DEMONSTRATION OF A GLYCOPROTEIN WHICH IS ASSOCIATED WITH A PURIFIED MYELIN FRACTION FROM RAT BRAIN

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Received March 17, 1972

SUMMARY - Rats were injected intracerebrally with radioactive fucose in order to label specifically the glycoproteins of brain. The radioactive glycoproteins were fractionated by polyacrylamide electrophoresis in the presence of sodium dodecyl sulfate. The purified myelin fraction contained a major radioactive glycoprotein with an apparent molecular weight of ~110,000. The glycoprotein could also be stained with periodic acid-Schiff reagent.

Glycoproteins are concentrated on the surfaces of most mammalian cells and there is experimental evidence indicating that they may mediate specific cellular interactions (1). For this reason glycoproteins could be of great importance in developing brain as many specific interneuronal connections are formed, and neuronal-glial relationships are established (2-5). However, an examination of the role of individual brain glycoproteins in specific functions has been hampered by the apparent extreme heterogeneity of brain glycoproteins (5-8). In this communication, we report the association of a particular glycoprotein with a purified myelin fraction from rat brain.

MATERIALS AND METHODS

Twenty-two-day old Sprague-Dawley rats were injected intracerebrally with 8 μCi of a ^{14}C-sugar or 20 μCi of a ^{3}H-sugar as previously described (8). All radioactive compounds were purchased from New England Nuclear Corporation, Boston, Massachusetts. The animals were sacrificed 16 h after injection and myelin was purified by the procedure of Norton and Poduslo (9) which is especially designed for obtaining myelin of high purity from developing brain. The discontinuous sucrose gradient in step 1 was repeated, and a continuous sucrose gradient from 0.32 to 1.2 M sucrose was used in step 5.

The radioactive glycoproteins were fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) (8). Proteins were dissolved in 0.05 M Na_2CO_3, 2.5% SDS, and 10% mercaptoethanol (10) and dialyzed against 0.01 M phosphate buffer (pH 7.2) containing 0.1% SDS, 2 M urea, and 0.05% dithiothreitol. Samples containing 1 mg or less of protein were electrophoresed. The gels were cut into 3 mm slices which were depolymerized by treatment with 0.3 ml of 30% hydrogen peroxide at 50° for 4 to 6 hours and counted in Aquasol (New England Nuclear Corp.). In some experiments, fucose-^{3}H-labelled glycoproteins from one sample were compared with fucose-^{14}C-labelled glycoproteins in another sample by solubilizing the samples together, electrophoresing them on the same gel, and comparing the distribution of ^{3}H and ^{14}C along the gel by double label counting techniques.

RESULTS

The activity of 2' 3'-cyclic nucleotide phosphohydrolase, which is considered to be a marker enzyme for myelin (9,11), was enriched 6 to 7 fold in the purified myelin fractions as compared to the whole homogenate (Table 1). In contrast, the total ATP'ase activity in the myelin fraction

TABLE 1 - PROPERTIES OF THE MYELIN FRACTION

	Homogenate	Myelin	Myelin/Homogenate
2',3'-cyclic nucleotide 3'-phosphohydrolase (μmoles/hr./mg protein)	184 ± 6.6 (6)	1,150 ± 49 (6)	6.3 ± 0.40 (6)
Na^+,K^+,Mg^{++}-ATP'ase (μmoles/hr./mg protein)	15.5 ± 1.38 (3)	3.5 ± 0.95 (3)	0.22 ± 0.050 (3)
Fucose-^{3}H (cpm/mg protein)	8,950 ± 920 (3)	4,350 ± 220 (3)	0.41 ± 0.064 (6)
Fucose-^{14}C (cpm/mg protein)	11,900 ± 290 (3)	4,180 ± 386 (3)	

Enzyme activities and the amount of radioactive fucose which was incorporated into glycoproteins are compared for the homogenate and the myelin fraction. The activity of 2',3'-cyclic nucleotide phosphohydrolase was determined by the procedure of Kurihara and Tsukada (11). ATP'ase was assayed with the use of adenosine-5'-triphosphate-γ-^{32}P in the presence of 5 mM $MgCl_2$, 80 mM NaCl, and 40 mM KCl (12). The amount of labelled fucoglycoprotein was determined after precipitation with 10% trichloroacetic acid-

0.5% phosphotungstic acid. The results are given as mean ± S.E.M. with the number of samples in parentheses.

was low compared to the whole homogenate (Table 1). When animals were injected with radioactive fucose, the specific radioactivity (cpm/mg protein) of the myelin fraction was about 40% of that of the whole homogenate (Table 1). Acid hydrolysis of the myelin fraction and paper chromatography showed that all of the radioactivity was in the fucose moieties of glycoproteins.

