Lectin-Binding Proteins in Central-Nervous-System Myelin

BINDING OF GLYCOPROTEINS IN PURIFIED MYELIN TO IMMOBILIZED LECTINS

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The capacities of immature and mature rat brain myelin, bovine myelin and human myelin to be agglutinated by soya-bean agglutinin, *Ricinus communis* agglutinin, wheat-germ agglutinin, and *Lotus tetragonolobus* agglutinin were examined. The first two lectins, which are specific for galactose and *N*-acetylgalactosamine, strongly agglutinated immature and mature rat myelin, weakly agglutinated bovine myelin, but did not affect human myelin. The other myelin and lectin combinations resulted in very weak or no agglutination. [^3H]Fucose-labelled glycoproteins of purified adult rat brain myelin were solubilized with sodium dodecyl sulphate and allowed to bind to concanavalin A-Sepharose and each of the other lectins mentioned above, which had been immobilized on agarose. About 60% of the radioactive fucose was in glycoproteins that bound to concanavalin A-Sepharose and these glycoproteins could be eluted with solutions containing methyl α -D-mannoside and sodium dodecyl sulphate. Periodate/Schiff staining or radioactive counting of analytical gels showed that most of the major myelin-associated glycoprotein (apparent mol.wt. approx. 100000) bound to the concanavalin A, whereas the glycoproteins that did not bind were mostly of lower molecular weight. Preparative polyacrylamide-gel electrophoresis of the glycoprotein fraction that was eluted with methyl α -D-mannoside yielded a relatively pure preparation of the myelin-associated glycoprotein. Similar results were obtained with each of the other lectins, i.e. the myelin-associated glycoprotein was in the fraction that bound to the immobilized lectin. Double-labelling experiments utilizing [^3H]fucose-labelled glycoproteins from adult myelin and [^{14}C]fucose-labelled glycoproteins from 14-day-old rat brain myelin did not reveal any difference in the binding of the mature and immature glycoproteins to any of the immobilized lectins. The results in this and the preceding paper [McIntyre, Quarles & Brady (1979) *Biochem. J.* 183, 205–212] suggest that the myelin-associated glycoprotein is one of the principal receptors for concanavalin A and other lectins in myelin, and that this property can be utilized for the purification of this glycoprotein.

In the preceding paper we showed that concanavalin A agglutinates purified rat, bovine and human myelin, although there are species differences in the degree of agglutination produced (McIntyre *et al.*, 1979). Binding of [^3H]concanavalin A to myelin proteins after separation on sodium dodecyl sulphate/polyacrylamide gels revealed several glycoproteins that bound concanavalin A. The major myelin-associated glycoprotein (Quarles *et al.*, 1973*a,b*; Quarles, 1979) was one of the principal concanavalin A-binding proteins in each species (McIntyre *et al.*, 1979). In the present paper, we report the results of experiments in which solubilized glycoproteins of purified myelin were bound to immobilized concanavalin A as a means of isolating the glycoproteins. We also studied the binding of glycoproteins in purified myelin to other immobilized lectins with differing sugar specificities and investigated the ability of other lectins to agglutinate purified myelin.

Experimental

Materials

Ricinus communis agglutinin-60, wheat-germ agglutinin, soya-bean agglutinin and fucose-binding protein (*Lotus tetragonolobus*) were obtained from Miles Laboratories, Elkhart, IN, U.S.A. The latter three lectins were also purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and the same results were obtained with the lectins from the two sources. Concanavalin A-Sepharose was obtained from Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A., and contained 8 mg of concanavalin A/ml of packed gel. All of the other agarose-linked lectins were obtained from Miles Laboratories, and contained 0.9–1.4 mg of lectin protein/ml of packed gel. L-[6- ^3H]Fucose and L-[1- ^{14}C]fucose were purchased from New England Nuclear, Boston, MA, U.S.A. Young-adult male Osborne-Mendel rats (200–250 g)

were obtained from the Veterinary Resources Branch of NIH; 13 day-old rats were from the same source.

