Lectin-Binding Proteins in Central-Nervous-System Myelin

DETECTION OF GLYCOPROTEINS OF PURIFIED MYELIN ON POLYACRYLAMIDE GELS BY [³H]CONCAVALIN A BINDING

By Laurence J. MCINTYRE, Richard H. QUARLES and Roscoe O. BRADY
Section on Myelin and Brain Development, Developmental and Metabolic Neurology Branch, NINCDS, NIH, Bethesda, MD 20014, U.S.A.

(Received 20 February 1979)

Concanavalin A strongly agglutinates purified fragments of immature and mature rat brain myelin, but only weakly agglutinates mature bovine and human myelin fragments. A sensitive method involving [³H]concanavalin binding to sodium dodecyl sulphate/polyacrylamide gels was used to detect the concanavalin A-binding proteins in purified myelin. When applied to mature rat brain myelin proteins that had been labelled *in vivo* with [¹⁴C]fucose, the distribution of the [³H]concanavalin A on the gel was very similar to that of [¹⁴C]fucose with the major peak corresponding to the major myelin-associated glycoprotein. The technique revealed that the immature form of the myelin-associated glycoprotein with a slightly larger apparent molecular weight also bound concanavalin A, and that in purified immature rat myelin the quantitative importance of some of the other glycoproteins in binding concanavalin A was increased relative to the myelin-associated glycoprotein. The separated proteins of bovine and human myelin bound more [³H]-concanavalin A than those of rat myelin. In these species, the myelin-associated glycoprotein was a major concanavalin A-binding protein, although two higher-molecular-weight glycoproteins also bound significant quantities of [³H]concanavalin A. The results indicate that there are receptors for concanavalin A on the surface of rat, bovine and human myelin membranes and suggest that the myelin-associated glycoprotein is one of the principal receptors.

Lectins have been widely used in the study of cell-surface membrane glycoproteins (Lis & Sharon, 1972; Nicolson, 1974). They are particularly useful in the study of brain tissue as cell-surface carbohydrates may have an important role in cell-cell interactions during the formation of the neuronal network. The most commonly used lectin in these studies is concanavalin A. Concanavalin A-binding glycoproteins and glycopeptides have been isolated from whole rat and human brain by affinity chromatography (Gombos *et al.*, 1972; Susz *et al.*, 1973; Brunngraber *et al.*, 1975; Zanetta *et al.*, 1977). Concanavalin A has also been shown to bind to several different structures in central-nervous-system synapses (Matus *et al.*, 1973; Bittiger & Schnebli, 1974; Zanetta *et al.*, 1975; Kelly & Cotman, 1977; Gurd, 1977). In the peripheral nervous system, concanavalin A binds to the intraperiod line of myelin (Wood & McLaughlin, 1975), and this also appears to be the case in central-nervous-system myelin (Matus *et al.*, 1973) although concanavalin A molecules have only been visualized on exposed surfaces of isolated myelin fragments. In addition, Matthieu *et al.* (1974) demonstrated that purified rat central-nervous-system myelin could be agglutinated with concanavalin A. A postulated receptor

molecule for concanavalin A in rat central-nervous-system myelin is the major myelin-associated glycoprotein (Quarles *et al.*, 1973a; Quarles, 1979).

Techniques have been developed for identifying molecules potentially involved in the binding of lectins to cell membranes. The membrane glycoproteins are separated by polyacrylamide/sodium dodecyl sulphate-gel electrophoresis and the lectin-binding proteins are identified by their reaction with radioactively- or peroxidase-labelled lectin, which is diffused into the gel (Wood & Sarinana, 1975; Robinson *et al.*, 1975; Tanner & Anstee, 1976; Gurd & Evans, 1976; Burridge, 1976; Rostas *et al.*, 1977).

