A SIMPLE METHOD FOR THE ISOLATION OF NEUTRAL GLYCOPEPTIDES BY AFFINITY CHROMATOGRAPHY

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1. Introduction

Membrane glycoproteins have been shown to contain both *O*-glycosidically- and *N*-glycosidically-linked carbohydrate moieties [1–3]. The latter are composed of neutral and acidic carbohydrate chains [3,4]. Because of the difficulties in the fractionation it has not been possible to isolate homogeneous preparations and to study the structure of the *N*-glycosidically-linked carbohydrate chains. Therefore, only little is known about the structures of the *N*-glycosidic carbohydrate moieties in membrane-bound glycoproteins [5,6].

This report describes a method for the isolation of neutral glycopeptides devoid of contaminating acidic glycopeptides. It was found that Concanavalin A binds specifically one type of acidic glycopeptides and neutral glycopeptides derived from rat brain glycoproteins. The neutral glycopeptides can be separated from the acidic ones by eluting the Concanavalin A-Sepharose column with a concentration gradient of methyl α-D-glucoside.

2. Materials and methods

2.1. *Preparation of* N-[^{3}H]*acetylated glycopeptides*

Whole brains from adult albino Wistar rats were used. Rat brain glycopeptides were prepared and purified as described previously [3]. In order to minimize the effect of the peptide chain of the glycopeptides on Con A-affinity chromatography the purified glycopeptides were extensively redigested with pronase [7]. The glycopeptides were *N*-[^{3}H] acetylated in their peptide moiety with [^{3}H]acetic anhydride (500 mCi/mmol, The Radiochemical Centre, England) in 0.1 M $NaHCO_3$ at room temperature for 30 min. The *N*-[^{3}H]acetylated glycopeptides were separated from free radioactivity by gel filtration on a column of Sephadex G-25 (2 × 45 cm).

2.2. *Affinity chromatography of glycopeptides on Con A-Sepharose*

Rat brain glycopeptides were fractionated by affinity chromatography on a column of Con A-Sepharose (1.8 × 16 cm, Pharmacia Fine Chemicals, Sweden) at 4°C. The glycopeptides dissolved in 0.005 M HEPES buffer (pH 6.9) containing $CaCl_2$, $MgCl_2$ and $MnCl_2$ (1 mM each) were applied to the column equilibrated with the same buffer. Elution was carried out first with 0.005 M HEPES buffer (pH 6.9) containing the divalent cations and 0.1 M NaCl and then with a continuous convex gradient of 0.0–0.3 M methyl α-D-glucoside in 480 ml of the starting buffer. The glycopeptides in the fractions eluted from the Con A-Sepharose column were separated from methyl α-D-glucoside by gel filtration on a column of Sephadex G-10 (3 × 70 cm).

2.3. *Analytical methods*

Monosaccharides were determined with gas-liquid chromatography after methanolysis [8] and after acid hydrolysis [3]. Methyl α-D-glucoside was assayed by a modification of the anthrone reaction [9]. *O*-Glycosidically linked carbohydrate units were analyzed as by Finne [2]. Neuraminic acid was also determined by the resorcinol method of Svennerholm [10], as modified by Miettinen and Takki-Luukkainen [11]. Tritium was counted in 5 ml of Bray's scintillation solution in a Packard Tricarb Liquid Scintillation Spectrometer.

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3. Results

Affinity chromatography of glycopeptides obtained from rat brain glycoproteins on a column of Con A-Sepharose produced three distinct fractions (fig.1). Glycopeptides with no affinity for the column were eluted with starting buffer (fraction A). Glycopeptides bound by Con A were eluted with a concentration gradient of methyl α-D-glucoside. Fractions B and C were eluted with about 15 mM and 100 mM methyl α-D-glucoside, respectively. Upon re-chromatography fractions B and C were eluted as a single peak in the same position as in the first run. The separation of the glycopeptides into three fractions on Con A-Sepharose was independent of the pH of the eluting buffer, since the elution profile was essentially the same as with 0.005 M sodium acetate buffer (pH 5.2).