Preparation of myelin

The procedure for labelling brain glycoproteins *in vivo* with radioactive fucose and purifying myelin are described in the preceding paper (McIntyre *et al.*, 1979). In some cases, mixed myelin preparations were purified by homogenizing [^{14}C]fucose-labelled 14 day-old brains and [^3H]fucose-labelled adult brains together and then continuing the myelin purification.

Agglutination of myelin fragments by lectins

Freshly prepared rat, human and bovine myelin preparations were tested for their capacity to be agglutinated by *Ricinus communis*, soya-bean, wheat-germ and *Lotus tetragonolobus* lectins by exactly the same procedure as described in the preceding paper (McIntyre *et al.*, 1979) for concanavalin A. In each case, portions (25–100 μg) of lectin were tested with 100 μg of total myelin protein.

Binding of glycoproteins to concanavalin A-Sepharose

The amount of myelin protein used varied from 10mg in pilot or analytical experiments to 400mg in preparative experiments. The procedure is described for 100mg portions of myelin protein, but the actual amount of concanavalin A-Sepharose and the volumes used were scaled up or down in proportion to the size of the myelin sample. Freeze-dried myelin was extracted with 50ml of chloroform/methanol (2:1, v/v). The insoluble residue was washed with diethyl ether and dried under N_2 . The residue was dissolved in 2ml of 1% sodium dodecyl sulphate containing 1.5% dithiothreitol and dialysed for 16h against 0.1M-Tris/HCl (pH6.9) containing 0.02% sodium dodecyl sulphate. The latter solution will henceforth be referred to as buffer/sodium dodecyl sulphate. The dialysed sample was then diluted 8-fold with buffer/sodium dodecyl sulphate and the glycoproteins were allowed to bind to the concanavalin A-Sepharose at 4°C either by passing the solution through a small column or by making a suspension of the agarose-lectin in the glycoprotein solution and gently stirring for 30min followed by low-speed centrifugation. The latter procedure will be referred to as the batch technique, and in either case 0.5ml of packed Sepharose was used per 100mg of myelin protein in the starting sample. The effluent or supernatant was then treated with a second equal portion of concanavalin A-Sepharose to ensure complete binding. The two portions of concanavalin A-Sepharose were washed with 5ml of buffer/

sodium dodecyl sulphate and the wash was added to the unbound fraction. The bound glycoproteins were then eluted from the concanavalin A-Sepharose with 10ml of 0.5M-methyl α -mannoside in buffer/sodium dodecyl sulphate followed by 0.5M-methyl α -mannoside in buffer containing 1% sodium dodecyl sulphate. Elution was performed either by passage of the solutions through a column or by the batch technique as described for the binding step. In the latter procedure, the concanavalin A-Sepharose was gently stirred for 30min in the eluting solution and removed by low-speed centrifugation. Although the same amount of glycoprotein was bound in the column and batch techniques, the latter procedure gave slightly better recoveries and was used for most experiments. The various glycoprotein fractions were dialysed against 0.02% sodium dodecyl sulphate and freeze-dried before examination on analytical sodium dodecyl sulphate/polyacrylamide gels as described below. In some cases, the major myelin-associated glycoprotein was further purified from the fraction that bound to the concanavalin A by preparative polyacrylamide-gel electrophoresis on gels of 1.5cm diameter as previously described (Quarles, 1975).

Binding of glycoproteins to other immobilized lectins

All of these experiments were done by the batch technique described for concanavalin A-Sepharose and used only 10 to 20mg of total myelin protein for starting material. However, two 0.5ml packed-volume portions of the agarose-lectins were used for binding since the amounts of lectin coupled to the agarose were less (see under 'Materials'). Elution was carried out with two 5ml portions of 0.5M-sugar hapten in buffer/sodium dodecyl sulphate followed by two 5ml volumes of 0.5M hapten in buffer containing 1% sodium dodecyl sulphate. The glycoprotein that was not eluted by this procedure could be recovered for analytical sodium dodecyl sulphate gels by heating the agarose-lectin in the latter solution at 100°C for 3min. The sugar haptens used for the various lectins were as follows: *R. communis*, galactose; soya-bean, galactose; wheat-germ, *N*-acetylglucosamine; *L. tetragonolobus*, fucose. In some cases, these experiments were done with mixed myelin preparations in which the glycoproteins of immature rats were labelled with [^{14}C]fucose and those in adult rats with [^3H]fucose. The various glycoprotein fractions were dialysed against 0.02% sodium dodecyl sulphate and freeze-dried.