An understanding of the surface structure of myelin at the molecular level is particularly important because of immune processes or viral interactions that may occur in pathological states such as multiple sclerosis. For this reason it was decided to identify potential concanavalin A receptors in rat central-nervous-system myelin by the binding of ³H-labelled lectin after separation of myelin proteins by polyacrylamide/sodium dodecyl sulphate-gel electrophoresis. It was also decided to investigate whether changes occurred in lectin binding to myelin during development and if there are species differences. A second reason for studying the binding of [³H]-

concanavalin A to myelin on polyacrylamide/sodium dodecyl sulphate gels was to determine whether this would provide a sensitive method of detecting myelin glycoproteins when extrapolating studies with rat myelin labelled *in vivo* with radioactive isotopically labelled sugars to unlabelled human myelin. Initially, agglutination assays were carried out on purified myelin fragments from several species to determine whether or not they had receptors for concanavalin A. The following paper (Quarles *et al.*, 1979) describes studies in which the agglutination assays were extended to other lectins with differing sugar specificities and in which the binding of solubilized myelin glycoproteins to immobilized concanavalin A and other immobilized lectins was investigated.

Experimental

Materials

Males (250g) and litters of 13 day-old Sprague-Dawley rats were obtained from Taconic Farms, Germantown, NY, U.S.A. Bovine adult brains were purchased from Frederick Products, Frederick, MD, U.S.A. Human brain samples were obtained at autopsy from the Department of Pathology, George Washington University Medical School, Washington, DC, U.S.A. L-[1-¹⁴C]Fucose (sp. radioactivity 53.2mCi/mmol) and [G-³H]concanavalin A (sp. radioactivity 63.0Ci/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A. Unlabelled concanavalin A was a product of Miles-Yeda, Elkhart, IN, U.S.A.

Radioactive labelling of myelin

Rats (13 day-old and mature) were injected intracranially with 8 μ Ci each of [¹⁴C]fucose in 5 μ l of 0.9% (w/v) NaCl under mild anaesthesia (Quarles *et al.*, 1973a). The rats were killed 16–20 h later and the brains were rapidly removed and taken for the purification of myelin.

Purification of myelin

In the purification of bovine and human myelin, areas rich in white matter were dissected from the brain and used as the starting material. In all cases myelin was prepared essentially by the procedure of Norton & Poduslo (1973). Freshly prepared myelin was used for the agglutination studies by the procedure described in the next section, or the myelin was freeze-dried and stored under vacuum at –20°C until used later for polyacrylamide-gel electrophoresis.

Agglutination of isolated myelin fragments

The ability of concanavalin A to agglutinate purified myelin was determined essentially as

described previously (Matthieu *et al.*, 1974). Freshly prepared myelin was suspended in 0.05M-sodium phosphate buffer (pH 7.4) containing 0.85% NaCl at a concentration of about 1 mg of protein/ml. A portion (100 μ l) of this solution was added to an equal volume of the same buffer with or without a potential sugar inhibitor. Finally, 25 μ l of a concanavalin A solution (2 mg/ml) was added, and the mixture was incubated at 37°C for 30 min. If present, the final concentration of the potential sugar inhibitor was 0.44M. At the end of the incubation, the degree of agglutination was rated visually on a scale of 0 to +++ as follows: +++, large clumps of myelin that rapidly settle to the bottom of the tube; ++, myelin is obviously agglutinated, but the clumps appear smaller; +, careful inspection reveals small aggregates of myelin that are absent from control; 0, no visual indication of agglutination. The relative sizes of the myelin aggregates were also examined by Nomarski differential-interference microscopy (Allen *et al.*, 1969). This procedure confirmed the agglutination evaluations done with the unaided eye. Light-micrographs of controls (0) and +++ agglutinations of myelin have been published previously (Matthieu *et al.*, 1974).

Polyacrylamide-gel electrophoresis

Before electrophoresis, the freeze-dried myelin was delipidated by extracting it three times with diethyl ether/ethanol (3:2, v/v) and once with diethyl ether (Greenfield *et al.*, 1971) or by treating it with chloroform/methanol (2:1, v/v; Quarles *et al.*, 1973a). The insoluble residue was centrifuged out of the chloroform/methanol at 105 000g, washed once with diethyl ether and dried under N₂. In both cases the proteins were solubilized in 1% sodium dodecyl sulphate by heating at 100°C for 3 min in the presence of 0.15M-dithiothreitol.

