J. Steroid Biochem. Molec. Biol. Vol. 42, No. 5, pp. 475–478, 1992
Printed in Great Britain.

0960-0760/92 $5.00 + 0.00

COMPARISON OF THE CARBOHYDRATE COMPOSITION OF RAT AND HUMAN CORTICOSTEROID-BINDING GLOBULIN: SPECIES SPECIFIC GLYCOSYLATION

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(Received 16 September 1991)

Summary—We have examined the carbohydrate composition of corticosteroid-binding globulin (CBG) obtained from rat and human serum. Rat CBG contained a carbohydrate composition that was strikingly different from that of human CBG. Like other glycoproteins that circulate in human plasma, human CBG had a carbohydrate composition that was consistent with the presence of biantennary and triantennary oligosaccharide structures. In contrast, the carbohydrate composition of rat CBG indicated the presence of more than one sialic acid residue per antenna. It is not clear whether rat CBG contains a carbohydrate structure with sialic acids attached to both galactose and *N*-acetylglucosamine on the same antenna, or a terminal disialylated structure (sialic acid linked α2-8 to sialic acid). These structural variations may play a role in the interaction of CBG with its receptor.

INTRODUCTION

Glycosylation of a specific protein may vary subtly or radically from one species to another, and this variation may result in functional differences between such proteins. For some molecules, such as the gonadotropins, the carbohydrate moiety is critical for receptor interaction and/or signal transduction [1–5]. Carbohydrate side chains may also be involved in antibody–antigen interactions. Alterations in glycosylation, for instance, have marked effects on the interaction of monoclonal antibodies with their respective antigens. Thus, it is apparent that species differences in glycosylation may have a dramatic impact on bioassays, radioimmunoassays, immune reactions, or physiology, and may limit the experimental use of a specific molecule in heterologous species. Frequently, studies are performed in animal models using human proteins, and, in some cases, the converse has been true, i.e. hormones obtained from animal sources are given to human subjects. Even in cases in which there is considerable peptide sequence homology, there may be dramatic species specific differences in glycosylation [6]. The effect of these differences in glycosylation on antigenicity, tissue targeting, and receptor interaction ranges from insignificant to total involvement, depending on the individual system. Thus, before embarking on experiments with glycoproteins in heterologous species, it is important to be aware of the carbohydrate composition of the molecules to be used.

Strel'chyonok *et al.* [7] showed that corticosteroid-binding globulin (CBG), isolated from human postpartum serum, contained a ratio of three biantennary to two triantennary oligosaccharide structures per polypeptide. Further, they also have isolated a variant of human CBG from pregnancy serum, that comprises approx. 10% of the total CBG, that appears to contain only triantennary oligosaccharides [8]. This variant CBG has a binding affinity different from non-variant CBG for binding sites on placental syncytiotrophoblast [9]. Murata *et al.* [10] have shown that although CBG is synthesized and secreted in the presence of tunicamycin, an inhibitor of *N*-linked glycosylation, the amounts are decreased relative to controls, indicating involvement of carbohydrate side chains in these two functions. Although CBG's carbohydrates are not necessary for the binding of cortisol [11], they are important for binding to membrane receptors [9].

In this study we have compared the carbohydrate composition of rat and human

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CBG and found them to be substantially different.

EXPERIMENTAL

Rat CBG was purified from pooled rat serum (Pel-Freez Biologicals, Rogers, AR) and human CBG from pooled pregnancy plasma as described previously [12–14]. Amino acid analyses were performed at the Howard Hughes Medical Institute Protein Chemistry Core Laboratory of the College of Physicians and Surgeons of Columbia University.