Analytical polyacrylamide-gel electrophoresis

The glycoprotein fractions were heated at 100°C for 3min in 1% sodium dodecyl sulphate, 1.5% dithiothreitol, and 8% sucrose and electrophoresed on sodium dodecyl sulphate/5% polyacrylamide gels

as previously described (Quarles *et al.*, 1973a). Proteins were stained with Fast Green (Greenfield *et al.*, 1971) and glycoproteins with periodate/Schiff reagents (Segrest & Jackson, 1972). The distribution of radioactive fucose on the gels was determined by Method B of Quarles *et al.* (1973a). In a few cases, 10% polyacrylamide gels were used as indicated.

Results

Agglutination of purified myelin by various lectins

Several lectins with differing sugar specificities were tested for their ability to agglutinate suspensions

of purified myelin obtained from different species (Table 1). The degree of agglutination was evaluated both by direct visual examination and with the aid of Nomarski phase microscopy as described in the preceding paper (McIntyre *et al.*, 1979). *R. communis* and soya-bean lectins that are specific for galactose and *N*-acetylgalactosamine produced a strong agglutination of mature and immature rat myelin similar to that described for concanavalin A in the preceding paper (McIntyre *et al.*, 1979). These lectins produced a weak agglutination of bovine myelin, but we could not detect an effect on human myelin. The wheat-germ lectin caused weak agglutination of mature and immature rat myelin and of human

Table 1. *Agglutination of isolated myelin by various lectins*

Agglutination tests were done and evaluated as described by McIntyre *et al.* (1979). Abbreviations: R.C., *R. communis* agglutinin for galactose or *N*-acetylgalactosamine; SB, soya-bean agglutinin for galactose or *N*-acetylgalactosamine; W.G., wheat-germ agglutinin for *N*-acetylglucosamine; L.T., *L. tetragonolobus* agglutinin for fucose. The specificities indicated for the lectins are from Sharon & Lis (1972) or Nicolson (1974).

Source of myelin	Agglutinin added	Sugar added	Degree of agglutination
Adult rat brain	None	—	0
	R.C.	—	+++
	R.C.	Galactose	0
	R.C.	Methyl α -mannoside	++
	SB	—	+++
	SB	Galactose	0
	SB	Methyl α -mannoside	+++
	W.G.	—	+
	W.G.	<i>N</i> -Acetylglucosamine	0
	W.G.	Galactose	+
	L.T.	—	0
14-day rat brain	None	—	0
	R.C.	—	++
	R.C.	Galactose	0
	R.C.	Methyl α -mannoside	++
	SB	—	++
	SB	Galactose	0
	SB	Methyl α -mannoside	++
	W.G.	—	+
	W.G.	<i>N</i> -Acetylglucosamine	0
	W.G.	Galactose	+
	L.T.	—	0
Adult bovine brain	None	—	0
	R.C.	—	+
	R.C.	Galactose	0
	R.C.	Methyl α -mannoside	+
	SB	—	+
	SB	Galactose	0
	SB	Methyl α -mannoside	+
	W.G.	—	0
	L.T.	—	0
Adult human brain	None	—	0
	R.C.	—	0
	SB	—	0
	W.G.	—	+
	W.G.	<i>N</i> -Acetylglucosamine	0
	W.G.	Galactose	+
	L.T.	—	0

Table 2. *Binding of [³H]fucose-labelled glycoproteins to immobilized lectins*

The third column shows the percentage of the total [³H]fucose-labelled glycoproteins in the sodium dodecyl sulphate solution that were retained by the immobilized lectin and not washed off by the 0.02% sodium dodecyl sulphate buffer. The numbers in the last column are the percentages of the bound [³H]fucose-labelled glycoproteins that could be eluted from the immobilized lectin by using a 0.5 M solution of the appropriate monosaccharide first in buffered 0.02% sodium dodecyl sulphate and then in 1% sodium dodecyl sulphate.

