PURIFICATION AND CHARACTERIZATION OF A SOLUBLE GLYCOPROTEIN FROM RAT BRAIN (GM 50-C)

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1. Introduction

When trying to determine appropriate labeling conditions for some behavioral experiments involving glucosamine incorporation into rat brain subcellular fractions in vivo, it was noticed that a substantial portion of the radioactivity present in a 105 000 *g* supernatant of a brain homogenate, exhaustively dialyzed against distilled water, migrated as a peak, in the front, upon discontinuous gel electrophoresis at pH 8.8 [1], in 9% polyacrylamide. When the 105 000 *g* supernatant was partitioned with chloroform–methanol [2], and the electrophoresis repeated on the upper phase material, it could be seen that the above front peak remained unchanged. While it was not possible to exclude the presence in this peak of either free labeled glucosamine or some of its metabolites [3], this finding prompted us to investigate the existence of some water-soluble, acidic, low molecular weight glycoproteins migrating to the upper phase (chloroform–methanol–water, 3:48:47, v/v/v) upon partition in a chloroform–methanol–water system (8:4:3, v/v/v) [2]. In this way we found out the existence of a water-soluble, 50% methanol-soluble, acidic glycoprotein, with affinity for Concanavalin A (GM 50-C).

2. Materials and methods

Except for the partition step, performed at room temperature, all other operations were carried out at 4°C. Whole brains from Sprague–Dawley rats (around 200 *g* of body weight) were homogenized in 6 vol of 0.32 M sucrose in 0.1 mM Tris–HCl, pH 7.1. This homogenate was centrifuged for 1 hr at 105 000 *g*, and the supernatant was exhaustively dialyzed against distilled water adjusted to pH 7.1 with Na_2CO_3. The dialysis residue was filtered through a 0.2 μm Millipore filter to get rid of any material precipitated during dialysis. This filtrate constitutes the water-soluble fraction. It was next partitioned with a mixture of chloroform–methanol so as to give final proportions of chloroform–methanol–water, 8:4:3, v/v/v [2]. The upper phase was collected and shaken twice with 1.5 vol of freshly-prepared lower phase. The final upper phase was dialyzed against distilled water, pH 7.1, and filtered again through a 0.2 μm Millipore filter. This filtrate will be referred to as the methanol-soluble fraction. Since we were specifically looking for glycoproteins, we then passed the methanol-soluble fraction, previously made up to 1 mM in Mg^{2+}, Mn^{2+} and Ca^{2+}, as chloride salts, through a ConA-Sepharose* (Pharmacia) column which specifically binds glycomacromolecules containing α-D-glucopyranosyl, α-D-mannopyranosyl or α-D-fructofuranosyl residues as oligosaccharide chain terminal units [4]. Elution of bound glycomacromolecules was performed by washing the column with a 2% (w/v) solution of methyl-α-D-mannopyranoside (Sigma Chemical Co.) in water. Fractions of 2 ml were collected, and protein was monitored by reading the absorbance at 280 nm. The fractions of the eluate showing significant absorbance at 280 nm were analyzed by either discontinuous polyacrylamide gel electrophoresis at pH 8.8 [1], or by gel electrophoresis at the same pH in presence of SDS [5]. Amino acid analysis was performed directly on stained gel bands [6, 7]. Complement fixation was carried out as previously described [8].

* *Abbreviations*: ConA-Sepharose, concanavalin A covalently attached to Sepharose 4B; SDS, sodium dodecyl sulfate.

3. Results and discussion

Fig. 1 shows the electrophoretic pattern of proteins present at different stages along the purification procedure. It can be seen that the so-called methanol-soluble protein fraction includes at least two protein species. When these methanol-soluble proteins are passed through a ConA-Sepharose column, and the bound material is eluted with a 2% (w/v) solution of methyl-α-D-mannopyranoside, the elution profile obtained is illustrated in fig. 2. Although this profile appears somewhat asymmetric, only one and the same protein band was obtained upon electrophoresis of any of the fractions 6–40. We have called this protein (fig. 1C) GM 50-C in consideration to its being a rat brain glycoprotein soluble in about 50% methanol and with affinity for concanavalin A. The recovery of GM 50-C protein is difficult to evaluate due to possible incomplete release of glycoprotein from the ConA-Sepharose column [9]. Nevertheless, the yield of GM 50-C in three different preparations ranged between 0.20 and 0.25% of the water-soluble proteins.

