Carbohydrate Research, 134 (1984) 133–140
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

PREGNANCY-ASSOCIATED MOLECULAR VARIANTS OF HUMAN SERUM TRANSCORTIN AND THYROXINE-BINDING GLOBULIN

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(Received March 18th, 1983; accepted for publication, April 24th, 1984)

ABSTRACT

Affinity chromatography on immobilised concanavalin A revealed that transcortin and thyroxine-binding globulin isolated from human postpartum serum contained ~10% of molecular variants that did not occur in these glycoproteins isolated from normal donor serum (both male and female). The chromatographic behaviour of the pregnancy-associated glycoprotein variants, their monosaccharide compositions, and the results of methylation analysis indicated that these variants contained only triantennary oligosaccharide chains of the *N*-acetyl-lactosamine type.

INTRODUCTION

Microheterogeneity is a characteristic[1] of the carbohydrate moieties of glycoproteins. Bayard and Kerckaert[2] suggested that a heterogeneous glycoprotein is a mixture of a few molecular variants differing to some extent in their carbohydrate structures. Such molecular variants have been found, for example, for human transferrin[3] and orosomucoid[2]. It was also suggested[2] that different variants of a glycoprotein are synthesised in different tissues or in different cells of one tissue. If this assumption is true, then the changes in the physiological state of the human organism might be accompanied by variations in the relative amounts and the appearance and/or disappearance of glycoprotein variants.

We now present data which suggest that, in the human, pregnancy is associated with the appearance of specific molecular variants of transcortin and thyroxine-binding globulin, two hormone-binding serum glycoproteins the blood levels of which increase significantly during pregnancy[4,5].

EXPERIMENTAL

Glycoproteins. — Transcortin and thyroxine-binding globulin (TBG) were isolated from normal donor serum (male or female, separately) and postpartum human serum by affinity chromatography on immobilised cortisol and thyroxine,

0008-6215/84/$ 03.00

respectively, and purified as earlier described[6,7]. The glycoprotein preparations used were homogeneous according to gel electrophoresis and *N*-terminal amino acid analysis. Their basic physicochemical properties and parameters of hormone binding were similar to those previously reported[7,8]. The solution concentrations of the glycoproteins were determined spectrophotometrically using the known absorbance coefficients[7,8].

The glycoproteins were radioiodinated according to the technique of Greenwood *et al.*[9]. The specific activity of the labelled glycoproteins was 20–40 μCi/μg, and >95% of the radioactivity migrated in the band of the corresponding parent glycoprotein on electrophoresis in polyacrylamide gel.

Analytical techniques. — The sialic acid and protein contents in chromatographic fractions were determined using, respectively, the techniques of Aminoff[10], after hydrolysis of glycoproteins (0.1M H_2SO_4, 1 h, 80°), and Lowry *et al.*[11] with bovine serum albumin as the reference protein.

To determine the monosaccharide compositions, glycoproteins were heated with methanolic 0.5M HCl for 24 h at 100°, and the resulting methyl glycosides were analysed by g.l.c.[12]. Methylation of glycoproteins was performed as earlier described[13]. The products were methanolysed under the above conditions, the methylated methyl glycosides were quantitatively transformed into the corresponding trimethylsilylated alditol and 2-deoxy-2-(*N*-methylacetamido)alditol derivatives[13], and quantified by using g.l.c.[14]. The methyl ethers of manno- and galactopyranosides used as standards were obtained in preparative quantities by column liquid-adsorption chromatography, as earlier described[15].

Affinity chromatography. — Columns (5 × 0.6 cm) were packed with a fresh portion of Con-A-Sepharose 4B (Pharmacia) and washed with 10 vol. of 0.02M Tris-HCl buffer (pH 7.0) containing 0.15M NaCl and 0.01M each of $CaCl_2$, $MgCl_2$, and $MnCl_2$ (the starting buffer) at 20–23°. All further operations were performed at 4°. A desalted, freeze-dried glycoprotein preparation was dissolved in the starting buffer to a final concentration of 5 mg/mL. An aliquot (1.0 mL) of this solution was applied to the column which, after a 15-min delay, was eluted at 5 mL/h, first with the starting buffer (10 mL) and then with 30 mL of the same buffer containing 0.02M methyl α-D-glucopyranoside. Fraction I (the first 12 mL of the eluate) contained material that did not interact with concanavalin A. Fraction II (the following 28 mL) contained glycoproteins that interacted with the lectin. Fractions I and II were desalted by gel filtration through a column of Bio-Gel P-6 (Bio-Rad Laboratories) and freeze-dried.

