. All three proteins are glycosylated at more than one site. The data indicate that the proteins may have more than one type of oligosaccharide on them. It is likely that differences in glycosylation are responsible for different physiological roles of the proteins.

INTRODUCTION

Androgen-binding protein (ABP) was originally detected in cytosol prepared from epididymides of rats [1] and rabbits [2] in the early 1970s. Since that time it has been reported to be present in epididymal and testicular extracts of several species including man [3–6]. It is now known that ABP is produced by the Sertoli cells of the testis [7] and makes its way into the epididymis via testicular fluid [8]. ABP is primarily confined to the epididymal lumen although some appears to be taken up by the epithelium of the epididymis [9–11]. Testosterone-binding globulin (TeBG), which is also referred to by several other names including sex-steroid-binding protein (SBP) and sex hormone binding globulin (SHBG), is found in the blood of humans [12] and several other species [13]. TeBG is produced by hepatocytes [14]. Like ABP, evidence for the uptake of TeBG by cells has been reported [14, 15] and the recent data from Rosner's group [16–17] provide evidence that TeBG uptake by the prostate is a receptor mediated event.

Both ABP and TeBG bind androgens with high affinity with the greatest affinity being for 5α-dihydrotestosterone (5α-DHT) [1, 2, 12]. They have very low affinity for the antiandrogens that inhibit binding to the androgen receptor [17, 18], and their affinity for estradiol varies with the species from which the proteins are isolated, but it is very low compared to that for androgens [2, 4, 5]. We have shown that monoclonal antibodies raised against rat ABP (rABP) can crossreact with human (h) and rabbit (rb) TeBG [20], emphasizing similarities between the proteins. The similarity between these molecules is further emphasized by the fact that there is a 76% sequence identity at the nucleotide level between the cDNA of rABP and hTeBG and a

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68% identity at the amino acid level [21, 22]. These latter data indicate that ABP and TeBG are the products of a highly conserved gene.

The major difference between ABP and TeBG is in the types of oligosaccharides attached to them. The original evidence concerning this phenomenon was provided by a study that revealed that the chromatographic elution pattern of the proteins from immobilized concanavalin A (Con-A) differed [6]. Since evidence from several sources indicated that glycosylation of glycoproteins is an important factor regulating their mechanism of action (for a review, see Ref. [23]), we undertook studies to analyze the glycans on ABP and TeBG as a preliminary step to studies on their structure/function relationships.

EXPERIMENTAL

Materials

Rat ABP and hTeBG and rbTeBG were purified using an affinity column consisting of 6-(5α-androstan-17β-hydroxy-17α-yl) hexanoic acid linked to Sepharose CL-4B as described previously [24, 25]. Nonpurified rABP, hTeBG and rbTeBG were also used in some studies. [1,2-^{3}H]17β-hydroxy-4-6,-androstadien-3-one (60 Ci/mmol) and [1,2-^{3}H]5α-androstan-17β-hydroxy-3-one (5α-DHT), (50.8 Ci/mmol), were purchased from DuPont/NEN (Boston, MA). Isoelectricfocusing, electrophoresis and column chromatography supplies were from Bio-Rad (Richmond, CA). Con-A conjugated to Sepharose 4B was obtained from Sigma (St Louis, MO); all other immobilized lectins were from E.Y. Laboratories, Inc. (San Mateo, CA). The endoglycosidases *N*-glycanase (peptide: *N*-glycosidase F) and *O*-glycanase (endo-α-*N*-acetylgalactosaminidase) were obtained from Genzyme (Boston, MA). The exoglycosidases were from Sigma. Other chemicals, reagents and supplies were from Sigma, Fisher (Fairlawn, NJ) or Sarstedt (Princeton, NJ).

Photolysis

Affinity purified rABP, hTeBG and rbTeBG were exposed to [1,2-^{3}H]17β-hydroxy-4,6-androstadien-3-one and u.v. light ($\lambda > 305$ nm) for 60 min and processed as described previously [26].

Electrophoresis, electrophoretic transfer, immunochemical localization and fluorography

Denaturing polyacrylamide gel electrophoresis (SDS–PAGE) was used to separate proteins according to molecular weight [27]. Two-dimensional gel electrophoresis was conducted according to the method of O'Farrell [28]. The first dimension consisted of isoelectricfocusing and the second dimension consisted of SDS–PAGE. In some cases after SDS–PAGE, proteins were transferred to nitrocellulose sheets [29]. The sheets were processed with a monoclonal antibody raised against rABP, appropriate secondary antibodies, peroxidase–antiperoxidase, and stained with diaminobenzadine [20]. Photolabeled samples were localized following fluorography [24].

Deglycosylation

Purified rABP, hTeBG and rbTeBG were deglycosylated using endo- and exoglycosidases or trifluoromethanesulfonic acid (TFMSA) as described previously [24, 25].