Lectin	Sugar specificity	Proportion of [³ H]fucose bound (%)	Proportion of bound [³ H]-fucose eluted (%)
Concanavalin A	Mannose, glucose	59	68
<i>R. communis</i>	Galactose, <i>N</i> -acetylgalactosamine	59	66
Soya-bean	Galactose, <i>N</i> -acetylgalactosamine	55	70
Wheat germ	<i>N</i> -Acetylglucosamine	72	66
<i>L. tetragonolobus</i>	Fucose	38	79

myelin, but no agglutination of bovine myelin was detected. A fucose-specific lectin did not agglutinate any of the myelin preparations tested. In each case where a lectin produced agglutination, it was inhibited by the specific sugar hapten, but not by an inappropriate sugar at the same concentration.

Binding of the glycoproteins in purified rat myelin to concanavalin A-Sepharose

The glycoproteins in purified myelin were labelled *in vivo* with radioactive fucose. The labelled glycoproteins were solubilized with sodium dodecyl sulphate for investigation of binding to concanavalin A-Sepharose. Binding to the immobilized concanavalin A was done at pH 6.9 in 0.02% sodium dodecyl sulphate by either a column or batch technique as described in the Experimental section. Essentially the same results were obtained by the two procedures. An average of 59% of the fucose-labelled glycoproteins were bound to the concanavalin A (Table 2) with a range of 42 to 67% in six separate experiments. Passage of the eluate through a second column or absorption with an additional portion of concanavalin A-Sepharose did not remove significantly more glycoprotein from the solution, indicating that the capacity of the concanavalin A-Sepharose had not been surpassed. Most of the labelled glycoproteins that bound to the lectin could be eluted by solutions containing 0.5M-methyl α -mannoside. About one-half of the bound glycoprotein was eluted by adding the methyl α -mannoside to the same buffer with 0.02% sodium dodecyl sulphate, but it was necessary to increase the sodium dodecyl sulphate concentration to 1% in the mannoside solution to elute the remainder. In general, more efficient and complete elution was obtained by the batch technique and this was used for most experiments.

Analytical sodium dodecyl sulphate/polyacrylamide gels revealed that the population of glycoproteins that bound to the concanavalin A was quite different from that which did not bind. Fig. 1(a)

shows that the glycoproteins that did not bind were enriched in relatively low-molecular-weight components, whereas the myelin-associated glycoprotein was almost exclusively in the fraction that did bind to concanavalin A. Figs. 1(b) and 1(c) show that the glycoprotein fractions eluted with methyl α -mannoside in 0.02% sodium dodecyl sulphate and 1% sodium dodecyl sulphate were similar with the myelin-associated glycoprotein being the principal component in both. The same separation of the glycoproteins in purified myelin was indicated by periodate/Schiff staining of analytical gels (Fig. 2). The myelin-associated glycoprotein was the principal Schiff-stained glycoprotein in the fraction that bound to concanavalin A, whereas some lower-molecular-weight glycoproteins were the major components in the fraction that did not bind. The myelin-associated glycoprotein could be further purified from the fraction that did bind to the concanavalin A by preparative polyacrylamide-gel electrophoresis. Schiff staining (Fig. 2c) and counting of radioactive fucose (Fig. 1d) on analytical gels suggested a single glycoprotein in the fraction obtained by these procedures. However, Fast Green staining revealed small amounts of other proteins in addition to the myelin-associated glycoprotein (Fig. 2d).