Samples (500 μ g) of protein were electrophoresed on 5% (w/v) polyacrylamide gels (6 mm $\times$ 100 mm) as previously described (Quarles & Brady, 1971). When it was required, gels were stained for glycoprotein by the periodate/Schiff method (Segrest & Jackson, 1972).

Concanavalin A binding

After electrophoresis, the 5% sodium dodecyl sulphate gels were reacted with [³H]concanavalin A by using basically the method of Gurd & Evans (1976). The gels were washed with 25% propan-2-ol/10% acetic acid for 8 h with one change and then with 10% propan-2-ol/10% acetic acid for 16 h. The gels were then washed with 5 mM-Tris/HCl, pH 7.6, for 4 h with four changes. All the washes were carried out at 4°C. Each gel was then incubated overnight at 24°C in 5 mM-Tris/HCl, pH 7.6, to which had been

added 100 μ l (16.8 μ g) of [3 H]concanavalin A. Unbound [3 H]concanavalin A was then removed by washing the gels three times with 250ml per gel of 5mM-Tris/HCl, pH7.6, at 4°C over at least a 3 h period. In the case of control gels 2.5% methyl α -D-mannopyranoside was dissolved in the labelling and washing solutions. The gels were then sliced in 2mm sections with a Mickle gel slicer and the radioactivity in each section was determined by using method B of Quarles *et al.* (1973a). In some experiments smaller amounts of both protein and [3 H]concanavalin A and shorter incubation times were used.

Results

Agglutination of purified myelin fragments

Addition of concanavalin A to suspensions of freshly prepared adult or immature rat brain myelin caused the formation of large clumps that rapidly settled to the bottom of the tube (Table 1). This strong agglutination was prevented by methyl α -mannoside, but not by methyl α -galactoside. Adult bovine and human brain myelin were also agglutinated by concanavalin A, and again the agglutination was only inhibited by the lectin-specific sugar methyl α -mannoside. However, in these cases the agglutination was much weaker than for the rat brain myelin (Table 1). The much lower capacity for bovine and human myelin to be agglutinated by concanavalin A was also evident when the samples were examined by Nomarski interference microscopy. Although there were some aggregates of bovine and human myelin fragments in the concanavalin A-treated samples that were larger than those in the controls, they were much smaller

than the very large aggregates seen in the similarly treated rat myelin.

Binding of concanavalin A to rat myelin proteins

To determine the optimum conditions for the binding of [3 H]concanavalin A, a number of 5% sodium dodecyl sulphate gels were run by using 500 μ g each of chloroform/methanol-extracted adult rat myelin proteins labelled with [14 C]fucose. [3 H]-Concanavalin A was bound to the gels under a variety of experimental conditions. It was found that increasing the time of incubation led to an increasing amount of binding of radioactive concanavalin A. Incubation for 16h was selected as the most convenient time period and binding did not appear to rise markedly beyond this point. A similar result was observed with increasing amounts of [3 H]concanavalin A up to 100 μ l (10 μ Ci), but not with higher amounts. Also the amount of [3 H]concanavalin A bound increased with increasing amounts of myelin protein on the gel. In most experiments 500 μ g of protein was used, but as little as 50 μ g would permit detection of the myelin-associated glycoprotein. Gels of adult rat myelin reacted with [3 H]concanavalin A under optimum conditions are shown in Fig. 1.