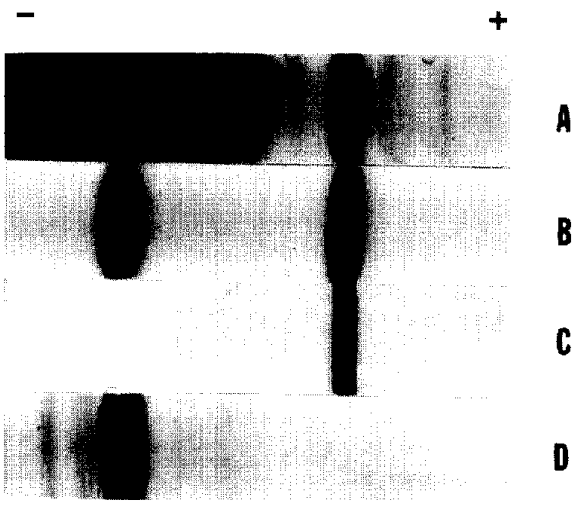

Fig. 1. Electrophoretic analysis of proteins present at different stages during the purification of rat brain glycoprotein GM 50-C. A: Water-soluble proteins. B: 50% Methanol-soluble proteins (methanol-soluble fraction). C: Purified GM 50-C glycoprotein eluted from the ConA-Sepharose column with methyl-α-D-mannopyranoside. D: Sample of calf brain glycoprotein GP 350 [11], shown as a reference. All the electrophoresis were performed in 12.5% polyacrylamide gels, at pH 8.8 (discontinuous) [1].

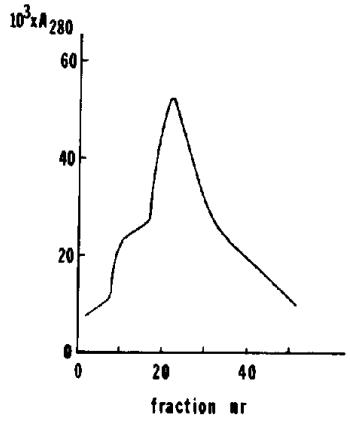

Fig. 2. Elution profile of rat brain 50% methanol-soluble glycoproteins bound to a ConA-Sepharose column. The elution was carried out with a 2% (w/v) solution of methyl-α-D-mannopyranoside in water. Two-ml fractions were collected.

Fig. 3 shows the electrophoretic behavior of glycoprotein GM 50-C in different electrophoretic systems. Interestingly, GM 50-C moves with the electrophoretic front (that is, superimposed to brain protein S 100) in gels up to 9% in polyacrylamide (discontinuous gel electrophoresis system of Davis [1], at pH 8.8), that relates well with the phenomenon described in Introduction. On the other hand, when the acrylamide con-

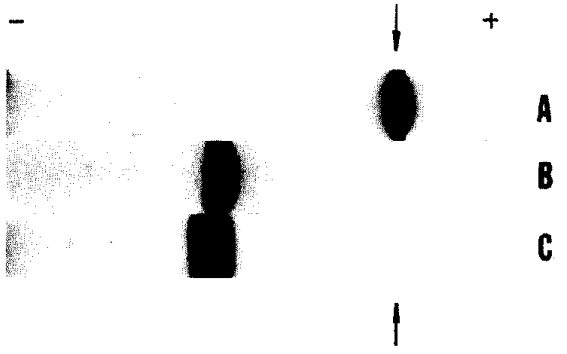

Fig. 3. Electrophoretic behavior of purified rat brain GM 50-C glycoprotein in different electrophoretic systems. A: Electrophoresis of GM 50-C in a 9% polyacrylamide gel, at pH 8.8 (discontinuous) [1]. B: Electrophoresis of GM 50-C in a 15% polyacrylamide gel, at pH 8.8 (discontinuous) [1]. C: Electrophoresis of GM 50-C in a 15% polyacrylamide gel, in the presence of sodium dodecyl sulfate, at pH 8.8 (discontinuous) [5]. The arrows point to the electrophoretic front for all three gels.