The binding of the myelin-associated glycoprotein from immature rat myelin to concanavalin A-Sepharose was also investigated. This was done because the immature myelin-associated glycoprotein is known to have a higher apparent molecular weight than the mature myelin-associated glycoprotein and this might be explained by a different carbohydrate composition. It was of interest, therefore, to determine if the immature and mature myelin-associated glycoprotein could be distinguished and possibly separated by differing affinities for concanavalin A. Myelin was purified from a mixed homogenate obtained by combining [¹⁴C]fucose-labelled 14 day-old rat brains and [³H]fucose-labelled adult brains. Binding of the doubly labelled glycoproteins to concanavalin A-Sepharose was studied as described above. The ¹⁴C/³H ratio was 0.70 in the whole

Fig. 1. [^3H]Fucose-labelled glycoproteins in fractions eluted from concanavalin A-Sepharose

Each curve represents the distribution of ^3H on an analytical sodium dodecyl sulphate/5% polyacrylamide gel. The gel was run from left to right, and the arrow indicates the position at which the myelin-associated glycoprotein would be in each panel. (a) Fraction that did not bind to concanavalin A; (b) fraction that was eluted with methyl α -mannoside in 0.02% sodium dodecyl sulphate; (c) fraction that was eluted with methyl α -mannoside in 1% sodium dodecyl sulphate; (d) material shown in (b) and (c) after combining and preparative polyacrylamide-gel electrophoresis.

glycoprotein extract and was unchanged in the fractions that did and did not bind to concanavalin A. Fig. 3 shows the distribution of the ^3H - and ^{14}C -labelled glycoproteins in the fraction that bound to concanavalin A on an analytical sodium dodecyl sulphate/polyacrylamide gel. The myelin-associated

Fig. 2. Periodate/Schiff and Fast Green staining of fractions eluted from concanavalin A-Sepharose

Gels (a)–(c) are 5% polyacrylamide and stained with periodate/Schiff reagents. Gel (d) is 10% polyacrylamide and stained with Fast Green. Gel (a), fraction that did not bind to concanavalin A; gel (b), combination of the fractions that were eluted from concanavalin A-Sepharose by methyl α -mannoside in 0.02% and 1% sodium dodecyl sulphate; gels (c) and (d), material shown on gel (b) after preparative polyacrylamide-gel electrophoresis. The position of the myelin-associated glycoprotein is indicated by the arrows. The four bands below the broken line on gel (a) are turbidity due to protein precipitated in the fixative and are not Schiff-positive glycoproteins.

glycoprotein peak is the major component in the fractions from both immature and mature myelin, and the difference in apparent molecular weight between the two peaks is as marked as in the whole myelin fraction. Additional experiments in which discontinuous gradients from 0.1 to 1.0M-methyl α -mannoside were used to elute the myelin-associated glycoprotein from concanavalin A-Sepharose columns failed to give any separation of immature and mature myelin-associated glycoprotein.

Fig. 3. Developmental comparison of the glycoproteins that bind to concanavalin A-Sepharose

The $[^3\text{H}]$ fucose-labelled glycoproteins from mature rat brain myelin (solid line) and the $[^{14}\text{C}]$ fucose-labelled glycoproteins from 14-day-old rat brain myelin (broken line) in the fraction that was eluted from concanavalin A-Sepharose with methyl α -mannoside were electrophoresed on a 5% polyacrylamide gel. The arrow shows the position of the myelin-associated glycoprotein.

Binding of glycoproteins in purified rat myelin to other immobilized lectins

The binding of $[^3\text{H}]$ fucose-labelled glycoproteins from rat brain to other immobilized lectins was also examined. The lectins studied in this respect were the same ones tested for their ability to agglutinate myelin fragments (Table 1). These experiments were done by the batch technique described for concanavalin A-Sepharose. Table 2 shows the percentage of $[^3\text{H}]$ fucose-labelled glycoproteins of myelin that bound to each of the immobilized lectins and the proportion of the bound glycoproteins that was eluted with the appropriate sugar hapten in sodium dodecyl sulphate solutions. The percentage of glycoproteins bound varied from a minimum of

