ISOLATION OF CONCANAVALIN A-BINDING GLYCOPROTEINS FROM RAT BRAIN

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1. Introduction

Gombos et al. [1] have shown that a fraction of the glycopeptides obtained by proteolysis of rat brain glycoproteins could be adsorbed on concanavalin A (Con A) polymerized with glutaraldehyde. These glycopeptides were rich in mannose and *N*-acetylglucosamine and stimulated neurite growth in tissue culture.

Our object was to determine whether the Con A-binding oligosaccharides are widely distributed among the brain glycoproteins or are confined to a few types. For this purpose, the fraction of brain glycoproteins containing the Con A-binding oligosaccharides was prepared from delipidated brain extract by affinity chromatography on Con A-Sepharose in presence of sodium deoxycholate (DOC) by the method of Allan et al. [2].

The results reported in this paper indicate that the fraction binding to Con A-Sepharose and eluted with α-methylmannoside represents a significant fraction of total brain glycoproteins; it contained 15% of the glycoprotein NANA and approx. one third of the glycoprotein hexose. Its separation into more than 8 bands by gel electrophoresis demonstrates its heterogeneity.

2. Materials and methods

Adult male rats were anesthetized with ether and perfused with saline. The whole brains were excised and the lipids were removed by extraction with chloroform–methanol [3]. Since the residue could not be completely solubilized in DOC, the following procedure was used. The residue was immediately homogenized at room temp. in 5% sodium dodecylsulfate (SDS) containing 1% disodium EDTA, 0.01 M Tris-HCl, pH 7.4 and NaOH to final pH 7.0; 20 ml of solvent per rat brain were used. The solution was dialyzed for 12 hr against 50 vol of 0.1% SDS in 0.0005 M Tris-HCl, pH 7.4, at room temp. The dialysis against fresh solution was repeated once. It was then dialyzed for 24 hr at room temp. against 100 vol of 1% DOC. This was repeated with fresh solution at 2–4°. Insolubilized material, representing 1% of the total protein, was removed by centrifugation at 100,000 *g* for 2 hr at 2°.

Affinity chromatography of this extract was performed on a column of Con A-Sepharose 4B obtained from Pharmacia, Fine Chemicals AB, Uppsala, Sweden. The column was prepared and utilized [2] as indicated under fig. 1. The ratio of brain protein in the extract to Con A bound to the resin was 1.5 to 2.5 (w/w).

The fractions were freed of detergent and methylated sugars by dialysis against 100 vol of 6 M urea in 0.0005 M Tris-HCl, pH 7.4, for 24 hr at 2–4°. This was repeated against fresh solution. They were further dialyzed two times against 200 vol of ice-cold 0.0005 M Tris-HCl, pH 7.4.

For gel electrophoresis, aliquots were lyophilized, redissolved in 2% SDS (3 to 7 mg protein per ml) and dialyzed at room temp. against 0.1% SDS in 8 M urea and 0.01 M Tris-HCl pH 7.4. Electrophoresis was carried out in polyacrylamide gel slabs, utilizing an Ortec No 4214 glass cell. The discontinuous buffer system of Grossfeld and Shooter [4] was used; 4 M urea and 0.1% SDS were added to both buffer chambers. 8 M urea and 0.1% SDS were added to each layer of the gel, which was polymerized from 8.4 ml of 10% acrylamide gel solution, 6.3 ml of 7.5% solution, 4.2 ml of 4% solution and 7.5 ml of stacking gel solution.

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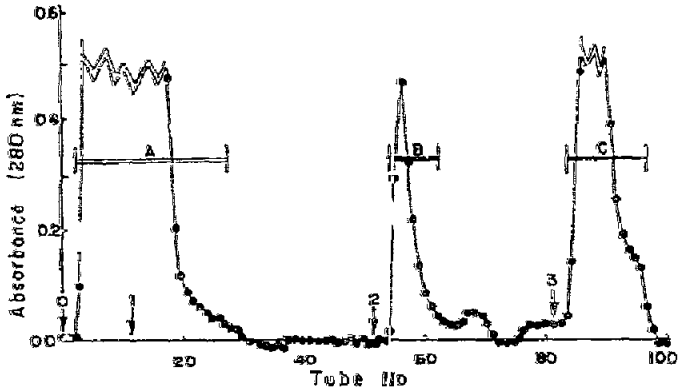

Fig. 1. Fractionation of rat brain extract. A column (1 cm diameter) containing 6.5 ml of packed Con A-Sepharose 4B was washed successively (2–4°) with 16 ml of 1% DOC, 23 ml of 5% α-methylglucoside in 1% DOC and 38 ml of 1% DOC. The brain extract (116 mg protein in 25 ml of 1% DOC) was introduced to the column at arrow 0. First 1% DOC, then 2% α-methylmannoside in 1% DOC and finally 8 M urea in 1% DOC were introduced as indicated by arrows 1, 2 and 3, respectively. 2.3 ml aliquots were collected. Fractions A, B and C were obtained by pooling tubes as indicated.

Samples contained 175 to 350 μg of protein in 50 μl. Electrophoresis was run at room temp.; starting conditions were 17 V and 10 mA; at end of run (bromophenol blue marker, 0.5 cm from anodic end of gel), conditions were 60 V and 15 mA. After overnight fixation in 100% acetic acid, the gels were stained by the periodic acid–Schiff technique [5] and with Buffalo Black NBR, obtained from Allied Chemical, Morristown, N.J.. Con A (Grade III from Jack Beans) was purchased from Sigma Chemical Co., St Louis, Mo., USA.