With each glycoprotein preparation (transcortin or TBG from either male, female, or postpartum serum), sextuplicate chromatography experiments were carried out. All of the material collected as Fraction I was used to determine the contents of protein or sialic acid (triplicate assays), and appropriate aliquots were withdrawn from a solution of Fraction II in distilled water for the same purposes. With the transcortin and TBG preparations from postpartum serum, triplicate assays of the content of the binding sites for cortisol (transcortin) or thyroxine

(TBG) in Fractions I and II were performed by the equilibrium dialysis technique[16], using either ^{3}H-cortisol or ^{125}I-thyroxine as tracers. The radioactivity of the samples was measured either with Tracor Europa Mark III liquid-scintillation or Tracor Europa RIA-300 gamma counters.

In special experiments, the radio-iodinated transcortin or TBG ($1–3 \times 10^7$ c.p.m. in each experiment) was added to a related unlabelled glycoprotein before its application to the affinity column. The affinity chromatography was then performed as described above. Fractions (1 mL) were collected in polystyrene gamma-counting tubes, and the ^{125}I-radioactivity in these fractions was measured.

RESULTS AND DISCUSSION

Recently, we elucidated the structures of the oligosaccharide chains of transcortin isolated from human postpartum serum[17,18]. This glycoprotein contains ~5 mol of *N*-linked oligosaccharides of the *N*-acetyl-lactosamine type[1] per mol of the glycoprotein, ~3 mol of which are biantennary glycans and ~2 mol are triantennary. Human TBG contains[19] 3 mol of biantennary and 1 mol of triantennary *N*-linked oligosaccharides of the *N*-acetyl-lactosamine type per mol of the glycoprotein.

Such structures of the transcortin and TBG carbohydrate moieties prompted the use of affinity chromatography on immobilised concanavalin A to search for molecular variants of these glycoproteins. This lectin binds[20] biantennary oligosaccharides of the *N*-acetyl-lactosamine type, in which both of the α-D-mannosyl residues are substituted only at position 2, but not triantennary oligosaccharides. Consequently, the affinity chromatography of transcortin and TBG might separate molecular variants on the basis of the relative content of bi- and tri-antennary sugar chains in the glycoprotein molecules.

Transcortin from normal donor serum (both male and female) was retarded by the affinity adsorbent and eluted with the buffer containing methyl α-D-glucopyranoside (Fig. 1, Table I). In contrast, transcortin from postpartum serum contained a molecular variant that did not interact with concanavalin A (Fig. 1, Table I). Based upon the data presented in Table I, the amount of this transcortin variant was calculated to be ~10% of the transcortin isolated from postpartum serum.

That this non-retarded component is a transcortin variant, and not an impurity, was established as follows. This variant was isolated from a transcortin preparation that was homogeneous according to the criteria conventionally used in protein chemistry and was obtained by using biospecific chromatography on immobilised cortisol. Also, this variant had the requisite biological activity of transcortin, *i.e.*, capacity to bind cortisol (Table I). The parameters of the cortisol and progesterone binding to the pregnancy-associated transcortin variant (the data will be published elsewhere) were close to those previously reported for transcortin[8].