38% with the fucose-specific lectin to a maximum of 72% for the wheat-germ lectin. Most of the bound glycoproteins could be eluted from the agarose-lectins with the appropriate sugar hapten in sodium dodecyl sulphate solutions. However, as was the case with concanavalin A-Sepharose, only a portion of the eluted glycoproteins was obtained by adding the sugar hapten to the buffer with 0.02% sodium dodecyl sulphate, and it was necessary to increase the sodium dodecyl sulphate to 1% to obtain the recoveries shown in Table 2. For example, with the wheat-germ lectin, about half of the recovered glycoproteins were obtained by just adding *N*-acetylglucosamine to the 0.02% sodium dodecyl sulphate/buffer, and with the *R. communis* lectin only 40% of glycoproteins were eluted by just adding galactose. For this reason, experiments were done in which sugar inhibitors were added to the buffers during the initial binding step to further test the specificity of the interactions with the immobilized lectins. Table 3 shows that the free sugars greatly inhibited, but did not entirely prevent, the binding of glycoproteins to the wheat

Fig. 4. Fucose-labelled glycoproteins of mature and immature rat brain myelin that were eluted from the immobilized fucose-specific lectin (*L. tetragonolobus*)

The glycoproteins were separated on sodium dodecyl sulphate/5% polyacrylamide gels. The solid lines represent the $[^3\text{H}]$ fucose-labelled glycoproteins of adult rat brain myelin and the broken lines represent the $[^{14}\text{C}]$ fucose-labelled glycoproteins of 14-day-old rat brain myelin. (a) Fraction that did not bind to the lectin; (b) fraction that bound to the lectin and was eluted with fucose in sodium dodecyl sulphate solutions. The position of the myelin-associated glycoproteins in each curve is indicated by the arrow.

Table 3. Inhibition of glycoprotein binding by monosaccharide inhibitors

Portions (5ml) of the solubilized glycoproteins in myelin containing 55900 d.p.m. each were treated with the indicated agarose-lectins by the regular batch technique described in the Experimental section. The only difference was that in some cases the sugar inhibitor was present with the glycoproteins before the immobilized lectin was added.

	Inhibitor	$[^3\text{H}]$ Fucose-labelled glycoprotein bound (d.p.m.)
Wheat-germ lectin-agarose	None	33300
	0.3M- <i>N</i> -Acetylglucosamine	12300
<i>R. communis</i> lectin-agarose	None	32100
	0.3M-Galactose	13200
Sephacrose 6B	None	3400

germ and *R. communis* lectins. Also, there was very little non-specific binding of glycoproteins to Sepharose 6B under the conditions of these experiments.

Results with the galactose- and *N*-acetylglucosamine-specific lectins and wheat-germ agglutinin were also similar to those with concanavalin A in that the glycoproteins that did not bind were primarily of low molecular weight, whereas the myelin-associated glycoprotein was in the fractions that were absorbed by the immobilized lectins. The only variation from this pattern was with the fucose-specific lectin as shown in Fig. 4. In this case, the distributions of labelled glycoproteins in the bound and unbound fractions were similar, with the myelin-associated glycoprotein constituting the principal peak in each. The failure to bind all of the myelin-associated glycoprotein peak to the fucose lectin was not due to saturation of binding sites, since, as in all the experiments, the completeness of binding was tested by adding extra immobilized lectin.

The high affinity of the myelin-associated glycoprotein for the various immobilized lectins was indicated by the substantial enrichment of this glycoprotein in the fraction that could not readily be eluted from the agarose-lectins by the appropriate sugar hapten in 0.02% or 1% sodium dodecyl sulphate and is indicated as uneluted in Table 2. In fact, most of this fraction could be recovered from the matrix by heating to 100°C with the 1% sodium dodecyl sulphate/sugar solutions and run on analytical gels. Fig. 5 shows the substantial enrichment of the myelin-associated glycoprotein in this fraction from the *R. communis* lectin, for example.