3. Results and discussion

Brain tissue, delipidated in chloroform–methanol, could not be completely solubilized in DOC; therefore it was first dissolved in SDS and then transferred to a DOC solution by dialysis. Traces of SDS remaining in the solution were not deleterious since Con A is fairly resistant to SDS denaturation [6]. Prior to the addition of brain extract, the column of Con A-Sepharose 4B had been equilibrated with α-methylglucoside in 1% DOC and washed with DOC. The fraction of the brain extract which did not adsorb to Con A was washed out with DOC (Fraction A, fig. 1). Elution of adsorbed glycoproteins with α-methylmannoside in DOC gave

Table 1
Analysis of fractions.

Fractions	Distribution of protein (%)	Distribution of NANA (%)	NANA (μM)*	Hexose (μM)*
Total extract in DOC	100	100		
Fraction A (not adsorbed)	86	57		
Fraction B (eluted with methylmannoside)	4	15	0.17	1.0–1.7
Fraction C (eluted with urea)	29	5		
Recovery in fractions A + B + C	110	77		

Proteins were determined by the method of Lowry et al. [8] or by reading the absorbancy in 1% DOC at 260 and 280 nm [9]. NANA was determined by the method of Warren [10] and hexose, by the anthrone method [11], using mannose as standard. Results represent the average values for two experiments. A range is given when large variations were recorded.
* Content expressed in μM per 100 mg of protein in the total extract.

Fraction B. As observed by Allan et al. [2], the elution of glycoproteins with α-methylmannoside was not complete; further elution with 8 M urea in DOC yielded a fraction (Fraction C, fig. 1) containing 5% of the NANA originally put on the column. This fraction was highly contaminated with denaturated Con A and was not studied further.

Fraction B, eluted with α-methylmannoside, was considerably enriched in glycoproteins and represented a sizable fraction of the brain glycoproteins. It contained 4% of the protein and 15% of the NANA applied to the column (table 1). It can be calculated from results reported previously [7] that approx. one third of the glycoprotein hexose applied to the column was present in this fraction.

The total extract in DOC and fractions A, B and C were subjected to electrophoresis in presence of SDS and urea (fig. 2). The heterogeneity of fraction B was indicated by the presence of 8 bands staining strongly for carbohydrate with the periodic acid–Schiff technique and several weaker staining bands. These bands

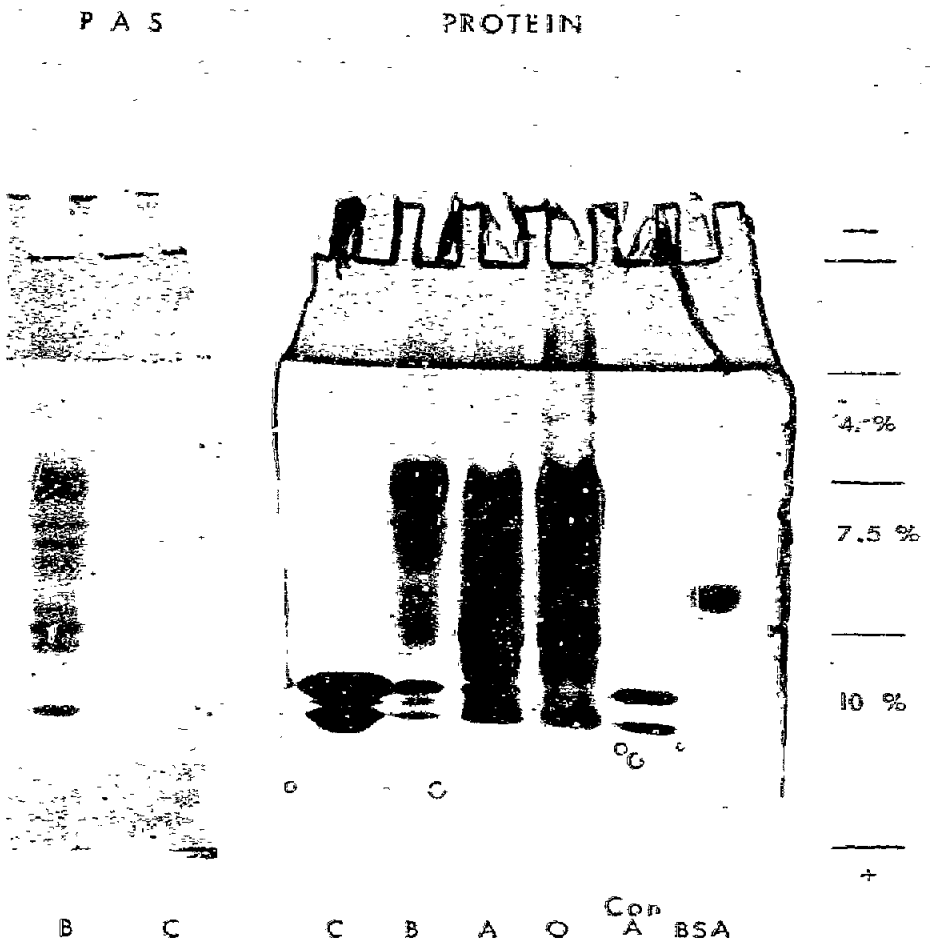

Fig. 2. Gel electrophoresis of fractions in 0.1% SDS and 8 M urea, as described in text. Acrylamide concentrations in the layers of the gel are indicated on the right side of the figure. Proteins were stained with Buffalo Black and carbohydrates, by the periodic acid–Schiff stain (PAS); O, total extract in DOC (350 μg of protein); A, fraction A of fig. 1 (320 μg of protein); B, fraction B (175 μg of protein); C, fraction C (280 μg of protein); Con A (35 μg); BSA, reduced bovine serum albumin (35 μg).

were also observed on staining for protein, although with different relative intensities. A slight contamination by Con A liberated from the column is likely (presence of the triple front bands).

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