A Rapid Procedure for Selectively Isolating the Major Glycoprotein from Purified Rat Brain Myelin

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Treatment of the chloroform/methanol-insoluble residue of rat brain myelin with lithium 3,5-di-iodosalicylate solubilized the major myelin-associated glycoprotein along with most other proteins and glycoproteins. Equilibration of the extract with phenol resulted in the selective partitioning of the major glycoprotein into the aqueous phase, whereas nearly all of the other proteins and glycoproteins went into the phenol phase.

Knowledge of the chemistry of membrane glycoproteins is limited, largely because of difficulties with solubilization and isolation. Highly purified myelin from the central nervous system contains a number of glycoproteins that are quantitatively minor components (Quarles *et al.*, 1973; Quarles & Everly, 1977; Poduslo *et al.*, 1977). The most prominent of these has an apparent mol. wt. of approx. 100 000 and is associated with the myelin–oligodendroglial unit in nervous tissue (Quarles *et al.*, 1973; Matthieu *et al.*, 1974). This glycoprotein appears to be particularly concentrated in membranes that are transitional between compact multilamellar myelin and the plasma membrane of the oligodendrocyte (Matthieu *et al.*, 1973; Waehneldt, 1975; Poduslo *et al.*, 1976; McIntyre *et al.*, 1977). The properties of this glycoprotein are of particular interest both with regard to cell–cell interactions during myelinogenesis and in connexion with demyelinating diseases (Quarles, 1975). We now report that extraction with lithium 3,5-di-iodosalicylate followed by phenol partitioning is an effective and easy procedure for isolating this glycoprotein. The technique was first developed for isolating the major sialoglycoprotein of the erythrocyte (Marchesi & Andrews, 1971). When applied to brain myelin, the procedure not only separates the glycoprotein from the other proteins, but is highly selective for the most prominent glycoprotein.

Experimental

Materials and general methods

Young adult Osborne–Mendel rats (225–275 g) were obtained from the Veterinary Resources Branch of the National Institutes of Health, U.S.A. They were injected intracranially with 25 μ Ci of L-[1,5,6-³H]fucose or 10 μ Ci of L-[1-¹⁴C]fucose in order to label specifically brain glycoproteins (Quarles *et al.*, 1973). The rats were killed after 16 h, and myelin was purified by the procedure of

Norton & Poduslo (1973). The myelin was freeze-dried immediately and stored desiccated at –20°C and under vacuum until used for the glycoprotein isolation procedure described below. In some experiments the freshly prepared wet myelin was extracted with lithium di-iodosalicylate immediately without freeze-drying or lipid extraction.

Lithium 3,5-di-iodosalicylate was obtained from the Eastman–Kodak Co. (Rochester, NY, U.S.A.). Phenol was Baker (Phillipsburg, NJ, U.S.A.) Reagent Grade. Analytical polyacrylamide–gel electrophoresis in the presence of sodium dodecyl sulphate was done as previously described (Quarles *et al.*, 1973). Samples to be electrophoresed were dialysed against 0.02% sodium dodecyl sulphate, freeze-dried and solubilized in 1% (w/v) sodium dodecyl sulphate, 1.5% (w/v) dithiothreitol and 8% (w/v) sucrose by placing them in a boiling-water bath for 3 min (Agrawal *et al.*, 1972). The gels were stained for protein with Coomassie Brilliant Blue (Weber & Osborn, 1969) and for carbohydrate with periodic acid/Schiff reagents (Segrest & Jackson, 1972). The distribution of radioactivity on gels was determined by Method B of Quarles *et al.* (1973) after slicing them into 2 mm fractions with a Mickle Gel Slicer (Mickle Laboratory Engineering Co., Gomshall, Surrey, U.K.).

Standard procedure for solubilization with lithium di-iodosalicylate

Freeze-dried myelin was suspended in chloroform/methanol (2:1, v/v) at a concentration of 10 mg dry wt./ml. The suspension was stirred at room temperature (22°C) for 30 min, and the insoluble residue was sedimented at 75 000g for 30 min. The residue was washed with diethyl ether and dried under N₂. The residue was then dispersed in 0.05 M-Tris/HCl (pH 7.4) containing 0.3 M-lithium di-iodosalicylate with a Dounce hand-operated homogenizer. A 1 ml

portion of this solution was used for each 50 mg dry wt. of the starting myelin. The suspension was stirred for 30 min. An equal volume of 50% (w/w) phenol was added to the supernatant, and the mixture was stirred at 4°C for 30 min. On low-speed centrifugation (4000g, 45 min) in a swinging-bucket rotor, the system separated into two phases. The upper aqueous phase accounted for about 80% of the total volume and contained the glycoprotein. It was dialysed exhaustively against water to remove lithium di-iodosalicylate and phenol and clarified by centrifugation at 100000g.

Results and Discussion

The first step in the isolation is the extraction of freeze-dried myelin with chloroform/methanol. Most of the major myelin proteins, proteolipid and basic protein, are solubilized with the lipids in the organic solvents, whereas most of the glycoproteins and other high-mol.wt. proteins remain with the insoluble residue. Treatment of the residue with lithium di-iodosalicylate solubilized an average of 70% of the fucose-labelled glycoproteins in four separate experiments. There was little selectivity in the solubilization itself, since analytical gel electrophoresis showed that essentially all of the high-mol.wt. proteins were solubilized (Plate 1, gel A). Furthermore the distribution of labelled glycoproteins on the analytical gels was characteristic of whole myelin (Fig. 1a). The pattern was similar to that obtained when myelin is solubilized with sodium dodecyl sulphate (Quarles *et al.*, 1973), consisting of a major peak with an apparent mol.wt. of 100000 plus a number of lower-mol.wt. components.