Table 1
Amino acid analysis of GM 50-C glycoprotein.

Glutamic acid	16.71	Leucine	5.37
Aspartic acid	13.30	Valine	4.85
Glycine	12.08	Isoleucine	4.51
Threonine	8.05	Phenylalanine	4.01
Alanine	7.63	Tyrosine	1.14
Arginine	7.46	Proline	0.98
Lysine	7.19	Histidine	0.81
Serine	6.92		

Amino acid analysis was performed directly on stained gel bands pooled from three different preparations [6, 7]. Results are given as residues/100 residues.

Table 2
Complement fixation of GM 50-C glycoprotein against brain protein S100, calf brain glycoprotein GP 350 and α_2-glycoprotein.

Antigen	Water-soluble fraction	Methanol-soluble fraction	Purified GM 50-C
S100	0.3	0.021	0
GP 350	0.3	24.6	0
α_2-Glycoprotein	N.D.	N.D.	0

Complement fixation was carried out as previously described [8]. The results are expressed as percent of total protein in the fraction assayed. N.D. = not determined.

centration, in the same electrophoretic system, is increased up to 15%, the mobility of GM 50-C goes down to 0.555 ± 0.008 (fig. 3B). At this same acrylamide concentration, in the presence of SDS [5], GM 50-C shows one single protein band with an apparent mol. wt. of 14 500 ± 1400. Nevertheless, the native molecular weight of GM 50-C remains to be determined.

As for the composition of GM 50-C, the results of the amino acid analysis are given in table 1. The apparent prevalence of acidic residues is in agreement with the electrophoretic behavior of the native protein at pH 8.8. The possible contamination of GM 50-C by ganglioside was ruled out by two different methods. First, ganglioside standards run in similar gels (in the absence of SDS) gave unique stained bands within 5 mm of the origin, with independence of the acrylamide concentration used. On the other hand, an aliquot of GM 50-C was taken up in 25 μl of chloroform–methanol (1:1, v/v) and chromatographed on a thin layer of Silica Gel G using propanol–water (3:1, v/v) as the ascending solvent. After development of the plate according to Svennerholm [10], no sialic acid was found in the position of the standard gangliosides. Sialic acid was however found in the origin, most likely representing protein-bound sialic acid. We are, at present, developing a batch procedure to prepare the protein in adequate amounts as to afford extensive chemical analyses of the carbohydrate moiety.

Table 2 shows the results of complement fixation of GM 50-C against brain proteins S 100, GP 350 [11], and α_2-glycoprotein [12]. Neither of these proteins seems to be immunologically related to GM 50-C. However, it is interesting to note that glycoprotein GP 350 is present in the 50% methanol-soluble fraction (compare fig. 1, gels B and D), of which it constitutes almost 25%. Thus, GP 350 shares with GM 50-C this special solubility property. The fact that GP 350, in spite of being a glycoprotein [11], is not apparently bound to the ConA-Sepharose column would suggest the absence of Concanavalin A-specific carbohydrate residues as oligosaccharide chain ends [4].

The study of brain soluble glycoproteins appears most interesting since they may represent soluble glycoprotein subunits to be later incorporated, in an insoluble form, into cell membranes [13]. In fact, preliminary work in our laboratory, using immunoprecipitation and complement fixation, strongly suggests the presence of both glycoproteins GM 50-C and GP 350 in the synaptic plasma membrane. The ease of the preparation of both proteins makes them specially suitable for functional studies involving changes in brain glycoprotein composition and, eventually, in membrane composition and structure.

Acknowledgements

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