The amount of rat CBG or human CBG in each purified preparation was assessed by amino acid analysis. The purified preparations were divided into aliquots for the separate analyses of sialic acid and of the neutral and amino sugars. The neutral and amino sugars were analyzed using a modification of the method of Hardy *et al.* [15] as previously described [16]. Briefly, the preparations were hydrolyzed in 5 M trifluoroacetic acid (Pierce, Rockford, IL) for 4 h at 100°C, and then lyophilized. The dried samples were redissolved in H_2O and injected directly into a Dionex CarboPac HPLC column (Dionex Corp, Sunnyvale, CA) and were eluted (1 ml/min) with 10 mM NaOH for 14.5 min, followed by a wash cycle with 200 mM NaOH for 6.5 min, and an equilibration period (10 min) with 10 mM NaOH before the next injection. The sugars were detected with a Dionex pulsed amperometric detector using previously established settings [15]. A postcolumn eluant of 300 mM NaOH (added via a mixing tee at 1 ml/min) was used to maintain a stable baseline at an output range of 100 nA. The output of the detector was plotted and integrated using the Waters Model 840 integration program (Waters Chromatography Division, Milford, MA). The sugars were quantified by using standard monosaccharides subjected to hydrolysis conditions identical to those used for CBG. The coefficients of variation of the procedure, as determined for each sugar, from 5 injections of sugar mixtures containing 1 nmol of each standard, were as follows: fucose, 2.2%; galactosamine, 2.1%; glucosamine, 2.2%; galactose, 3.1%; glucose, 2.0%; and mannose, 3.2%.

To determine the sialic acid content, each preparation was subjected to mild acid hydrolysis, in 0.8 M trifluoroacetic acid, for 1 h at 80°C. Under these conditions, a standard neuraminlactose preparation was converted quantitatively to free sialic acid and lactose. The sialic acid in the hydrolysate was quantified using the HPLC apparatus described above. The column was eluted with 100 mM sodium acetate in 150 mM NaOH. As before, a postcolumn eluant of 300 mM NaOH (1 ml/min) was added to maintain a stable baseline at an output range of 100 nA.

RESULTS

Preparations of purified human and rat CBG were divided into three aliquots for analysis of neutral sugars, sialic acid, and amino acid content. Multiple analyses were performed on two different preparations each of rat and human CBG. The results of these analyses are summarized in Table 1. The carbohydrate composition of human CBG was similar to that found by Akhrem *et al.* [17]. The molar ratio of mannose to protein in human CBG suggested the presence of 5–6 *N*-linked oligosaccharides, most likely consisting of a mixture of biantennary and triantennary forms. The greater amounts of *N*-acetylglucosamine, galactose, and sialic acid, relative to mannose, in the compositional analysis of our preparations of human CBG suggested the possibility of a somewhat higher ratio of triantennary to biantennary forms than that which was found by Strel'chyonok *et al.* [7].

Rat CBG had a carbohydrate composition that was markedly different from that of human CBG. Most striking was the fact that the composition indicated that rat CBG contained one less oligosaccharide than did human

Table 1. Carbohydrate composition of rat and human CBG

Source	nmol of monosaccharide per nmol protein[a]				
	Sialic acid	Fucose	Glucosamine	Galactose	Mannose
Rat CBG	21.3 ± 1.2	1.9 ± 0.2	24.3 ± 1.6	12.9 ± 1.0	14.2 ± 0.9
(molar ratios)	(4.5)	(0.4)	(5.1)	(2.7)	(3.0)*
Human CBG	13.6 ± 1.7	1.4 ± 0.2	27.4 ± 1.2	16.3 ± 0.8	17.7 ± 0.9
(molar ratios)	(2.3)	(0.2)	(4.7)	(2.8)	(3.0)*

[a] Mean ± SEM of 4 values obtained from two separate analyses of two preparations of CBG from each species. *The values in parentheses represent the molar ratios of the sugars relative to a mannose content of 3.0 residues per oligosaccharide.

CBG. Although compositional analysis does not distinguish between biantennary, triantennary, or tetraantennary structures, the high ratio of galactose and glucosamine to mannose in rat CBG was consistent with the presence of predominately multiantennary oligosaccharides. Although the average structure suggested by the sugar ratios in Table 1 would be triantennary, the presence of only triantennary structures on rat CBG was inconsistent with its lectin binding properties. Because the molecule binds to Concanavilin A (ConA) [18], one or more biantennary structures must have been present. Either tetraantennary structures (in combination with biantennary structures) or biantennary structures containing a repeating galactose-*N*-acetylglucosamine sequence could be present. Both types of molecules would bind to ConA [19, 20].