Serial lectin chromatography

Affinity purified or nonpurified rABP, hTeBG and rbTeBG preparations were subjected to chromatography on immobilized lectins as described previously [30, 31]. The proteins in the column fractions were identified by their ability to specifically bind [^{3}H]5α-DHT [30, 31]. The androgen-binding proteins that were eluted from a given lectin with a haptenic saccharide were rechromatographed on other lectins with different carbohydrate specificities.

RESULTS

The effect of deglycosylation on rABP, hTeBG and rbTeBG

One-dimensional PAGE. When native or photolabeled ABP is subjected to SDS–PAGE, two bands with M_r of ~48,000 and 43,000 can be detected (Fig. 1); these bands are present in a ratio of about 3:1. The fact that both of the promoters can be photoaffinity labeled indicates that they both bind steroid. These data suggested that ABP is a heterodimer composed of two dissimilar subunits. This phenomenon of two dissimilar subunits in different proportions appears to be a characteristic of androgen-binding proteins as it is also seen with hTeBG and rbTeBG [25, 32]. The unusually broad nature of the two protein bands (especially the heavier one) suggested that the protomers might be glycosylated and that differences in glycosylation might account for their different molecular weights.

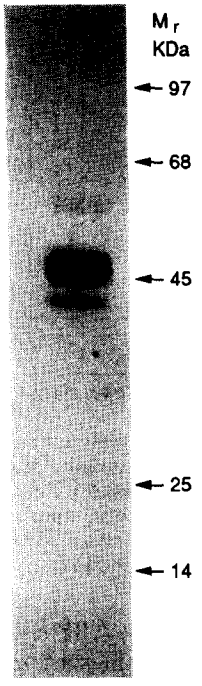

Fig. 1. SDS–PAGE of photoaffinity-labeled rABP. Purified rABP was photoaffinity-labeled and analyzed by SDS–PAGE and fluorography. M_r, relative molecular weight; kDa, kilodaltons. The standard proteins used were phosphorylase-B, 97 kDa; bovine serum albumin, 68 kDa; ovalbumin, 45 kDa; α-chymotrypsin, 25 kDa; and lysozyme, 14 kDa.

To explore this possibility, we enzymatically deglycosylated rABP and hTeBG and rbTeBG using neuraminidase, to remove sialic acid, and with *N*-glycanase, to remove Asn-linked glycans, and with *O*-glycanase, to remove *O*-linked oligosaccharides. The proteins were also chemically deglycosylated using TFMSA. Neuraminidase treatment resulted in an increased mobility of both the heavy and light subunits of all three proteins, indicating the presence of sialic acid on them. *N*-glycanase treatment of the proteins resulted in the greatest decrease in molecular weight of the protomers. In the case of hTeBG and rbTeBG only one subunit of approx. 40 kDa was detected after *N*-glycanase treatment. However, with rABP two subunits persisted; these had M_rs of 42,700 and 39,100. Treatment of the proteins with neuraminidase and *O*-Glycanase caused a somewhat greater reduction in apparent molecular weight than treament with neuraminidase alone, suggesting that, if *O*-linked sugars are present on the proteins, they make a minor contribution to their M_r. Deglycosylation with neuraminidase, *N*-glycanase and *O*-glycanase resulted in a single protomer for the TeBGs, but two protein bands remained with rABP. Deglycosylation of rABP and hTeBG and rbTeBG with TFMSA resulted in the appearance of a single subunit having an M_r of about 40,000. This should represent the completely deglycosylated form of the proteins. These data indicate that rABP, hTeBG and rbTeBG are actually composed of dimers made up of a single protomer that is differentially glycosylated. These data are presented in Refs [24, 25, 32].

Two-dimensional PAGE. To further evaluate the microheterogeneity of rABP, hTeBG and rbTeBG, the affinity purified proteins were analyzed by two-dimensional PAGE. Charge variants were separated by isoelectricfocusing, and molecular weight variants were separated by SDS–PAGE. These procedures were conducted on native proteins and on proteins that had been deglycosylated as described above. Both nonlabeled and photoaffinity-labeled proteins were used in these studies. Figure 2 summarizes data on the isoelectric points of rABP isoforms. rABP purified from two sources was used in this study, i.e. from the epididymis (eABP) and from the serum of immature rats (sABP). We had previously shown [24] that sABP had an apparent molecular weight greater than eABP. This figure shows that ABP from the two sources also varies in the number and charge of their isoelectric variants. Although ABP from the two sources is affected by the deglycosylating enzymes, in no case did treatment result in identical isoforms for eABP and sABP suggesting that the oligosaccharides on them differed. None of the treatments, including chemical deglycosylation, resulted in a single isoform suggesting that substituents other than charged sugars might be contributing to charge heterogeneity of the proteins. Data similar to that obtained with ABP was obtained when hTeBG and rbTeBG were analyzed by two-dimensional SDS–PAGE. That is, several charge variants were seen with the native proteins and none of the methods of deglycosylation produced a single charged species [24, 25, 32].