The pattern of 14 C radioactivity seen in Fig. 1(a) is typical of that obtained previously with fucose-labelled rat myelin (Quarles *et al.*, 1972). There is one major peak with an apparent mol.wt. of approx. 100000, which corresponds to the myelin-associated glycoprotein, and there are several minor fucose-labelled peaks of both higher and lower molecular weight. When [3 H]concanavalin A is permitted to bind to this gel, the pattern of lectin binding is very

Table 1. Agglutination of isolated myelin by concanavalin A
The capacity of concanavalin A to agglutinate the myelin fragments from the species indicated was tested and evaluated as described in the Experimental section.

Source of myelin	Concanavalin A present	Glycoside present	Degree of agglutination
Adult rat brain	—	—	0
	+	—	+++
	+	Methyl α -mannoside	0
	+	Methyl α -galactoside	+++
14-day-old rat brain	—	—	0
	+	—	+++
	+	Methyl α -mannoside	0
	+	Methyl α -galactoside	++
Adult bovine brain	—	—	0
	+	—	+
	+	Methyl α -mannoside	0
	+	Methyl α -galactoside	+
Adult human brain	—	—	0
	+	—	+
	+	Methyl α -mannoside	0
	+	Methyl α -galactoside	+

Fig. 1. Binding of [^3H]concanavalin A (^3H radioactivity; solid line) to mature rat myelin proteins after separation by polyacrylamide/sodium dodecyl sulphate-gel electrophoresis

The acrylamide concentration used was 5%. The gels were cut in 2 mm segments and analysed for radioactivity. The direction of migration is from left to right. (a) Chloroform/methanol-extracted myelin labelled *in vivo* with [^{14}C]fucose (^{14}C radioactivity; broken line). (b) Diethyl ether/ethanol-extracted myelin. The position of the myelin-associated glycoprotein determined from an identical periodate/Schiff-stained gel is indicated by the arrow.

similar to that of fucose-containing glycoproteins, suggesting that the myelin-associated glycoprotein is the major concanavalin A-binding site in myelin. The greatest divergence of ^3H and ^{14}C labelling occurred in the high-molecular-weight region of the gel, where the amount of concanavalin A bound relative to [^{14}C]fucose radioactivity increased. Although this was a common finding on all gels, the amount of concanavalin A that bound in this region varied from preparation to preparation, suggesting that these minor concanavalin A-binding proteins may not be true components of myelin.

Although most of the glycoproteins in myelin are insoluble in chloroform/methanol, a large amount of the myelin protein, including the proteolipid, most of the basic protein, and small amounts of glycoproteins, are removed in this extraction process (Quarles *et al.*, 1973a; Quarles & Everly, 1977). For this reason the [^3H]concanavalin A binding was repeated by using diethyl ether/ethanol-delipidated myelin. Little protein is lost during this extraction process. In this case unlabelled rat myelin was used

as insufficient ^{14}C radioactivity could be put on the gel to give meaningful results. The position of the myelin-associated glycoprotein was determined from identical gels stained by the periodate/Schiff method. (It was found that this stain could not be used on the same gel as the concanavalin A binding.) As with chloroform/methanol-extracted myelin, it is apparent that the major concanavalin A-binding protein in diethyl ether/ethanol-extracted myelin is the myelin-associated glycoprotein (Fig. 1b). Because it permits the use of [^{14}C]fucose labelling and it leads to a higher degree of [^3H]concanavalin A binding, chloroform/methanol-extracted myelin was used in the remaining experiments.

Specificity of concanavalin A binding to rat myelin

To show that the [^3H]concanavalin A was binding specifically to glycoproteins in myelin, the treatment of 5% gels of adult rat myelin was repeated in the presence of 2.5% methyl α -D-mannopyranoside, an inhibitor of concanavalin A binding (Sharon & Lis, 1972). All the binding of [^3H]concanavalin A to the glycoproteins on the gel was prevented by the presence of this specific inhibitor of concanavalin A binding.