Fig. 5. Fraction that required heating in the presence of galactose and 1% sodium dodecyl sulphate for elution from the immobilized *R. communis* agglutinin

The Figure shows the distribution of [^3H]fucose-labelled glycoproteins on a 5% polyacrylamide gel, and the arrow indicates the position of the myelin-associated glycoprotein.

A comparison of the binding of the [^3H]fucose-labelled glycoproteins of mature myelin and the [^{14}C]fucose-labelled glycoproteins of immature myelin to the various lectins was also done by the method described for concanavalin A-Sepharose. Furthermore, the results were the same as for concanavalin A in that there was no change in the $^3\text{H}/^{14}\text{C}$ ratio for any of the fractions obtained and no separation of the immature and mature myelin-associated glycoprotein peaks was observed. This is illustrated for the fucose lectin in Fig. 4 where the ^{14}C peak of immature myelin is shifted towards a higher molecular weight in both the unbound and bound fractions.

Discussion

Since central-nervous-system myelin is formed as an extension of the surface membrane of the oligodendrocyte, it is not surprising that the myelin membrane contains lectin receptors. However, the results described in the present paper show that although there are numerous glycoproteins in the myelin fractions that can bind to each of the lectins studied, not all of the lectins cause agglutination of the purified myelin fragments. Furthermore, this is in spite of the finding that the myelin-associated glycoprotein, which is known to be in myelin and myelin-related membranes (Quarles, 1975, 1979; Sternberger *et al.*, 1979), bound to each of the lectins investigated after it had been solubilized in sodium dodecyl sulphate solutions. The most likely explanation for this is that not all of the sugar receptors are accessible to the lectin when the glycoprotein is in its native site in the membrane. Thus it is likely that the fucose and *N*-acetylglucosamine residues are not accessible on the surface of the intact myelin membrane of any of the species studied, since the *L. tetragonolobus* and wheat-germ lectins produced little or no agglutination. Similarly, in a previous study (Matthieu *et al.*, 1974b), the fucose-specific lectin from *Ulex europaeus* and the *N*-acetylglucosamine-specific lectin from *Dolichos lablab* failed to agglutinate adult rat myelin. By contrast, both of the galactose- and *N*-acetylglucosamine-specific lectins tested in the present study produced a strong agglutination of rat brain myelin. It is possible that the large amount of galactocerebroside in myelin is involved in this agglutination. However, an argument against this possibility is that bovine and human myelin also contain large amounts of this galactolipid, but bovine myelin was only weakly agglutinated and human myelin was not agglutinated by these lectins. In fact, this species variation in agglutinability was similar to the findings with concanavalin A described in the preceding paper (McIntyre *et al.*, 1979) in which mature and immature rat myelin were strongly

agglutinated, whereas the agglutination of bovine and human myelin was much weaker. This suggests that similar glycoproteins may function as receptors for concanavalin A and the galactose- or galactosamine-specific lectins in the intact myelin membranes. The preceding paper (McIntyre *et al.*, 1979) showed that there is not a correlation between the total amount of concanavalin A-binding proteins in the purified myelin fractions of various species and the degree of agglutination. Rather the degree of agglutination probably reflects the relative exposure of the appropriate sugar residues on the surface of the myelin fragments.

