O-GLYCOSIDIC CARBOHYDRATE UNITS FROM GLYCOPROTEINS OF DIFFERENT TISSUES: DEMONSTRATION OF A BRAIN-SPECIFIC DISACCHARIDE, α-GALACTOSYL-(1→3)-*N*-ACETYLGALACTOSAMINE

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1. Introduction

A number of soluble glycoproteins contain oligosaccharide units linked to the protein with an alkali-labile *O*-glycosidic linkage between *N*-acetylgalactosamine and serine or threonine [1–6]. A common structural feature in the carbohydrate units of these glycoproteins is the disaccharide β-galactosyl-(1→3)-*N*-acetylgalactosamine which occurs in these glycoproteins with or without additional neuraminic acid or fucose residues.

Much less is known about the structures of carbohydrate units in membrane-bound tissue glycoproteins. The erythrocyte membrane glycoproteins contain oligosaccharide units similar to those of the soluble glycoproteins [7]. These neuraminic acid-containing carbohydrate units are involved in MN blood-group activity [8]. The neuraminic acid-free oligosaccharide core is also an integral part of the T (Thomsen-Friedenreich) antigen [9,10], which recently has been reported to occur also in cancerous mammary tissue as a specific tumour-associated antigen [11].

In a previous communication [12], the structures of the *O*-glycosidic carbohydrate units of rat-brain glycoproteins were studied. Four of the five carbohydrate units were similar to those of the soluble and erythrocyte membrane glycoproteins. However, a previously unknown carbohydrate unit, α-galactosyl-(1→3)-*N*-acetylgalactosamine was also detected. In the present work, the *O*-glycosidic carbohydrate units of different tissues were studied. It was found that the disaccharide β-galactosyl-(1→3)-*N*-acetylgalactosamine occurs as a core in the carbohydrate units of glycoproteins in all the tissues studied. In contrast to this, the new carbohydrate unit α-galactosyl-(1→3)-*N*-acetylgalactosamine was detected in brain tissue only, and is thus the first tissue-specific carbohydrate unit of glycoproteins to be described.

2. Materials and methods

2.1. *Purification of glycopeptides*

Adult albino Wistar rats were killed by decapitation. The blood was collected and the erythrocytes were washed by centrifugation [13]. Gastric and small intestinal mucosae were obtained by scraping with a glass slide. Brain samples were also taken from adult rabbits and hens. The tissue samples were delipidated by homogenization in chloroform–methanol [14]. The lipid-free residues were subjected to papain digestion and the solubilized glycopeptides were purified by cetyl pyridinium chloride precipitation and gel filtration as described earlier [14]. The neuraminic acid content of the glycopeptides was determined as described by Miettinen and Takki-Luukkainen [15].

2.2. *Preparation of oligosaccharides*

Glycopeptide samples (from 5–20 mg of dry lipid-free tissue) were treated with 0.4 ml of 0.05 M NaOH in 1 M $NaBH_4$ for 16 h at 45°C. The $NaBH_4$ was

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destroyed with 3.6 ml of 0.2 M acetic acid and the samples were passed through small columns (bed volume 2 ml) of Dowex-50 (H^+) ion exchange resin. The columns were washed with 6 ml of H_2O and the samples were taken to dryness under reduced pressure. Boric acid was removed by repeated evaporations with acetic acid–methanol (1:1000, v/v).

2.3. *Fractionation of oligosaccharides*

The oligosaccharide samples were dissolved in 5 ml of 5 mM pyridine-acetic acid buffer (pH 5.0) and applied to columns (0.4 × 2 cm) of DEAE-Sephadex A-25 equilibrated with the same buffer. The neutral oligosaccharides were eluted with 15 ml of the buffer. The fractions containing oligosaccharides with one or two neuraminic acid residues were obtained by subsequent elution first with 15 ml of 50 mM and then with 15 ml of 250 mM pyridine–acetic acid buffer. The columns were finally eluted with 2 M buffer to collect any oligosaccharides carrying more than two neuraminic acid residues.

In control experiments, the step-wise elution method was found to result in the same degree of separation as did the elution with a continuous linear gradient [12].

2.4. *Identification of the disaccharide cores*

The internal standard (2–4 nmol lactose) was added and the samples were taken to dryness. The neuraminic acid residues were cleaved by mild acid hydrolysis (0.1 M HCl, 80°C, 1 h) or incubation with 10 units of *Vibrio-cholerae* neuraminidase (grade B, Calbiochem) in 0.4 ml of 10 mM Tris-acetate (pH 6.8) for 20 h at 37°C. The liberated disaccharides and the free disaccharides of the neutral fraction were analyzed as their trimethylsilyl derivatives by gas-liquid chromatography and mass spectrometry [12].