The great selectivity in the method lies in the phenol partitioning step. The most prominent glycoprotein partitioned into the upper aqueous phase, whereas nearly all of the other proteins and glycoproteins went into the phenol phase. On the average, only about one-third of the solubilized radioactive fucose was in the aqueous phase, but it was almost exclusively in the major glycoprotein (Fig. 1b). The phenol phase was rich in lower-mol.wt. glycoproteins (Fig. 1c), although it also contained two peaks at a position close to the major peak in the aqueous phase. Careful inspection of the gels suggested that the peak in the aqueous phase fell between the two components of the high-mol.wt. doublet in the phenol phase. This was confirmed by double-labelling experiments in which the aqueous phase from [³H]fucose-labelled myelin was electrophoresed on the same gel as the phenol phase from [¹⁴C]fucose-labelled myelin. This finding suggests that the two glycoproteins in the phenol phase are distinct from that in the aqueous phase, and that there is heterogeneity in the major peak obtained when total myelin glycoproteins are electrophoresed as in Fig. 1(a).

Coomassie Brilliant Blue and periodic acid/Schiff staining of the material in the aqueous phase on analytical gels revealed a single major glycoprotein band and two minor glycoproteins of slightly higher and lower mol.wt. respectively (Plate 1, gels B and C). The presence of these minor glycoproteins was also evident from the shoulders on the fucose-labelling pattern (Fig. 1b). Some periodic acid/Schiff-staining material in the preparations appeared as a smear at the bottom of the gels (Plate 1, gel C), but did not stain with Coomassie Brilliant Blue.

Fig. 1. Distribution of [³H]fucose-labelled glycoproteins on polyacrylamide gels

The [³H]fucose-labelled glycoproteins in the whole lithium di-iodosalicylate extract (a), aqueous phase (b) and phenol phase (c) were separated on 5% polyacrylamide gels in the presence of sodium dodecyl sulphate. The radioactive peak corresponding to the most prominent myelin-associated glycoprotein is farther down the gel than the stained glycoprotein in Plate 1 because of the lower polyacrylamide concentration in the gels.

EXPLANATION OF PLATE I

Polyacrylamide-gel electrophoresis of myelin extracts

The proteins in the whole lithium di-iodosalicylate extract and in the aqueous phase were electrophoresed on 10% (w/v) polyacrylamide gels in the presence of sodium dodecyl sulphate. Gels A and B were stained with Coomassie Brilliant Blue, and gel C was stained with periodic acid/Schiff reagents. A, whole lithium di-iodosalicylate extract; B and C, aqueous phase. Both of the minor bands that stained with Coomassie Brilliant Blue on gel B also stained with Schiff reagent, although this is difficult to see on the photograph.

Lipids migrate to this position during electrophoresis, and much more of this material was obtained when the myelin was not extracted with chloroform/methanol before solubilization. Since this staining was not eliminated by two additional extractions of the myelin with chloroform/methanol before treatment with lithium di-iodosalicylate, it may be due to a fraction of lipid which is very tightly bound to protein. On the other hand, the amount of this material could be decreased substantially by treatment of the final preparation with chloroform/methanol (1:1, v/v).

About 10–15% of the total radioactive fucose in the myelin was recovered in the aqueous phase. However, since the most prominent glycoprotein accounts for only 20–30% of the total fucose, the overall recovery of this glycoprotein in the procedure appears to be about 50%. Part of the loss occurs because of partial solubilization of the glycoprotein when the myelin is extracted with chloroform/methanol. We also applied the procedure to freshly prepared whole myelin without lipid extraction, and somewhat better yields of the glycoprotein were obtained in this manner. However, the preparations were not as pure as those obtained by using the chloroform/methanol-extracted myelin. Not only did they contain much more lipid, but they were significantly more contaminated by lower-mol.wt. proteins and glycoproteins. This was indicated both by more stained protein bands and fucose-labelled glycoproteins in the lower part of the analytical gels. Therefore chloroform/methanol delipidation of myelin before extraction with lithium di-iodosalicylate appears to be preferable for most purposes.

The highly selective partitioning of the most prominent glycoprotein into the aqueous phase may be due to its high negative charge. Labelling experiments with *N*-[³H]acetylmannosamine and [³⁵S]sulphate indicated that this glycoprotein has a higher content of sialic acid and sulphated sugars than do the other glycoproteins in purified myelin (Quarles *et al.*, 1973; Matthieu *et al.*, 1975). The two minor glycoproteins in the aqueous phase may have similar properties, rendering their separation from the major component difficult. We have already determined they are not removed by re-extraction of the aqueous phase with phenol or passage of the extract

through a phosphocellulose column at pH 3.5, as was described for the erythrocyte sialoglycoprotein (Marchesi & Andrews, 1971). However, it is clear that the very simple procedure of solubilization with lithium di-iodosalicylate followed by phenol partitioning gives a good yield of the most prominent myelin-associated glycoprotein in a relatively pure form. This is a significant advance towards determination of the chemical and immunological properties of this membrane glycoprotein and elucidation of its structural and functional roles in the myelin-oligodendroglial unit.

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