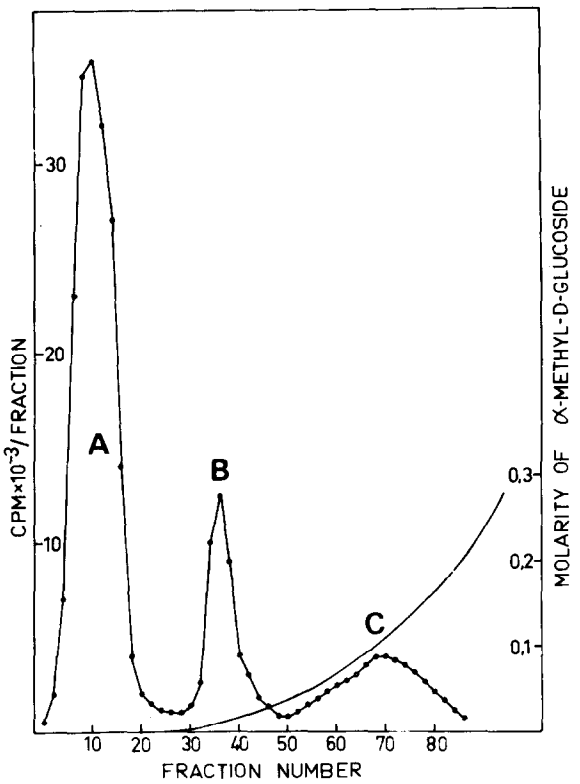

Fig.1. Fractionation of rat brain glycopeptides on Con A-Sepharose (1.8 × 16 cm). A sample corresponding to 500 mg of lipid-free brain residue was dissolved in 40 ml of 0.005 M HEPES buffer (pH 6.9) containing $CaCl_2$, $MgCl_2$ and $MnCl_2$ (1 mM each) and applied to the column equilibrated with the same buffer. Elution was carried out with 0.005 M HEPES buffer (pH 6.9) containing 0.1 M NaCl and the divalent cations and then with a gradient of 0.0–0.3 M methyl α-D-glucoside in 480 ml of the starting buffer (solid line). Fractions of 5.5 ml were collected and counted for radioactivity (o). The fractions are labelled A, B and C in order of their emergence from the column (see text).

The neutral glycopeptides could also be isolated by stepwise elution of the Con A-Sepharose column. Glycopeptides bound by the lectin were obtained by subsequent elution first with 20 mM methyl α-D-glucoside and then with 200 mM methyl α-D-glucoside in the starting buffer. The purity of the fractions obtained by stepwise elution was checked by subjecting the fractions to re-chromatography using a continuous gradient.

About 75% of the carbohydrate applied to the column was recovered and there were no marked differences in the yields of the different sugars. One ml of Con A-Sepharose was able to bind the glycopeptides obtained from about 40 mg of lipid-free brain residue.

The three glycopeptide fractions were quite different in sugar composition (table I). The glycopeptides in fraction A were composed of fucose, mannose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine and *N*-acetylneuraminic acid. In sugar composition these glycopeptides resembled the acidic glycopeptides obtained by DEAE-chromatography [3]. All *O*-glycosidically-linked carbohydrate units were found in this fraction, since no *N*-acetylgalactosamine or peptide-bound α- or β-galactosyl-(1→3)-*N*-acetylgalactosamine were present in fractions B and C [2]. The glycopeptides in fraction B were composed of fucose, mannose, galactose, *N*-acetylglucosamine and *N*-acetylneuraminic acid and they were less acidic than glycopeptides in fraction A. The ratio of *N*-acetylneuraminic acid to fucose was 2:1 in fraction A and 1:2 in fraction B. Mannose and *N*-acetylglucosamine were enriched relative to other sugars in fraction B as compared to fraction A. Fraction C was composed entirely of glycopeptides containing mannose and *N*-acetylglucosamine. The ratio of mannose to *N*-acetylglucosamine was 4:2. Fractions A, B and C contained 70%, 19% and 11% of the glycopeptide carbohydrate, respectively.