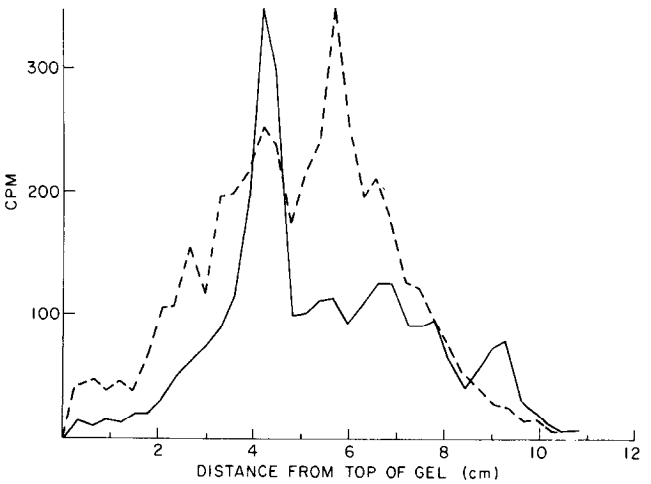
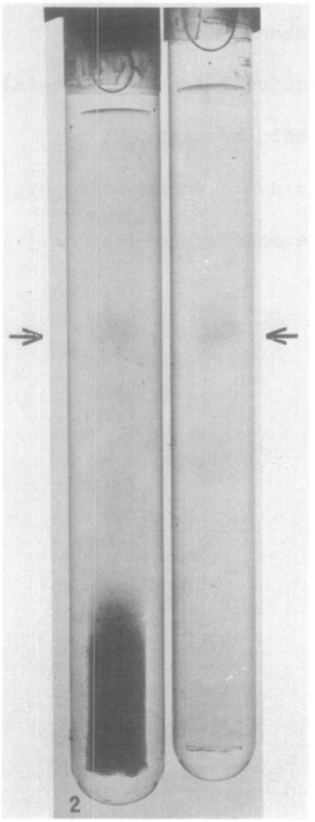


FIGURE 1 - The fucose-^{14}C-labelled myelin fraction from a group of 22-day-old rats (——) was electrophoresed on the same gel with the fucose-^{3}H-labelled whole 100,000 x g particulate fraction (– – –) from another group of rats. The major radioactive peak which is characteristic of the myelin fraction migrated 4.2 cm down the gel.

Figure 1 shows an experiment in which a myelin fraction from animals which had been injected with fucose-^{14}C was electrophoresed together with the whole 100,000 x g particulate fraction from other animals which had been injected with fucose-^{3}H. Although the glycoproteins in the whole particulate fraction were quite heterogeneous as previously described (7,8), the myelin fraction was characterized by a major, discrete, radioactive peak with an apparent molecular weight of ∿110,000. This was also the major radioactive peak when animals were injected with N-acetylmannosamine-^{3}H which primarily labels the sialic acid moieties of glycoproteins (8).



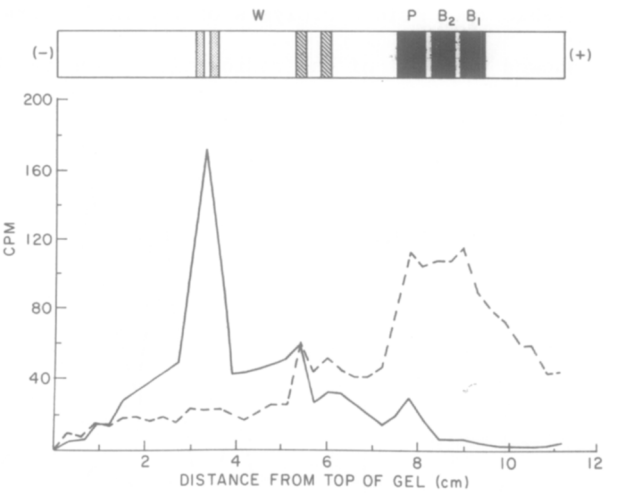


fig.3

FIGURE 2 - The intact myelin fraction was electrophoresed on the gel shown on the left, whereas the insoluble residue from myelin treated with 2:1 (v/v), chloroform:methanol was electrophoresed on the gel shown on the right. Both gels were stained for carbohydrate with periodic acid-Schiff reagents. The position of the glycoprotein in the myelin fraction is indicated by the arrows.