The isoelectric point data on rABP, hTeBG and rbTeBG are summarized in Table 1. As can be noted, rABP has the most acidic charge variants and hTeBG has the least. Whether there is any functional significance to this observation remains to be evaluated.

The effects of complete deglycosylation on the molecular weight of rABP, hTeBG and rbTeBG are summarized in Table 2. As can be seen, the heavy subunit has a greater decrease in molecular mass following complete deglycosylation

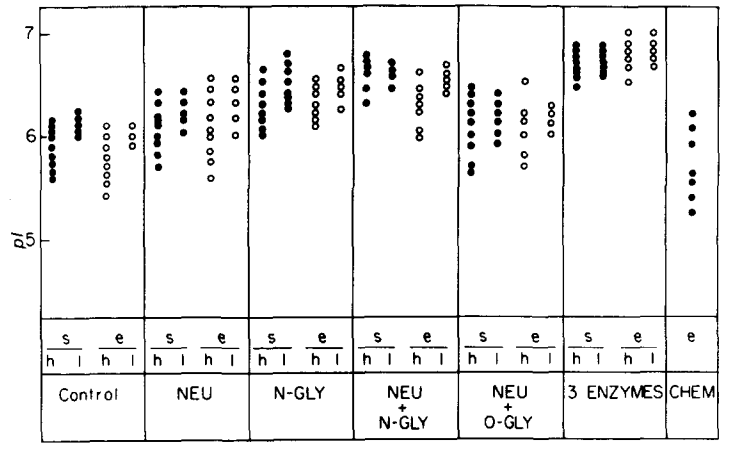

Fig. 2. Isoelectric points of ABP isoforms. The pH values of the isoelectric variants determined by two-dimensional PAGE of control and enzyme-treated ABPs are plotted here. Control, untreated; NEU, neuraminidase; N-GLY- *N*-glycanase; *O*-GLY, *O*-glycanase; 3 enzymes, neuraminidase + *N*-glycanase + *O*-glycanase; chem, chemical deglycosylation; s, serum ABP; e, epididymal ABP; h, heavy subunit; and l, light subunit. Reprinted with permission from Ref. [24].

Table 1. Summary of isoelectric point data on ABP and TeBG

Sample	Native pI range		Deglycosylated[b] pI range, single protomer
	Heavy[a]	Light	
ABP	5.61–6.12	6.01–6.12	5.25–6.20
hTeBG	5.87–6.55	6.14–6.55	6.37–7.02
rbTeBG	5.89–6.10	6.10–6.24	6.23–6.55

[a]Heavy and light refer to the heavy and light subunits.
[b]ABP and hTeBG were treated with TFMSA; rbTeBG was treated with neuraminidase, *O*-glycanase and *N*-glycanase.

than the light subunit, indicating that the former is more glycosylated than the latter. The molecular weight of the completely deglycosylated protomer approximates the 40,000 predicted from the cDNA of rABP and hTeBTG [21, 22]. Based on the molecular weight of the protein core, carbohydrate constitutes 16–22% of the molecular mass of the heavy subunit and 8–14% of that of the light subunit. The heavy subunit of sABP appears to be the most heavily glycosylated with about 22% of its weight being carbohydrate. The heavy subunit of rbTeBG is the least glycosylated with carbohydrate accounting for only about 16% of its weight. The light subunit of hTeBG and rbTeBG contains 14 and 11% carbohydrate, respectively, while the light subunit of sABP and eABP is about 8% carbohydrate. These estimates of molecular weight are only approximations since glycoproteins run anomolously on SDS–PAGE [33].

Serial lectin chromatography

To further evaluate the oligosaccharides present on rABP, hTeBG and rbTeBG, we took advantage of the carbohydrate-binding specificities of a variety of lectins to probe the nature and structures of the carbohydrate units present on the proteins [34]. Figure 3 shows a flow diagram of the fractionation procedure. The proteins were first chromatographed on Con-A, a lectin that binds Asn-linked complex biantennary, high-mannose type and hybrid type glycans. The former can be eluted from the lectin with 10 mM methyl-α-D-glucopyranoside (glucoside) and the latter two types can be eluted with 500 mM methyl α-D-mannopyranoside (mannoside). Asn-linked tri- and tetraantennary complex oligosaccharides, *O*-linked oligosaccharides, and nonglycosylated proteins are not retained by Con-A [34].

