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PURIFICATION, AND PARTIAL CHARACTERIZATION OF TWO GLYCOPROTEINS IN BOVINE PERIPHERAL NERVE MYELIN MEMBRANE

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SUMMARY

Two major glycoproteins of bovine peripheral nerve myelin were isolated from the acid-insoluble residue of the myelin by a procedure involving delipidation with chloroform/methanol (2:1, v/v) and chromatography on Sephadex G-200 column with a buffer containing sodium dodecyl sulfate. The separation patterns of the proteins on the gel were affected considerably by the dodecyl sulfate concentration in the elution buffer. At above 2% dodecyl sulfate concentration in the elution buffer, the glycoproteins could be separated clearly on the gel and were purified. The purified proteins, the BR protein (mol. wt. 28 000) and the PAS-II protein (mol. wt. 13 000), were homogeneous on dodecyl sulfate-polyacrylamide gel electrophoresis. The NH_2-terminal amino acids of the BR and the PAS-II proteins were isoleucine and methionine, respectively. The BR protein contained glucosamine, mannose, galactose, fucose and sialic acids and the PAS-II protein contained glucosamine, mannose, galactose, fucose and glucose. Neither the BR protein nor the PAS-II were a glycosylated derivative of a basic protein of bovine peripheral nerve myelin, a deduction based on the results of amino acid analysis. The two major glycoproteins were observed commonly in the peripheral nerve myelin of cows, pigs, rabbits and guinea pigs, using dodecyl sulfate-polyacrylamide gel electrophoresis.

INTRODUCTION

Myelin proteins have attracted attention, not only as membrane proteins but also as the source of the antigenic factor which induces demyelinating diseases, experimental allergic encephalomyelitis in the central nervous system [1], and experimental allergic neuritis in the peripheral nervous system [2]. Until recently, however, there has been considerable confusion over the nature and the composition of the myelin proteins of the peripheral nerve, which have been studied less extensively than those of the central nervous system, as reviewed by Carnegie and Dunkley [3].

Previously, we reported the purification and some properties of acid-soluble myelin basic proteins in pig and bovine peripheral nerves [4–6]. During these studies, we observed that bovine peripheral nerve myelin contains an acid-insoluble protein which is distinct from the basic proteins and which accounts for a

high proportion of the total myelin protein. Similar evidence in various species have been reported independently and verified by the introduction of sodium dodecyl-sulfate or phenol-acid electrophoresis [7–11]. On the other hand, it was shown that the major myelin proteins in the peripheral nerves of humans [12], rats [13–15] and rabbits [16] were glycoproteins.

In this report, we describe procedures for purifying two major glycoproteins from the acid-treated residue of bovine peripheral nerve myelin, and note some of their chemical properties Preliminary accounts of this work have appeared [17, 18].

MATERIALS AND METHODS

Preparation of the acid-insoluble protein fraction from bovine peripheral nerve myelin

The myelin fractions were prepared from the intradural spinal roots of cows by sucrose density gradient centrifugation as described by Uyemura et al. [5]. The myelin fractions from pig intradural spinal roots and from the sciatic nerves of rabbits and guinea pigs were also obtained by a similar procedure. The bovine myelin fraction was homogenized in 0.03 M HCl at pH 2.0, stirred for 13 h at 4 °C and centrifuged, and the BF protein was purified from the supernatant as previously reported by Kitamura et al. [6]. The insoluble residue was washed twice with 0.03 M HCl. The final residue after acid extraction of the myelin was used as a starting material for purification of myelin glycoproteins. About 85 % of the total myelin protein was recovered from this residue.

The residue of the acid extraction was homogenized with 10 vol. of distilled water per wet weight in a Teflon-glass homogenizer by five strokes at 0 °C, then the pH was adjusted to 7.0 with 0.2 M NaOH. The suspension was stirred for 10 min at 0 °C and centrifuged. The pellet was brought to 20 vol. of its wet weight with chilled chloroform/methanol (2:1, v/v) and homogenized with a Teflon-glass homogenizer. The homogenate was stirred for 30 min at 0 °C and then centrifuged at $7000 \times g$ for 30 min at 4 °C.