FIGURE 3 - The lower portion of the figure shows the electrophoretic pattern of fucose-^{3}H (——) and leucine-^{14}C (– – –) in the myelin fraction from 22-day-old rats which had been injected with a mixture of the two isotopes. At the top is shown a schematic representation of the major proteins in myelin which are stained with amido black-Coomassie blue. B_1,B_2=Basic protein; P=Proteolipid; W=Wolfgram fraction (13).

When gels were stained with periodic acid-Schiff reagent one, major, high molecular weight glycoprotein band was revealed in the myelin preparations corresponding to the position of the radioactive peak (Figure 2). The myelin also contained small molecular weight material

migrating close to the electrolyte front which was intensely stained with Schiff reagent. However, this material may be largely lipid since it is removed if the myelin is extracted with chloroform-methanol prior to electrophoresis. Treatment of fucose-^{3}H-labelled myelin with 2:1 (v/v) chloroform:methanol solubilized all of the lipid and most of the protein, but over 90% of the radioactivity remained in the insoluble residue. Electrophoresis of the residue followed by periodic acid-Schiff staining revealed only the high molecular weight glycoprotein (Figure 2).

Figure 3 shows the electrophoretic pattern of purified myelin from animals which had been injected with a mixture of leucine-^{14}C and fucose-^{3}H. Most of the leucine-^{14}C was in the major myelin proteins (basic protein and proteolipid) which ran close to the electrolyte front. The major fucose-^{3}H-labelled peak ran close to a high molecular weight doublet which could be stained with amido black-Coomassie blue. However, the glycoprotein is probably different from the two stained bands since the radioactivity did not correspond exactly with them, and periodic acid-Schiff staining revealed only one band (see above).

The preceding experiments were all done with 22-day-old rats which are very rapidly myelinating. However, the glycoprotein was also prominent in myelin preparations obtained from older animals (175-300 g body weight).

DISCUSSION

The major proteins in myelin are proteolipid and basic protein (9). In addition myelin contains some less well characterized high molecular weight proteins (13,14). We have now demonstrated that among the high molecular weight proteins in a purified myelin fraction from rat brain, there is glycoprotein with an apparent molecular weight of ∿110,000. Although this protein is minor with respect to the total protein in the fraction, it is probably the major glycoprotein with respect to protein-bound carbohydrate. It contains the highest proportion of radioactivity after labelling with fucose or N-acetylmannosamine. Furthermore, this glycoprotein is the major Schiff-positive material in the myelin preparations,

except for the intensely staining material near the electrolyte front which is probably mostly lipid. The relationship of the glycoprotein in the myelin fraction to the brain specific glycoprotein which Warecka and Bauer (15,16) demonstrated by immunological methods in aqueous extracts of white matter is unknown at this time. Gagnon, Finch, Wood, and Moscarello (17) have recently reported that a major protein from human myelin, with properties similar to proteolipid, contains a small amount of glucosamine and fucose and is probably a glycoprotein. It is interesting that rat myelin preparations labelled with fucose have a small radioactive peak whose electrophoretic migration corresponds to proteolipid (Figure 3). However, the glycoprotein which we demonstrate in this communication is clearly distinct from proteolipid.

Purified myelin fractions have been shown to have a low glycoprotein content compared to other membrane fractions from brain (6). Our finding, that the specific radioactivity (cpm/mg protein) of the fucose-labelled myelin fraction from rat brain is less than that of whole brain, is consistent with this observation. As a result, it is very difficult to determine whether the glycoprotein which is enriched relative to other glycoproteins in the myelin fraction is in myelin itself, or in some other subcellular component which has a very high content of this glycoprotein and which is enriched along with myelin during the purification procedure. Experiments to distinguish between these possibilities are in progress. In either case, our findings indicate the existence of a structure in brain with a high proportion of this particular glycoprotein. Other subcellular fractions which have been examined, including microsomes and synaptosomes, do not contain a high proportion of this glycoprotein. If the glycoprotein is in myelin, the association of the carbohydrate moieties with the myelin membrane could be of considerable importance in processes of myelination and demyelination.

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