Binding of concanavalin A to immature rat myelin

In previous studies with [^{14}C]fucose-labelled immature rat myelin and [^3H]fucose-labelled adult rat myelin it has been shown that there is a change in the apparent molecular weight of the myelin-associated glycoprotein during development (Quarles *et al.*, 1973b). Because this could reflect a change in the carbohydrate moieties of the myelin-associated glycoprotein, the binding of [^3H]concanavalin A was examined on gels of myelin samples prepared from 13 day-old rat brains that had been labelled with [^{14}C]fucose. The pattern obtained is shown in Fig. 2. In terms of concanavalin A binding there appears to be a greater proportion of other glycoproteins in immature myelin that interact with the lectin than in mature myelin. However, the immature myelin-associated glycoprotein is still one of the main lectin-binding sites.

Detection of molecular-weight differences

As mentioned above, labelling with radioactive fucose *in vivo* can be used to show a change in apparent molecular weight of the myelin-associated glycoprotein during development. As an initial step in investigating this change in unlabelled tissue, especially human myelin, it was decided to test whether it could also be detected by using [^{14}C]fucose-labelled immature rat myelin and [^3H]concanavalin A binding to unlabelled adult rat

myelin. A mixture of 500 μg of unlabelled mature and 50 μg of [^{14}C]fucose-labelled immature rat myelin was electrophoresed as before and [^3H]concanavalin A was bound to the gel. Fig. 3 shows that the peaks of [^3H]concanavalin A binding and [^{14}C]fucose radioactivity are separated as expected since most of the myelin-associated glycoprotein on the gel is of the smaller adult form, whereas the ^{14}C is in traces of the larger immature myelin-associated glycoprotein. This gel shows that [^3H]concanavalin A binding to unlabelled myelin is sufficiently sensitive to demon-

Fig. 2. Binding of [^3H]concanavalin A (^3H radioactivity; solid line) to immature rat myelin proteins after separation by polyacrylamide/sodium dodecyl sulphate-gel electrophoresis

The experimental conditions are as described in the legend to Fig. 1. The glycoproteins in the myelin had been labelled *in vivo* with [^{14}C]fucose (^{14}C radioactivity; broken line).

Fig. 3. Demonstration of a developmental change in the myelin-associated glycoprotein by binding of [^3H]concanavalin A (^3H radioactivity; solid line)

A mixture of 500 μg of unlabelled mature and 50 μg of [^{14}C]fucose-labelled immature rat myelin (^{14}C radioactivity; broken line) was electrophoresed as described in the legend to Fig. 1.

strate molecular-weight differences between the myelin-associated glycoprotein of a particular sample and a radioactively labelled marker myelin-associated glycoprotein.

The binding of concanavalin A to bovine and human myelin

The binding of [^3H]concanavalin A was also carried out on 5% polyacrylamide gels of bovine and human myelin that had been delipidated with chloroform/methanol. Fig. 4(a) shows the pattern of radioactivity obtained with bovine myelin. As with adult rat myelin the myelin-associated glycoprotein binds more [^3H]concanavalin A than any of the other glycoproteins in bovine myelin. In this case, however, the minor concanavalin A-binding components with a molecular weight higher than the myelin-associated glycoprotein are more pronounced. The results of binding [^3H]concanavalin A to a gel of human myelin are shown in Fig. 4(b). Human myelin appears to have three glycoproteins that bind concanavalin A in about equal amounts. One of the peaks coincides with the position of the myelin-associated glycoprotein. The two other peaks electrophorese in about the same area as the two high-molecular-weight minor components observed in bovine and rat myelin. A further feature of the human myelin reaction with concanavalin A is that there appears to be a much

Fig. 4. Binding of [^3H]concanavalin A to (a) bovine and (b) human myelin proteins after separation by polyacrylamide/sodium dodecyl sulphate-gel electrophoresis

The experimental conditions are as described in the legend to Fig. 1. The positions of the myelin-associated glycoprotein determined from identical periodate/Schiff-stained gels are indicated by the arrows.

higher general background of concanavalin A binding in the case of the human sample. It is of interest that both the bovine and human myelin samples bind much more [^3H]concanavalin A/mg of total protein than does the rat myelin.