In more recent experiments we modified the procedure as follows. The acid-insoluble residue was homogenized directly with 20 vol. of chloroform/methanol (2:1, v/v) per wet weight. By this procedure, the residue was completely dissolved in acidic chloroform/methanol. The clear solution was adjusted to pH 7.0 with alkali and then the insoluble materials were collected by centrifugation.

This removal of lipids was repeated, and the fat-free precipitate was stirred well with a glass rod under air until dry. About 78 % of the total myelin protein was recovered from the final fat-free precipitate after these procedures.

Gel chromatography

70 mg fat-free precipitate were dissolved in 3 ml of 0.1 M sodium phosphate buffer, pH 7.2, containing 10 % sodium dodecyl sulfate and the solution was incubated for 1 h at 45 °C; the dodecyl sulfate must be present in at least a 1.4-fold weight excess over protein. A small amount of insoluble material was removed by centrifugation at $26\,000 \times g$ for 15 min at 25 °C. Practically all of the protein remained in the supernatant. The supernatant was applied on a Sephadex G-200 (40–120 μm) column equilibrated and eluted with 0.1 M sodium phosphate buffer, pH 7.2, containing various concentrations of dodecyl sulfate. The flow rate of the column (2.64×94 cm)

was maintained at 15 ml/h. Chromatographic patterns were monitored by absorbancy at 280 nm. This was followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Suitable fractions were collected and the protein in the fraction was precipitated by the addition of one-fifth vol. of saturated NaCl according to the method of Wood and Dawson [19]. The mixtures were cooled in an ice-bath for more than 30 min and centrifuged at $18\,000 \times g$ for 15 min at 4 °C. The pellets were washed twice with acetone/water (9:1, v/v) and once with water. The precipitates were resuspended in water and lyophilized. The lyophilized protein was rechromatographed on a Sephadex G-200 column as described above in order to obtain the purified protein.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Electrophoretic analysis of the total myelin protein and the fractions were carried out according to the method described previously [6]. Samples were treated in 0.1 M sodium phosphate buffer, pH 7.2, containing 1 % sodium dodecyl sulfate, 1 % 2-mercaptoethanol and 0.01 % Malachite green as tracking dye at 45 °C for 60 min before electrophoresis. 10 % polyacrylamide gels (0.5×6 cm) in 0.1 M sodium phosphate buffer, pH 7.2, containing 1 % dodecyl sulfate were used. The electrode buffer consisted of the same phosphate buffer. The electrophoresis was carried out at 4 mA per gel for 5–6 h until the marker moved 4.5 cm in the gels. The gels were stained with Coomassie brilliant blue R-250 for proteins and periodic acid-Shiff reagent for glycoproteins, respectively, according to the methods of Fairbanks et al. [20].

Amino acid analysis

0.5–1 mg of the protein sample was hydrolyzed in 1 ml of constant-boiling HCl at 110 °C for 22 h in an evacuated sealed tube. Amino acid analysis was carried out with a JEOL JLC-6AH automatic amino acid analyzer. Through a column (0.8×50 cm) packed with type LC-R2 resin (JEOL Ltd., Japan), all amino acids were separated at 55 °C with the following three buffers: (a) 0.2 M Na^+, pH 3.25, containing 8.5 % *n*-propanol; (b) 0.2 M Na^+, pH 4.25; (c) 1.6 M Na^+, pH 7.02, including 0.7 M NaCl.

NH_2-terminal amino acid analysis

The NH_2-terminal amino acids were determined by the Edman procedure as described by Blombäck et al. [21]. Phenylthiohydantoin-amino acids were separated on a polyamide layer (Chen Chin Trading Co., Taiwan) according to the method of Summers et al. [22]. The phenylthiohydantoin-amino acids were also hydrolyzed by constant-boiling HCl for 4 h at 150 °C, and the constituent amino acids were analyzed by an automatic amino acid analyzer.