Biantennary complex oligosaccharides can be separated into those that are and those that are

Table 2. Percentage of ABP and TeBG as carbohydrate

Sample	M_r Untreated		M_r Deglyc.[a]	ΔM_r[b]		% Carboh.[c]	
	Heavy	Light		Heavy	Light	Heavy	Light
eABP	48,600	43,000	39,600	9000	3400	19	8
sABP	50,900	43,300	40,100	10,800	3200	22	8
hTeBG	52,200	48,600	42,000	10,200	6600	20	14
rbTeBG	44,400	42,700	38,000	6400	4700	16	11

[a]Deglycosylated with TFMSA.
[b]ΔM_r, change in M_r.
[c]% Carbohydrate.

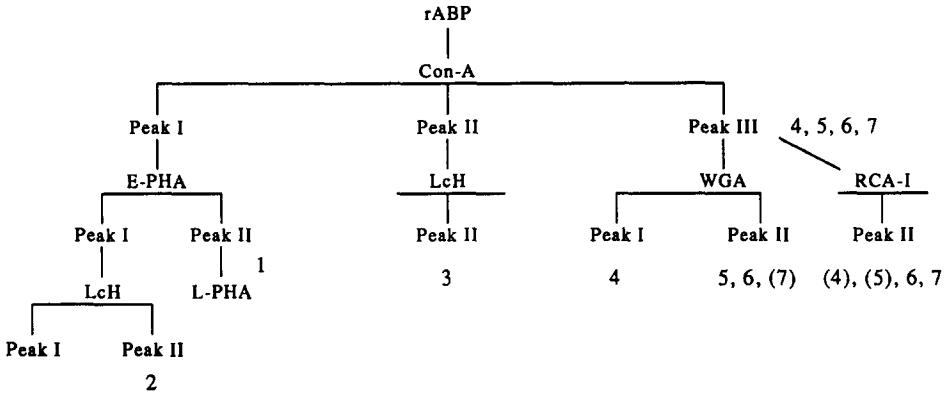

Fig. 3. Flow diagram of the serial lectin chromatographic procedure for the fractionation of rABP. Peak 1 refers to rABP that was weakly retained or not retained by a given lectin. Peaks II and III refer to rABP species that were retained and that could be eluted with additional buffer (chromatography on E-PHA and L-PHA) or with haptenic sugars (the remaining lectins). The numbers refer to oligosaccharide structures [shown in Fig. 5(a)] that would be present on the rABP in a given peak fraction. The numbers in parentheses indicate that these oligosaccharides may or may not be present on the rABP molecule together with the other oligosaccharide structures indicated. The abbreviations are defined in the text. Reprinted with permission from Ref. [31].

not fucosylated on the chitobiose core by chromatography on lentil lectin (LcH). The former bind to the lectin whereas the latter do not [35]; the bound fraction can be eluted with mannoside. High mannose oligosaccharides can be separated from hybrid oligosaccharides by chromatography on wheat germ agglutinin (WGA). Glycopeptides containing high mannose glycans are not retained by WGA, while those with hybrid glycans are, and they can be eluted with *N*-acelyglucosamine (GlcNAc) [36, 37]. Galactosylated glycans are retained on Ricinis communis I (RCA-I) lectin and can be eluted with lactose [36, 37].

Figure 4 shows the elution profile obtained when rABP was chromatographed on Con-A. Three peaks of androgen-binding activity were detected. The first peak, representing rABP that was not adsorbed to the lectin, comprised about 42% of the radioactivity. We were subsequently able to demonstrate by chromatography *Phaseolus vulgaris E*-agarose that about 28% of the rABP in this fraction contained bisected galactose-containing glycans. Chromatography on LcH indicated that rABP in this fraction also contained triantennary oligosaccharides with an internal fucose residue. We were unable to detect *O*-linked glycans in Con-A peak I of rABP.