Discussion

Previous studies have shown that ferritin-concanavalin A binds to fragments of rat central-nervous-system myelin (Matus *et al.*, 1973) and that concanavalin A strongly agglutinates suspensions of myelin purified from adult rat brain (Matthieu *et al.*, 1974). The current studies show that myelin from immature rat brain is also strongly agglutinated, and that mature bovine and human myelin are agglutinated but less effectively than rat myelin (Table 1). It was previously suggested that the myelin-associated glycoprotein could be involved in the binding of lectins to central-nervous-system myelin (Matthieu *et al.*, 1974). The present paper identifies the myelin-associated glycoprotein as a major concanavalin A-binding glycoprotein in myelin, when either chloroform/methanol or diethyl ether/ethanol-extracted myelin was examined (Fig. 1). It is interesting to note that the amount of concanavalin A radioactivity bound/mg of protein in diethyl ether/ethanol-extracted myelin was 20–25% of that obtained with chloroform/methanol-extracted myelin. This correlates well with the fact that the latter method of extraction removes 75–80% of the myelin protein, but little of the glycoproteins (Quarles *et al.*, 1973a).

The binding of concanavalin A to the myelin-associated glycoprotein indicates that the glycoprotein contains mannose or glucose residues. It has also been shown to contain galactose (Poduslo *et al.*, 1976) and is the major myelin protein component that is labelled when radioactive Na_2SO_4 (Matthieu *et al.*, 1975), fucose, glucosamine or *N*-acetylmannosamine, a sialic acid precursor, are incorporated *in vivo* (Quarles *et al.*, 1973a).

The facts that the myelin-associated glycoprotein appears to be a major concanavalin A receptor and that isolated myelin can be agglutinated by using the lectin suggest that carbohydrate moieties of the glycoprotein are exposed to some extent in the disrupted fragments of myelin that are purified. Previous surface-labelling studies with galactose oxidase led to the conclusion that the myelin-associated glycoprotein is also at least partially exposed on the external surface of the myelin sheath or closely related membranes *in situ* (Poduslo *et al.*, 1976), although a recent immunocytochemical investigation indicates that this glycoprotein is particularly concentrated in the portion of the myelin sheath adjacent to the axon (Sternberger *et al.*, 1979). It is likely that the myelin-associated glycoprotein in

the latter location would also be exposed in the isolated fragments of myelin. In the periaxonal location the myelin-associated glycoprotein is well situated to be involved in glial-axonal interactions occurring during myelinogenesis, and it is conceivable that such an interaction could involve a lectin-like molecule of the type now being detected in mammalian tissues (Simpson *et al.*, 1978). When [^3H]concanavalin A was bound to immature rat myelin on polyacrylamide/sodium dodecyl sulphate gels it was found that the myelin-associated glycoprotein was no longer the only major lectin receptor, but there were several principal concanavalin A-binding proteins present in the membrane (Fig. 2). Lectin labelling of sodium dodecyl sulphate gels has also been used to detect changes in membrane-surface glycoproteins during transformation (Burridge, 1976) and differentiation (Light & Tanner, 1977). In the course of development, changes in membrane glycoproteins have been demonstrated in chick erythrocytes (Weise & Ingram, 1976). The differences in concanavalin A binding between immature and mature myelin may be significant in understanding the mechanism of progression from myelin, which has one or two loose turns around the axon, to a highly compact multilamellar structure.

In the peripheral nervous system there is also a glycoprotein in purified myelin, although, in this case, it is a major component of the membrane (Everly *et al.*, 1973; Wood & Dawson, 1973). This glycoprotein also binds concanavalin A and peroxidase-tagged concanavalin A can be visualized at the intraperiod line of the myelin sheath (Wood & McLaughlin, 1975). Recent immunocytochemical studies indicate that a small amount of the myelin-associated glycoprotein, or an antigenically similar glycoprotein, is also present in peripheral myelin (Sternberger *et al.*, 1979).