The chromatographic behaviour of the pregnancy-associated transcortin

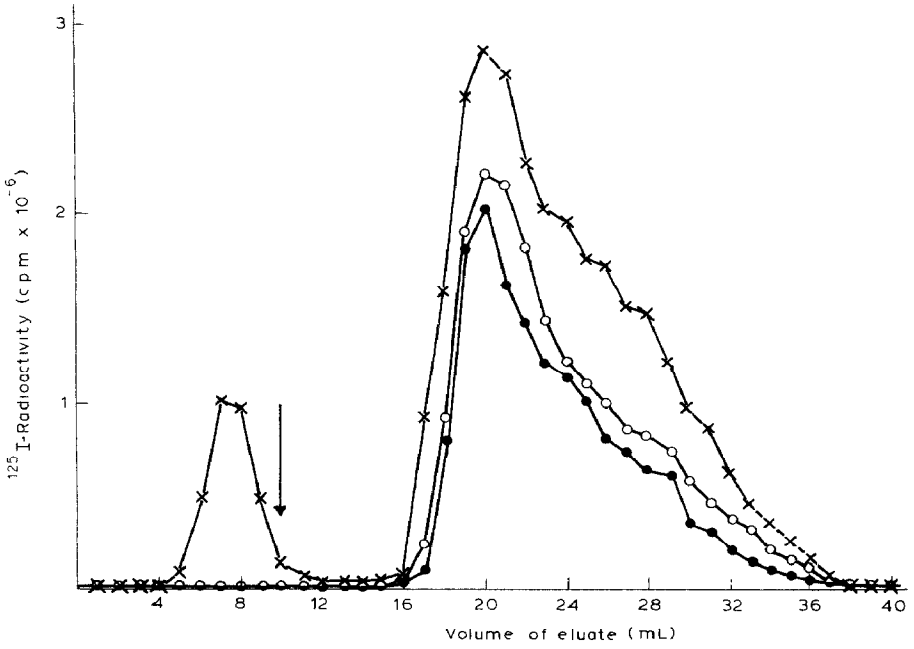

Fig. 1. Affinity chromatography of transcortin from male (—●—), female (—○—), and postpartum (—×—) human serum on immobilised concanavalin A. Each sample contained 5 mg of unlabelled transcortin and ~4 μg (1–3 × 10^7 c.p.m.) of ^{125}I-transcortin prepared by radioiodination of transcortin isolated from the same biological source. The arrow indicates the beginning of the elution with the buffer containing methyl α-D-glucopyranoside. Other chromatography conditions are given in the text.

TABLE I

AFFINITY CHROMATOGRAPHY[a] OF TRANSCORTIN ISOLATED FROM MALE (m), FEMALE (f), AND POSTPARTUM (r) HUMAN SERUM ON IMMOBILISED CONCANAVALIN A

Measured parameter	*Source of transcortin preparation*	*Fraction I*	*Fraction II*
Protein content (mg)	m	0	3.61 ±0.04
	f	0	3.72 ±0.04
	r	0.47 ±0.06	3.09 ±0.10
Sialic acid content (μmol)	m	0	1.03 ±0.02
	f	0	1.02 ±0.05
	r	0.13 ±0.03	0.87 ±0.01
Content of the cortisol-binding sites (nmol)	m		n.d.[b]
	f		n.d.
	r	9.0 ±0.8	81.2 ±4.3

[a]See Experimental for details. Values represent means ±S.E.M. of three experiments. [b]Not determined.

variant suggested that it did not contain the biantennary oligosaccharides or that their interaction with concanavalin A was sterically hindered. The data on the monosaccharide composition of this variant and the results of methylation analysis (Table II) proved that it contained only triantennary sugar chains. This conclusion

TABLE II

MONOSACCHARIDE COMPOSITION OF THE PREGNANCY-ASSOCIATED TRANSCORTIN VARIANT AND METHYLATED MONOSACCHARIDES DERIVED THEREFROM

Monosaccharide	*Total content*[a]	*Methylated derivatives*	
		Positions of methoxyl groups	*Yield*[a]
Fucose	1.2	n.d.[b]	
Mannose	15.0	3,4,6	5.1
		3,6	5.1
		2.4	4.9
Galactose	14.8	2,3,4,6	3.2
		2,4,6	5.7
		2,3,4	5.6
2-Acetamido-2-deoxyglucose	24.3	3,6	22.1
		3	0.9
N-Acetylneuraminic acid	12.7	n.d.[b]	

[a]Mol/mol of glycoprotein. [b]Not determined.

TABLE III

AFFINITY CHROMATOGRAPHY[a] OF TBG ISOLATED FROM MALE (m), FEMALE (f), AND POSTPARTUM (r) HUMAN SERUM ON IMMOBILISED CONCANAVALIN A

Measured parameter	*Source of TBG preparation*	*Fraction I*	*Fraction II*
Protein content (mg)	m	0	3.78 ±0.08
	f	0	3.86 ±0.06
	r	0.41 ±0.03	3.31 ±0.05
Sialic acid content (μmol)	m	0	0.68 ±0.07
	f	0	0.68 ±0.03
	r	0.09 ±0.01	0.59 ±0.05
Content of the thyroxine-binding sites (nmol)	m	n.d.[b]	
	f	n.d.	
	r	6.2 ±0.5	70.8 ±2.6

[a]See Experimental for details. Values represent means ±S.E.M. of three experiments. [b]Not determined.

stems from the equal contents of the mannosyl and galactosyl residues, and by the formation of equal amounts of the 3,4,6-tri-*O*-methyl and 3,6-di-*O*-methyl derivatives of mannose. The formation of the increased amount of 2,4,6-tri-*O*-methylgalactose, as compared with the yield from the whole transcortin from postpartum serum[17], also favoured the presence of triantennary oligosaccharides only, since they are characterised[18] by NeuAc-(2→3)-Gal linkages.