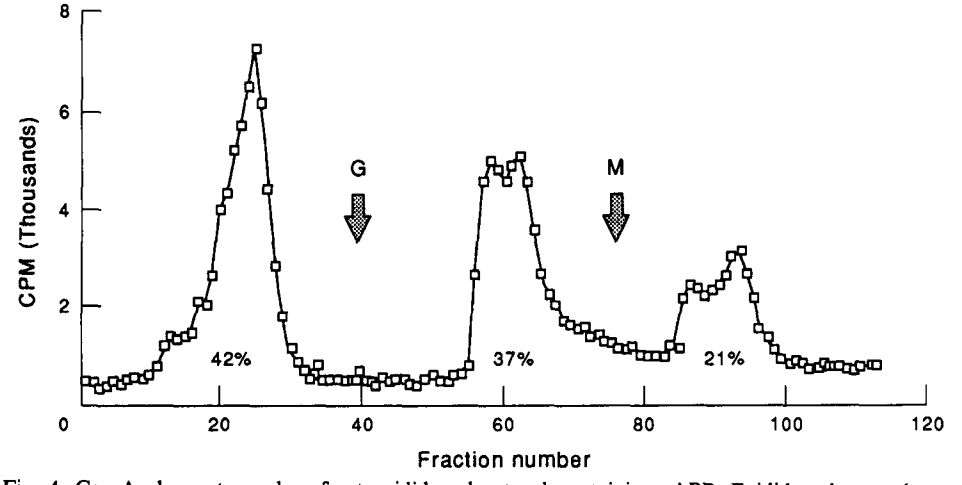

Fig. 4. Con-A chromatography of rat epididymal cytosol containing rABP. Epididymal cytosol was prepared and chromatographed on a 1.5 × 15 cm column containing approx. 25 ml immobilized Con-A Sepharose. Con-A buffer was used as eluant. Two milliliter fractions were collected; 25-μl aliquots of the fractions were incubated with 2 nM [^{3}H]5α-DHT to assay for the presence of rABP. The arrows indicate the addition of glucoside (G) and mannoside (M) in Con-A buffer. The numbers in the peaks indicate the percentage of the total radioactivity present in the peak. Additional details on all lectin chromatography procedures are given in Ref. [31]. Reprinted with permission from Ref. [31].

Table 3. Percentage of oligosaccharide types on ABP and TeBG

Sample	Complex[a] multiantennary	Complex biantennary	High mannose	Hybrid
rABP	44	34(100)[b]	10	13
hTeBG	20	43(8)	37[c]	
rbTeBG (male)	9	52(0)	39[c]	
rbTeBG (pregnant)	21	47(ND)	32[c]	

[a]This fraction may also contain *O*-linked and nonglycosylated forms.
[b]The numbers in parentheses indicate the percentage fucosylated on the chitobiose core.
[c]These may contain both high mannose and hybrid-type oligosaccharides. ND = not determined.

In contrast to rABP, lesser amounts of hTeBG and rbTeBG were not retained by Con-A. About 20% of the hTeBG and the TeBG from pregnant rabbits appeared in the void volume of the Con-A column. Only 9% of the TeBG from male rabbits was not absorbed into the lectin. The difference in the amount of TeBG from the pregnant rabbit vs the male rabbit that was not adsorbed to the gel suggests possible physiological differences in the TeBG from the two sources. There was not sufficient hTeBG or rbTeBG present in the nonretained fraction to further evaluate their oligosaccharide composition.

The second peak, which was eluted from the Con-A column with glucoside, represented 37% of the total rABP and 40–50% of the TeBGs. Subsequent evaluation of this fraction by chromatography on LcH indicated that 100% of the rABP in this fraction was fucosylated on the chitobiose core. Only 8% of the hTeBG was fucosylated in this manner and none of the male rabbit TeBG was fucosylated internally.

About 21% of the rABP and 30–40% of the TeBGs were present in the mannoside-eluted peak of the Con-A column. Subsequent rechromatography of this peak from rABP on WGA indicated that about 44% of this fraction represented rABP to which high mannose-type oligosaccharides were attached and 56% represented rABP to which hybrid-type glycans were attached. All of the Con-A peak 3 was retained by RCA-I and it could be eluted with lactose, indicating the presence of galactosylated chains. When the Con-A peaks 3 of hTeBG and rbTeBG were chromatographed on WGA, 100% was retained and could be eluted with GlcNAc. Thus, no data were obtained on the presence of high mannose-type glycans, which would not have been retained by WGA. It is likely, however, that high mannose-type oligosaccharides are present on the proteins, but that the complete interaction with WGA is owing to glycosylation of all the TeBG on another site with hybrid-type glycans. All of the Con-A peak 3 fraction of TeBG interacted with RCA-I, indicating the presence of galactosylated moieties.

Table 4. Terminal sugars on ABP and TeBG

	SA	GAL	MAN	FUC[a]	GlcNAc
ABP	+	+	+	++++	+
hTeBG	+	+	+	+	+
rbTeBG	+	+	+		+

[a]Fucose on the chitobiose core. The other abbreviations are given in the text or figure legends.

The most salient features of the serial lectin chromatography data are summarized in Table 3. Additional data are in our previously published work [30, 31]. Table 4 summarizes data on the terminal sugars on rABP, hTeBG and rbTeBG. These determinations were made by evaluating data on lectin interaction and by exoglycosidase treatment of the glycoproteins.

Figure 5a presents the minimal structure of the oligosaccharides present on rABP and Fig. 5b presents the minimal structure of the oligosaccharides on TeBG. We refer to these as minimal structures because additional saccharide moieties and/or structures may be present on the glycans that do not affect the interaction of the proteins with the lectins used. These studies do, however, provide a detailed analysis of the major oligosaccharides on rABP, hTeBG and rbTeBG.