There are two points worth noting on the methodology of binding radioactively labelled concanavalin A to myelin on sodium dodecyl sulphate gels. First, in all studies published to date on the binding of lectins to gels, the lectin was labelled with ^{125}I or ^{131}I . The experiments reported in the present paper demonstrate that commercially available [^3H]concanavalin A will work equally well in many cases. Secondly, the gel profiles all show a background of concanavalin A binding the length of the gel, which is eliminated by the presence of methyl α -mannoside. This could be due to many minor glycoprotein components. However Tanner & Anstee (1976) noted that with the erythrocyte membrane the amount of specific background labelling on gels tended to be higher with concanavalin A than with other lectins, but no explanation has been found for this as yet.

During the study of the optimum conditions for [^3H]concanavalin A binding it was found that less

than 50 μg of myelin protein would bind sufficient lectin to permit identification of the myelin-associated glycoprotein. When this is taken in conjunction with the fact that [^3H]concanavalin A can also detect the developmental shift in the myelin-associated glycoprotein (Fig. 3), it provides a sensitive method of studying the myelin-associated glycoprotein in tissues that cannot be labelled *in vivo* with radioactive sugar precursors, especially human myelin.

The differences in the pattern of binding of [^3H]concanavalin A to gels of bovine and human myelin in comparison with rat myelin (Fig. 4) are similar in some respects to the differences observed previously by periodate/Schiff staining of such gels (Everly *et al.*, 1977). Both techniques indicate that human myelin has a higher proportion of other glycoproteins relative to the myelin-associated glycoprotein. Both methods indicate that bovine myelin is intermediate between human and rat myelin in this respect. However, in absolute terms, human and bovine myelin-associated glycoprotein bound more radioactive concanavalin A than rat myelin-associated glycoprotein, whereas they stained less intensely with periodate/Schiff reagents (Everly *et al.*, 1977). This might be explained by a lower content of sialic acid-rich terminal oligosaccharide units in the human and bovine myelin-associated glycoprotein, which would decrease the periodate/Schiff staining, but could increase the concanavalin A binding to internal mannose residues. The greater binding of concanavalin A to the solubilized proteins of human and bovine myelin than to those of rat myelin does not correlate directly with substantially weaker agglutination of human and bovine myelin, but this might be explained by inaccessibility of the binding sites in the intact myelin membranes of these species.

In conclusion, these studies have shown that concanavalin A-binding sites are exposed on purified myelin membranes of three different species, including humans. [^3H]Concanavalin A binding to myelin proteins separated on sodium dodecyl sulphate gels has shown that the myelin-associated glycoprotein is likely to be a major receptor for this lectin in each species. In addition, it appears that this method may prove very useful in the study of glycoproteins in human myelin samples when material is limited. The following paper (Quarles *et al.*, 1979) considers the binding of rat myelin glycoproteins to immobilized concanavalin A as well as the interactions of other lectins with purified myelin fractions and solubilized myelin proteins.