To characterize the glycopeptide fractions obtained by affinity chromatography on Con A, the molecular sizes of the glycopeptides in the fractions were

Table 1
Carbohydrate composition of rat brain glycopeptide fractions obtained by affinity chromatography on Con A

Fraction	Fucose	Mannose	Galactose	GalNAc[a]	GlcNAc	NeuNAc	Total
(A)							
μmol/100 mg LFR	0.400	0.611	0.758	0.174	1.13	0.713	3.79
mol/100 mol	10.6	16.1	20.0	4.6	29.9	18.8	100
(B)							
μmol/100 mg LFR	0.151	0.265	0.135	n.d.	0.454	0.082	1.09
mol/100 mol	13.9	24.4	12.4		41.7	7.6	100
(C)							
μmol/100 mg LFR	n.d.	0.403	n.d.	n.d.	0.173	n.d.	0.576
mol/100 mol		69.9			30.1		100

The fractions were prepared by affinity chromatography on Con A-Sepharose as shown in Fig.1. Values are given as μmol sugar/100 mg of lipid-free residue (LFR) and are the means of 5-8 determinations.

[a] GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; NeuNAc *N*-acetylneuraminic acid; n.d., not detected.

estimated by subjecting samples to gel filtration on Sephadex G-50 under standardized conditions (fig.2). For comparison blue dextran and ovalbumin glycopeptides were run on the same column. The acidic glycopeptides not bound by Con A (fraction A) were clearly larger than the less acidic glycopeptides with low affinity for the lectin (fraction B) and these were slightly larger than the neutral glycopeptides (fraction C). The last-named were of about the same molecular size as the ovalbumin glycopeptides.

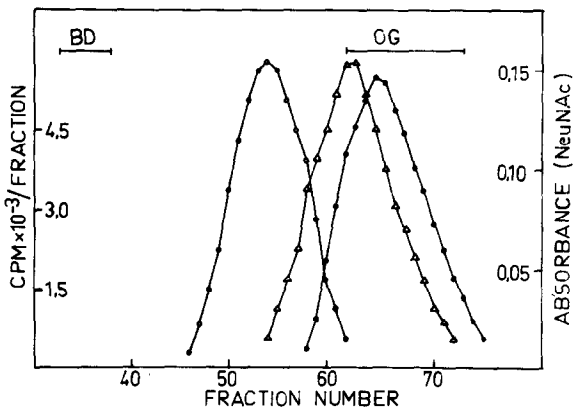

Fig.2. Rat-brain glycopeptide fractions obtained by affinity chromatography on Con A-Sepharose (fig.1) were subjected to gel filtration on Sephadex G-50 (2 × 75 cm). The column was eluted with 0.1 M pyridine/acetic acid buffer (pH 5.0). Fractions of 3.2 ml were collected. Glycopeptide fraction A (○) was analyzed for *N*-acetylneuraminic acid and glycopeptide fractions B (△) and C (●) for radioactivity. Each glycopeptide fraction was run separately under standardized conditions on the same column. The elution volumes of blue dextran (BD) and ovalbumin glycopeptides (OG) are shown for comparison.

4. Discussion

Neutral glycopeptide fractions have been previously isolated by DEAE anion-exchange chromatography [3,12], gel filtration [13] and dialysis followed by electrophoresis [14]. However, these fractions were not composed of neutral glycopeptides only, since small amounts of galactose, fucose and *N*-acetylneuraminic acid also were present. Javaid et al. have prepared Con A-binding glycopeptides from rat brain [15]. Their fraction was composed of both neutral and acidic glycopeptides. After leucine aminopeptidase treatment, gel filtration and coupled anion- and cation-exchange chromatography, a fraction of neutral glycopeptides was obtained [15].

The experiments described in the present report demonstrate that Con A interacts specifically with two types of glycopeptides of rat brain. It binds one type of acidic glycopeptides with low affinity and binds neutral glycopeptides with high affinity. Because of the difference in their affinity for Con A these two types of glycopeptides can readily be separated from each other by concentration gradient elution with methyl α-D-glucoside.

Gradient elution with a specific displacer in lectin affinity chromatography seems to be a useful addition to the methodological arsenal for the isolation of carbohydrate structures. A similar technique may be equally succesful in the fractionation of glycoproteins and glycopeptides using other lectins.

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