The oligosaccharide data on the proteins can be summarized as follows: (1) rABP has a greater percentage of tri- and possibly tetraantennary glycans than the TeBGs, (2) all of the complex biantennary oligosaccharides on rABP are fucosylated on the chitobiose core, whereas only 92% of these glycans on hTeBG and none of them on rbTeBG are fucosylated in this manner, and (3) serial lectin chromatographic data indicate that all three proteins are Asn-glycosylated at more than one site. Our data indicating more than one site for Asn-glycosylation is in agreement with the biochemical data of Avvakumov *et al.* [38] and the more recent data of Hammond *et al.* [22] who showed that there are consensus sequences for the attachment of two Asn-linked glycans on hTeBG. No clear evidence for *O*-glycosylation of any of the proteins was obtained in our studies.

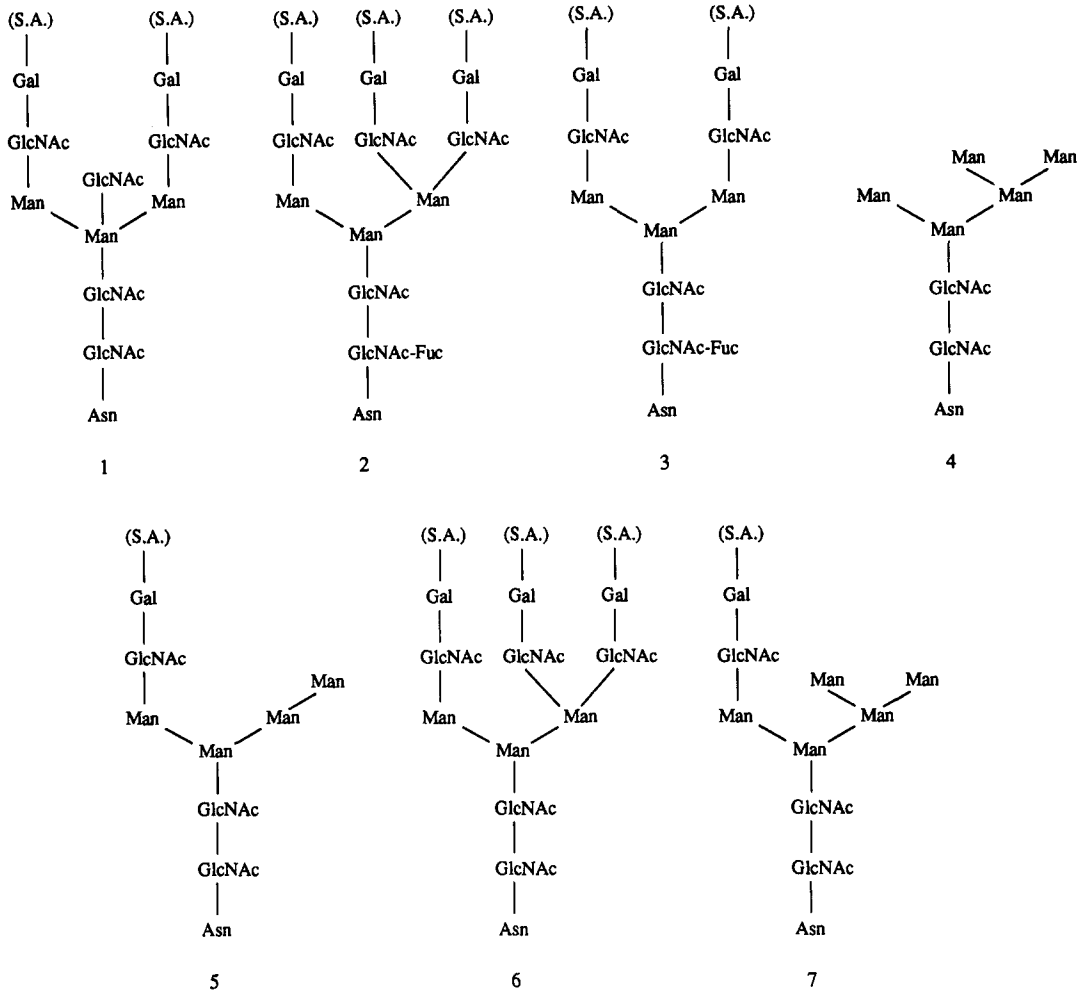

Fig 5a. Proposed minimal structures of the resolved oligosaccharides attached to rABP. Structure 1 is a bisected biantennary galactose containing oligosaccharide; it would be present in E-PHA Peak II (see Fig. 3 for an indication of where all the structures would be on the flow diagram). Structure 2 is a triantennary-complex chain containing an internal fucose residue. Structure 3 is a biantennary-complex oligosaccharide containing a fucose on the innermost GlcNAc. Structure 4 is a high mannose-type oligosaccharide; it may contain between 5 and 9 mannose residues. Structure 5 is a hybrid-type oligosaccharide. Structure 6 is a complex-type oligosaccharide. Structure 7 is derived from Varki and Kornfeld [46]; it could bind to Con-A and RCA-1. The moieties in parentheses may or may not be present in the glycans. All abbreviations are defined in the text except S.A., sialic acid; Fuc, fucose; Man, mannose. Reprinted with permission from Ref. [31].