The results in the present and the preceding paper (McIntyre *et al.*, 1979) show that there are a number of glycoproteins in purified myelin fractions that are capable of binding to concanavalin A and other lectins and that are, therefore, potential receptors for lectins in the myelin membrane. However, only the glycoprotein with a mol.wt. of about 100 000 (myelin-associated glycoprotein) has definitively been shown to be in myelin or associated structures and not in an unrelated contaminating membrane (Quarles, 1975, 1979; Sternberger *et al.*, 1979). Nevertheless, it is likely that some of the other glycoproteins are also in myelin-related membranes in the tissue, and there is some evidence that this is the case (Quarles *et al.*, 1973a). Therefore, binding of the myelin-associated glycoprotein and other glycoproteins to the immobilized lectins not only provides information about their possible roles as lectin receptors, but provides a procedure for the isolation and further characterization of various glycoprotein fractions. Thus preparative gel electrophoresis of the fraction that bound to concanavalin A-Sepharose yielded a fraction highly enriched in the myelin-associated glycoprotein. A paper by Zanetta *et al.* (1977) that appeared while this work was in progress reported on the sequential use of several immobilized lectins to fractionate glycoproteins in rat myelin. They also found that the major glycoprotein in purified myelin (myelin-associated glycoprotein or component A in their study) bound to concanavalin A and to a fucose-specific lectin. It is noteworthy that by using a general protein stain on gels of the glycoprotein fraction isolated by binding to these two lectins, they also found a major band with a second fainter band just behind it, which is similar to our result in Fig. 2(d). Also similar to our results, they found that with certain of the lectins it was necessary to increase the sodium dodecyl sulphate concentration to elute some of the glycoproteins from the columns, presumably because the monosaccharides were not able to effectively compete with the glycoproteins for lectin-binding sites. In contrast with our results, they found only a very small proportion of the fucose-containing glycoproteins bound to wheat-germ lectin or a galactose-specific lectin, and the major

glycoprotein (myelin-associated glycoprotein or component A) did not bind. The substantial binding of glycoproteins to such lectins, which we measured, was specific since it was significantly inhibited by including the appropriate sugar hapten in the buffer (Table 3), and some of the bound glycoproteins could be eluted from the immobilized lectins by simply adding the appropriate sugar inhibitor to the buffer. The reasons for these discrepancies are not known, but are probably related to the technical details of the experiments. Nevertheless, both studies show that immobilized lectins are useful for the separation and characterization of the many glycoproteins in purified myelin fractions and should enhance efforts to elucidate the roles of glycoproteins in myelinogenesis and myelin maintenance.

With regard to myelinogenesis, the developmental change in the apparent molecular weight of the myelin-associated glycoprotein (Quarles *et al.*, 1973b) is noteworthy, since it correlates well with the maturation of myelin under a variety of experimental circumstances (Mattieu *et al.*, 1974a, 1975a,b; Zimmerman *et al.*, 1976; McIntyre *et al.*, 1978). As previously discussed (Quarles *et al.*, 1973b; Quarles, 1976), this change in molecular weight could be due to a change in the carbohydrate or polypeptide portion of a single glycoprotein or to a change in the relative amounts of two different glycoproteins electrophoresing to about the same position on sodium dodecyl sulphate gels. The recent demonstration that a specific antiserum prepared to the smaller mature myelin-associated glycoprotein also reacts with the immature glycoprotein (Sternberger *et al.*, 1979), argues for the chemical modification of a single glycoprotein rather than the involvement of two distinct glycoproteins. Although the very similar binding of mature and immature myelin-associated glycoprotein to the various lectins does not provide any support for the difference being due to an alteration of sugar residues, it does not rule out this possibility. The slightly larger protein revealed by protein staining of the fraction that binds to concanavalin A (Fig. 2d) could be an altered form of the same glycoprotein or a different glycoprotein, and its relationship to the developmental change remains to be determined.

The agglutination or binding experiments described in the present paper did not reveal any developmental differences in the glycoproteins of immature or mature rat myelin. However, quantitative differences in the concanavalin A-binding proteins of immature and mature rat myelin were detected by binding of [³H]concanavalin A to glycoproteins after separation on analytical gels as described in the preceding paper (McIntyre *et al.*, 1979). Both studies show that lectin receptors are exposed on the surface of immature and mature myelin membranes and suggest that the myelin-associated glycoprotein is probably

one of the important receptor molecules. In view of recent reports of developmentally regulated lectins in vertebrate tissues including brain (Simpson *et al.*, 1977; Eisenbarth *et al.*, 1978), the lectin receptors associated with myelin may function during myelino-genesis. Furthermore, these exposed carbohydrate groups on myelin membranes may be relevant to potential antibody- or virus-attachment sites in the pathogenesis of demyelinating diseases.

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