References

- Allen, R. D., David, G. B. & Nomarski, G. (1969) *Z. Wiss. Mikrosk. Und Mikrosk. Tech.* **69**, 193–221
- Bittiger, H. & Schnebli, H. P. (1974) *Nature (London)* **249**, 370–371
- Brunngraber, E. G., Susz, J. P., Javaid, J., Aro, A. & Warecka, K. (1975) *J. Neurochem.* **24**, 805–806
- Burridge, K. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4457–4461
- Everly, J. E., Brady, R. O. & Quarles, R. H. (1973) *J. Neurochem.* **21**, 329–334
- Everly, J. E., Quarles, R. H. & Brady, R. O. (1977) *J. Neurochem.* **28**, 95–101
- Gombos, G., Hermetet, J. C., Reeber, A., Zanetta, J. P. & Treska-Ciesielski, J. (1972) *FEBS Lett.* **24**, 247–250
- Greenfield, S., Norton, W. T. & Morell, P. (1971) *J. Neurochem.* **18**, 2119–2128
- Gurd, J. W. (1977) *Brain Res.* **126**, 154–159
- Gurd, J. W. & Evans, W. H. (1976) *Can. J. Biochem.* **54**, 477–480
- Kelly, P. T. & Cotman, C. W. (1977) *J. Biol. Chem.* **252**, 786–793
- Light, N. D. & Tanner, M. J. A. (1977) *Biochem. J.* **164**, 565–578
- Lis, H. & Sharon, N. (1972) *Annu. Rev. Biochem.* **42**, 541–574
- Matthieu, J.-M., Daniel, A., Quarles, R. H. & Brady, R. O. (1974) *Brain Res.* **81**, 348–353
- Matthieu, J.-M., Quarles, R. H., Poduslo, J. F. & Brady, R. O. (1975) *Biochim. Biophys. Acta* **392**, 159–166
- Matus, A., DePetris, S. & Raff, M. C. (1973) *Nature (London)* **244**, 278–280
- Nicolson, G. (1974) *Int. Rev. Cytol.* **39**, 89–190
- Norton, W. T. & Poduslo, S. E. (1973) *J. Neurochem.* **21**, 749–758
- Poduslo, J. F., Quarles, R. H. & Brady, R. O. (1976) *J. Biol. Chem.* **251**, 153–158
- Quarles, R. H. (1979) in *Complex Carbohydrates of Nervous Tissue* (Margolis, R. K. & Margolis, R. U., eds.) pp. 209–233, Plenum Press, New York
- Quarles, R. H. & Brady, R. O. (1971) *J. Neurochem.* **18**, 1809–1820
- Quarles, R. H. & Everly, J. L. (1977) *Biochim. Biophys. Acta* **466**, 176–186
- Quarles, R. H., Everly, J. L. & Brady, R. O. (1972) *Biochem. Biophys. Res. Commun.* **47**, 491–497
- Quarles, R. H., Everly, J. L. & Brady, R. O. (1973a) *J. Neurochem.* **21**, 1177–1191
- Quarles, R. H., Everly, J. L. & Brady, R. O. (1973b) *Brain Res.* **58**, 506–509
- Quarles, R. H., McIntyre, L. J. & Pasnak, C. F. (1979) *Biochem. J.* **183**, 213–221
- Robinson, P. J., Bull, F. G., Anderton, B. H. & Roitt, I. M. (1975) *FEBS Lett.* **58**, 330–333
- Rostas, J. A. P., Kelly, P. T. & Cotman, C. W. (1977) *Anal. Biochem.* **80**, 366–372
- Segrest, J. P. & Jackson, R. L. (1972) *Methods Enzymol.* **28**, 54–63
- Sharon, N. & Lis, H. (1972) *Science* **177**, 949–959
- Simpson, D. L., Thorne, D. R. & Loh, H. H. (1978) *Life Sci.* **22**, 727–748
- Sternberger, N. H., Quarles, R. H., Itoyama, Y. & Webster, H. deF. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1510–1514
- Susz, J. P., Hof, H. I. & Brunngraber, E. G. (1973) *FEBS Lett.* **32**, 289–292
- Tanner, M. J. A. & Anstee, D. J. (1976) *Biochem. J.* **153**, 265–270

- Weise, M. J. & Ingram, V. M. (1976) *J. Biol. Chem.* **251**, 6667-6673
- Wood, J. G. & Dawson, R. M. C. (1973) *J. Neurochem.* **21**, 717-719
- Wood, J. G. & McLaughlin, B. J. (1975) *J. Neurochem.* **24**, 233-235
- Wood, J. G. & Sarinana, F. O. (1975) *Anal. Biochem.* **69**, 320-322
- Zanetta, J. P., Morgan, I. G. & Gombos, G. (1975) *Brain Res.* **83**, 337-348
- Zanetta, J. P., Sarlieve, L. L., Mandel, P., Vincendon, G. & Gombos, G. (1977) *J. Neurochem.* **29**, 827-838