DISCUSSION

Extensive data have accumulated indicating that the carbohydrate portion of glycoproteins is often crucial for their biological function (for a review, see [23]). Early studies on this topic used human chorionic gonadotropin (hCG) as a model protein. These experiments showed that when sialic acid or galactose was removed from hCG, it was able to bind to its receptor as effectively as the intact hormone, but it was less effective in stimulating steroidogenesis and cyclic adenosine 3′,5′-monophosphate production [39, 40]. There are also compelling data indicating that carbohydrates play a crucial role in the signal-transduction process of glycoprotein hormones [23]. Recent studies using site-directed mutagenesis indicate that specific glycosylation sites are responsible for regulating specific functions of hCG, for example, its secretion and subunit assembly [41]. Oligosaccharides have been shown to be involved in the secretion of renin [42], to have specific effects on the biosynthesis, secretion, and biological function of erythropoietin [43], and to be necessary for the function of the epidermal growth factor receptor [44].

Since oligosaccharides are intimately involved in the structure and function of a wide variety of glycoproteins, we considered it likely that they play an important role in regulating the

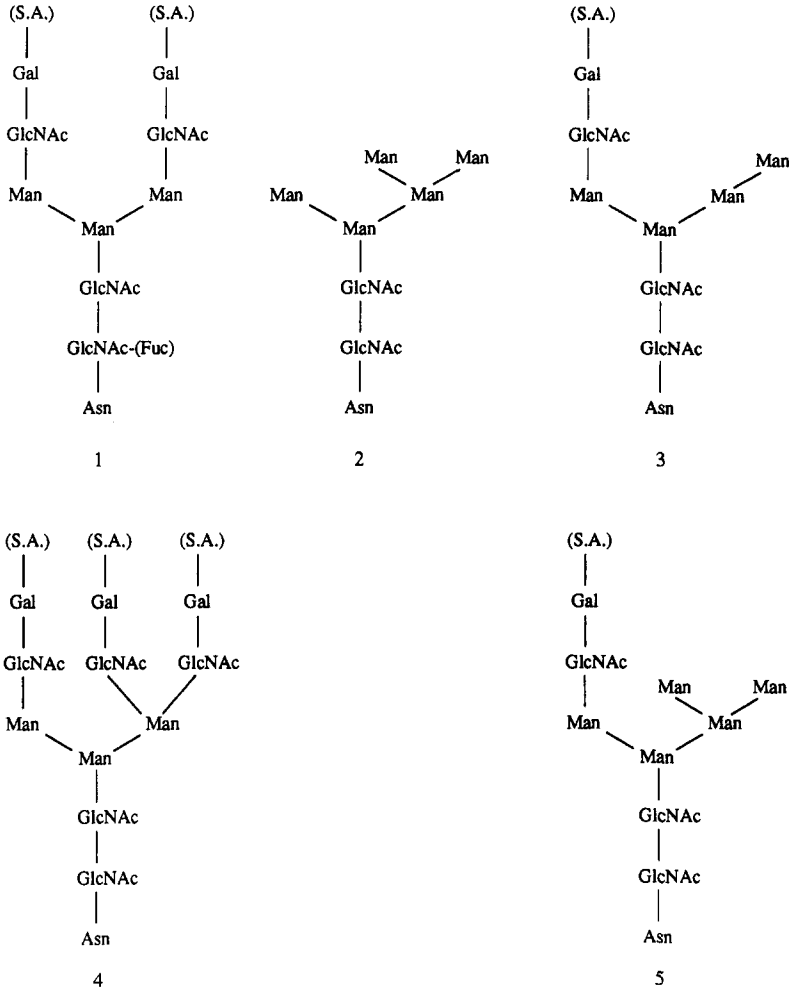

Fig. 5b. Minimal structures of the lectin-binding oligosaccharides present on rbTeBG and hTeBG. Structure 1 is a biantennary complex oligosaccharide; it would be present in Con-A Peak II. The innermost GlcNAc of this glycan is fucosylated on about 8% of the hTeBG present in this peak; none of the rbTeBG appears to be fucosylated in this position. The fact that the TeBG in this fraction also binds to RCA-1 indicates the presence of galactose. Structure 2 is a high mannose-type oligosaccharide; it may contain between 5 and 9 mannose residues. Glycans of this type could be present in Con-A Peak III. Structure 3 is hybrid-type and would also be present in Con-A Peak III. Conformation of the presence of this type of oligosaccharide was obtained by chromatography on WGA to which hybrid-type glycans bind. Structure 4 is a complex-type oligosaccharide; TeBG containing this type of oligosaccharide was identified by its ability to bind to RCA-1. Structure 5 is derived from Varki and Kornfeld[46]; it would be capable of binding to Con-A and RCA-1. The moieties in parentheses may or may not be present on the glycans. All abbreviations are given in the text or in the legend of Fig. 5a. Reprinted with permission from Ref. [30].