3. Results and discussion

The amounts of dry lipid-free residue obtained from 1 g (wet weight) of tissue were: liver, 230 mg; kidney, 160 mg; gastric mucosa, 85 mg; small intestinal mucosa, 60 mg; packed erythrocytes, 210 mg; brain, 100 mg. The amounts of glycopeptide-bound neuraminic acid varied considerably (table 1). The highest amount was observed in the small intestinal mucosa, whereas liver and erythrocytes contained the lowest amounts.

$NaOH–NaBH_4$ treatment of the glycopeptides released the *O*-glycosidic carbohydrate units as reduced oligosaccharides. In all the tissues studied, the same disaccharide unit was detected. The retention times on gas-liquid chromatography were the same as that of β-galactosyl-(1→3)-*N*-acetylgalactosaminitol isolated from rat brain [12], and the mass spectra showed a fragmentation pattern characteristic of a hexosyl-(1→3)-*N*-acetylhexosaminitol [12]. The disaccharide

Table 1
O-Glycosidic oligosaccharides of glycopeptides in different tissues and animals

Tissue	Total glycopeptide neuraminic acid	α-Gal-(1→3)-GalNAcol	β-Gal-(1→3)-GalNAcol			Total oligosaccharides
			Free	Monosialyl-	Disialyl-	
Rat						
liver	380	n.d.[a]	1.5	5.6	7.7	14.9
kidney	1080	n.d.	46	29	26	100
gastric mucosa	940	n.d.	84	21	19	124
small intestinal mucosa	1470	n.d.	21	26	15	62
erythrocytes	130	n.d.	1.3	5.1	18	24.5
brain	1010	16	24	33	60	134
Rabbit brain	1020	18	28	36	55	137
Hen brain	1060	19	31	33	55	138

[a]n.d. = not detected, less than 1% of the value of free β-Gal-(1→3)-GalNAcol.

The oligosaccharides were obtained by $NaOH–NaBH_4$ treatment of the purified glycopeptides and fractionated by ion-exchange chromatography. After mild acid hydrolysis, the disaccharides were analyzed by gas-liquid chromatography. Gal-(1→3)-GalNAcol = galactosyl-(1→3)-*N*-acetylgalactosaminitol. All values given as nmol/100 mg dry lipid-free tissue.

has previously been found in a number of soluble glycoproteins [1–6] and in glycoproteins of human erythrocyte membrane [7] and rat brain [12]. Now the disaccharide is shown to occur in glycoproteins of several other tissues.

The disaccharide unit was found in three oligosaccharide fractions: as free disaccharide and as a core in tri- en tetrasaccharides containing one and two neuraminic acid residues (table 1). Oligosaccharides of higher neuraminic acid content were not observed in any tissue. This is at variance with the suggestion that rabbit brain glycoproteins would contain as a major *O*-glycosidic carbohydrate unit a pentasaccharide consisting of the core disaccharide and three neuraminic acid residues [16]. No sign of such an oligosaccharide was detected in this study. Artifactual neuraminic acid cleavage was not observed during the preparation and fractionation procedures. The amounts of these oligosaccharides varied considerably from tissue to tissue. The highest level of oligosaccharides was found in brain. Erythrocytes and brain contained the highest proportion of dineuraminyl oligosaccharides, whereas in kidney and gastric mucosa the neutral form predominated. The similarity in the amounts of oligosaccharides in brain glycoproteins of different animal species (table 1) suggests that the oligosaccharide patterns observed in different tissues of rat might be similar also in other animal species.

The presence of β-galactosyl-(1→3)-*N*-acetylgalactosaminitol in several tissues is of special interest, since this disaccharide is an integral part of the T antigen [9,10]. This antigen has been reported to be specific for cancerous mammary tissue [11]. In non-malignant mammary tissue the antigen is detected only after removal of neuraminic acid residues, which suggests that mammary tissue glycoproteins also contain sialylated oligosaccharide units similar to those present in other tissues.

In contrast to β-galactosyl-(1→3)-*N*-acetylgalactosaminitol, which occurred in all the tissues studied, the new disaccharide α-galactosyl-(1→3)-*N*-acetylgalactosaminitol was detected only in brain. The disaccharide occurred in similar amounts in brains of different animal species. The disaccharide was present only in a non-sialylated form, suggesting that an α-linked galactose moiety cannot serve as a neuraminic acid acceptor. The same seems to be true also for glycolipids, since the known α-galactose-containing glycolipids do not contain neuraminic acid [17].

α-Galactosyl-(1→3)-*N*-acetylgalactosaminitol is, to our knowledge, the first reported tissue-specific carbohydrate unit in glycoproteins. A few brain-specific glycoproteins are known [18–20], but the structures of the carbohydrate units have not been studied. Therefore it is not known which part of the molecule is tissue-specific. Further studies are indicated to characterize the glycoprotein(s) in which the new brain-specific disaccharide occurs.

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