As has been noted previously [17], our preparation of human CBG had a sialic acid content that was lower than that of galactose, suggesting that a small amount of terminal galactose may have been present on the human protein. This was not true for rat CBG which had an excess of sialic acid over galactose. This observation was consistent with our previous finding that rat CBG did not bind to the hepatic asialoglycoprotein receptor unless it was first treated with neuraminadase [21], whereas human CBG did (Maitra U. S., Khan M. S. and Rosner W., unpublished). It was also consistent with experiments demonstrating that the clearance of human CBG from plasma was prolonged by the coinjection of asialofetuin, whereas that of rat CBG was not [22].

Galactosamine was undetectable in both human and rat CBG, indicating the absence of *O*-glycosylation in both. Further, the lack of galactosamine ruled out the possibility that the additional sialic acid content in rat CBG could have been due to the presence of *O*-linked glycosylation. The most likely arrangement that would account for the high sialic acid content would be that several antennae contained more than one sialic acid residue.

DISCUSSION

We have examined the carbohydrate composition of CBG purified from the plasma of rats and from humans. The relative amounts of carbohydrate on each type of CBG were very different. Based on quantitative analyses of sugar relative to amino acid composition, human CBG had enough carbohydrate to account for oligosaccharides on 5 to 6 glycosylation sites; its amino acid sequence indicates 6 consensus sequences for *N*-linked glycosylation [23]. Although, it has been postulated that only 5 of the sites are actually glycosylated [7], the absence of glycosylation on a specific site has not been demonstrated. In contrast to human CBG, based on quantitative analyses of sugar relative to amino acid composition, the carbohydrate composition of rat CBG suggests one less oligosaccharide. The amino acid sequence of rat CBG, like that of human CBG, contains 6 consensus sequences for *N*-linked glycosylation [24]; however, the first potential glycosylation site in human CBG, asparagine 9, is glycosylated [25], whereas the homologous site on rat CBG, asparagine 3, appears not to be [26].

The carbohydrate composition of human CBG was consistent with the presence of biantennary and triantennary oligosaccharide structures as reported previously [7]. These structures are similar to those commonly found on other glycoproteins circulating in human serum. The ratio of sialic acid, galactose, and *N*-acetylglucosamine, relative to mannose, that we observed in human CBG, suggested a somewhat higher ratio of triantennary to biantennary forms than was previously found [7]. However, the relative proportions of triantennary and biantennary and possibly tetraantennary structures are commonly variable from one laboratory to another, due to differences in source material or purification procedures. Indeed, a CBG variant has been described (accounting for approx. 10% of the CBG obtained from pregnancy serum) that contained only triantennary oligosaccharide forms [8].

Rat CBG had a carbohydrate composition that was quite different from that of human CBG. That composition indicated the presence of more than one sialic acid residue per antenna. It was not clear whether rat CBG contained a structure with sialic acids attached to both galactose and *N*-acetylglucosamine on the same antenna, or a terminal disialosyl structure (sialic acid linked α2-8 to sialic acid). Previous analyses of rat serum glycoproteins have demonstrated instances of the presence of two sialic acid residues per antenna. Yoshima *et al.* [27] reported a structure in which one sialic acid was attached to galactose(β1-3)*N*-acetylglucosamine, and a second was in α2-6 linkage to the underlying *N*-acetylglucosamine. This

type of structure also has been found in bovine, but not in the corresponding human glycoproteins [28]. An alternative arrangement, that could account for two sialic acid residues per antenna, would be sialic acid linked α2-8 to sialic acid. Polysialosyl structures containing multiple α2-8 linked sialic acid residues attached to an antenna via galactose have been reported in rat tissues [29].

These studies set out the oligosaccharide composition of rat CBG and emphasize the necessity of carefully justifying the use of human CBG for physiological studies in rodents.

Acknowledgement—This work was supported in part by National Institutes of Health Grant DK 36714.

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