The investigation of thyroxine-binding globulin (TBG) gave results similar to those for transcortin. As shown in Fig. 2 and Table III, TBG from postpartum

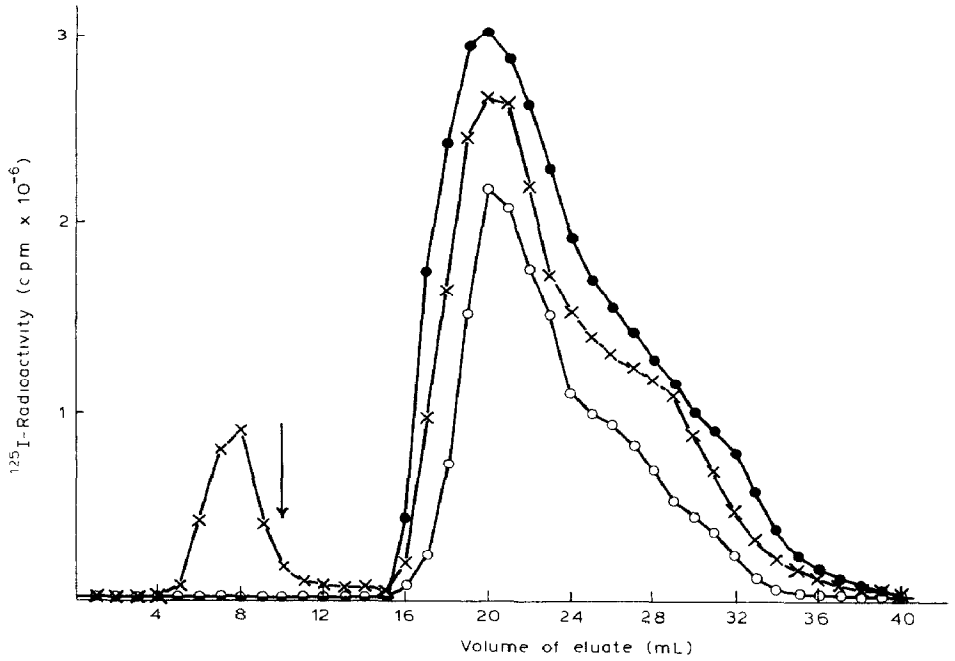

Fig. 2. Affinity chromatography of TBG from male (—●—), female (—○—), and postpartum (—×—) human serum on immobilised concanavalin A. The samples were prepared as described in the legend to Fig. 1.

serum also contained ~10% of a molecular variant that did not interact with the affinity column and did not occur in the glycoprotein from normal donor serum. Table III shows that the pregnancy-associated TBG variant binds thyroxine. The parameters of the thyroid-hormone binding to this TBG variant (data not shown) were close to those reported[7] for TBG.

TBG contains[19] both *N*-linked oligosaccharides of conventional structures and those with GlcNAc-(1→6)-Man linkages, which is unusual for the glycoprotein carbohydrate moieties[1]. That the pregnancy-associated TBG variant was not retarded in the affinity column might have resulted from substitution at position 6 of the α-D-mannosyl residues in its biantennary oligosaccharide chains[20]. However, methylation of this TBG variant gave no derivative appropriate for 6-substituted α-D-mannosyl residues (Table IV). Presumably, as for transcortin, the pregnancy-associated molecular variant of TBG contained only triantennary oligosaccharide chains. The data on its monosaccharide composition (equimolar content of mannose and galactose) and the results of the methylation analysis (the formation of equal amounts of 3,6-di-*O*-methyl- and 3,4,6-tri-*O*-methylmannose together with a relatively large amount of 2,4,6-tri-*O*-methylgalactose; see Table IV) supported this assumption.

We have shown with ovalbumin[13] and human transcortin[17] that it is possible to obtain reliable information on the carbohydrate structures of a glycoprotein by

TABLE IV

MONOSACCHARIDE COMPOSITION OF THE PREGNANCY-ASSOCIATED TBG VARIANT AND METHYLATED MONOSACCHARIDES DERIVED THEREFROM

Monosaccharide	*Total content*[a]	*Methylated derivatives*	
		Positions of methoxyl groups	*Yield*[a]
Mannose	11.8	3,4,6	4.0
		3,6	4.0
		2,4	4.1
Galactose	12.1	2,3,4,6	1.9
		2,4,6	3.4
		2,3,4	6.7
2-Acetamido-2-deoxyglucose	19.5	3,6	19.4
N-Acetylneuraminic acid	11.1	n.d.[b]	

[a]Mol/mol of glycoprotein. [b]Not determined.

methylation analysis of the intact biopolymer. More recently, this approach was used effectively in a study of the carbohydrate structures of human sex hormone-binding globulin[21]. The results of the present work prove that it is also useful for investigating the molecular variants of glycoproteins.

To the best of our knowledge, ours are the first data (part has been published previously[22]) on the relation between the physiological state of the human organism and the structure of the carbohydrate moieties of serum glycoproteins. The formation of specific molecular variants of the two hormone-binding serum glycoproteins during pregnancy, when such organs as uterus, placenta, and some others develop, may be circumstantial evidence supporting the assumption[2] that the biosynthesis of glycoprotein molecular variants occurs in different locations.

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