function of rABP, hTeBG and rbTeBG. The only known role of these proteins is the binding and transport of androgens. Since we were able to detect various glycosylated forms of the proteins in lectin column eluates by their ability to bind [^{3}H]5α-DHT, it seems unlikely that oligosaccharides are involved in their steroid-binding function. Further evidence mitagating against a role for carbohydrate in steroid-binding is the fact that various glycosylated states of the proteins can be photoaffinity-labeled [24, 25, 32]. These data, however, do not exclude the possibility that a specific glycosylated state may be required to confer the highest affinity of the proteins for their ligand or to yield the greatest steroid specificity.

Two observations we have made point to a physiological role for the oligosaccharides on rABP, hTeBG ane rbTeBG. The first observation is that serum ABP is glycosylated differently from epididymal ABP. Since the carbohydrate portion of glycoproteins has been implicated in protein sorting [45], it is reasonable to assume that the differences in oligosaccharides may reflect differences in targeting of the ABP. That is, the ABP which is destined to

be secreted into the lumen of the seminiferous tubule (presumably from the apical surface of the Sertoli cell) is glycosylated in one manner while ABP that is to be secreted into the bloodstream (presumably from the basal portion of the Sertoli cell) is glycosylated in a different fashion. The second observation is that TeBG from pregnant rabbits is glycosylated differently from TeBG from male rabbits. Whether this difference is a reflection of the different hormonal melieus of the two groups or is a reflection of varied function remains to be elucidated.

Uptake of rABP by the epdidymal epithelium has been demonstrated by immunochemical [9, 10] and autoradiographic methods [11]. Recent unpublished data from our laboratory indicate that receptors for rABP are present in cell membranes from the epididymis and liver. Data also indicate that TeBG can be identified in the cells of a variety of tissues [14, 15] and evidence for the presence of membrane receptors for it has been presented [16, 17]. These observations raise the possibility that rABP and TeBG may themselves be effector molecules. Since oligosaccharides have been shown to be involved in the function of other glycopeptides, it is likely that they play a similar role for rABP, hTeBG and rbTeBG.

One of the interesting features of rABP, hTeBG and rbTeBG is that all three proteins are composed of a single protein core that becomes differentially glycosylated to produce the heterodimeric proteins. Differential glycosylation of a single protomer seems to be a characteristic of this class of proteins and leads to the presence of a heavy and a light subunit. The mechanisms regulating the differential glycosylation of the single protomer remain unknown. Furthermore, it is not known whether differential glycosylation confers specific functions to each of the subunits. For example, specific glycosylation states of a given subunit might be required for receptor recognition or signal transduction. Although chemical crosslinking studies have been conducted on rABP, hTeBG and rbTeBG [24, 25, 32], the resolution of the system was not sufficient for us to determine if the dimer band consisted of a single crosslinked species or if it was composed of a family of dimers representing heavy and light subunits linked in various ratios. Thus, the actual structure of the native proteins under physiological conditions has yet to be determined.

The fact that rABP is glycosylated differently from the TeBGs is probably of physiological significance. Since oligosaccharides are known to protect glycoproteins from degradation, the differences in glycosylation between rABP and TeBG may reflect the different environments into which they are secreted. That is, ABP is primarily secreted into the relatively protected environment of the testis-epididymis compartment while TeBG is secreted into the general circulation. These differences in glycosylation may also reflect a differential ability of the proteins to interact with the same or similar receptor systems.

In conclusion, rABP and hTeBG and rbTeBG are products of an evolutionarily conserved gene. Thus, the protein core of the proteins has a high degree of identity. The protein core consists of a single protomer of about 40 kDa. This core is glycosylated to yield two subunits that differ in the amount and in the type of attached oligosaccharides. These oligosaccharides contribute to molecular weight and charge heterogeneity of the subunits. The types of oligosaccharides on ABP and TeBG differ. Although glycosylation does not affect the ability of the proteins to bind androgen, it may affect their affinity and specificity for ligands. The oligosaccharides may affect intracellular trafficking of the proteins and/or may serve a protective function *vis à vis* extracellular degradation. It is likely that the glycans affect receptor recognition and/or signal transduction. The fact that rABP and the TeBGs possess different oligosaccharide substituents probably reflects differences in their roles as cellular effector molecules.

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