Carbohydrate determinations

The individual amounts of glucosamine and galactosamine were obtained after hydrolysis with 4 M HCl at 100 °C for 4, 6 and 8 h on an amino acid analyzer using LC-R2 resin. The elution was carried out with the citrate buffer (b) described above. The 6-h hydrolysis revealed a maximum yield of hexosamine and was used in routine operations.

Quantitative determination of individual neutral sugars was carried out by

JEOL automated liquid chromatography. A 15-cm column of LC-R3 resin was used, and the elution was carried out with three buffers: (i) 0.13 M borate, pH 7.5; (ii) 0.25 M borate, pH 9.0; (iii) 0.35 M borate, pH 9.6. Samples for this assay were hydrolyzed with 1 N H_2SO_4 at 100 °C for 6, 8 and 10 h. The 8-h hydrolysis which revealed a maximum yield of neutral sugars was used in routine operations. The neutral sugar fractions were obtained by passage of the diluted hydrolysate through coupled columns of Dowex 50-X4, 200-400 mesh (H^+ form) and Dowex 1-X8, 200-400 mesh (formate form) according to the methods of Spiro [23].

A thiobarbituric acid assay was used to determine the sialic acids in the proteins according to the method of Warren [24] after the samples had been hydrolyzed in 0.1 N H_2SO_4 at 80 °C for 1 h.

RESULTS

Fractionation of the myelin proteins

The myelin proteins in bovine peripheral nerve migrated as two major protein bands (BR and BF) on sodium dodecyl sulfate-polyacrylamide gel electrophoresis as shown in Fig. 1A and as previously reported [6]. When the gel was stained by periodic

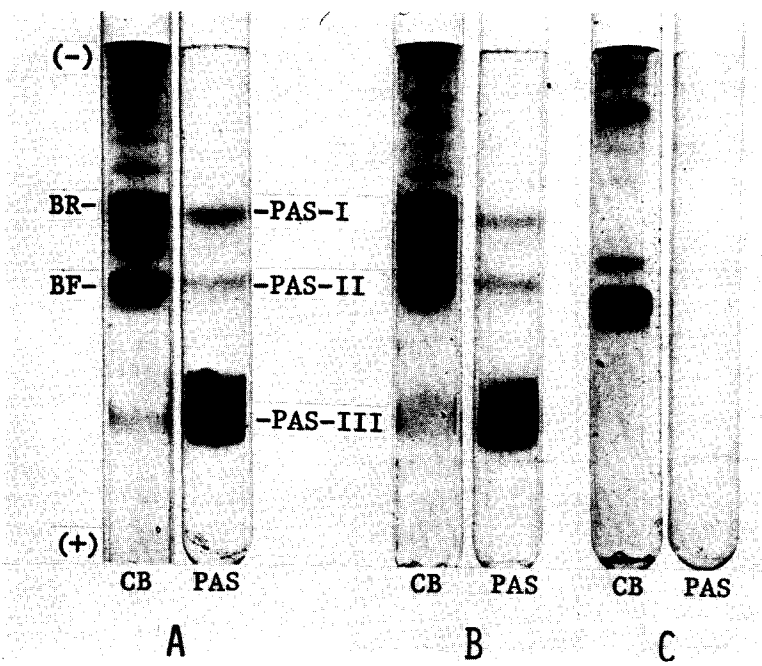

Fig. 1. Comparison of acid-soluble and acid-insoluble proteins of bovine peripheral nerve myelin on 1 % dodecyl sulfate-10 % polyacrylamide gel electrophoresis. Samples were dissolved in 0.1 M sodium phosphate buffer, pH 7.2, containing 1 % dodecylsulfate, 1 % 2-mercaptoethanol and 0.01 % Malachite green. The gels were stained with Coomassie blue (CB) or with periodic acid-Shiff reagent (PAS). A, whole myelin proteins; B, acid-insoluble proteins of myelin; C, acid extracts of myelin. From left to right, the quantities of protein applied to the gels were 30, 90, 30, 60, 20, 60 μg, respectively. For labeling details see in Results.

acid-Shiff reagent, three distinct bands (PAS-I, PAS-II and PAS-III) were observed (Fig. 1A, right gel). Migration rates of the PAS-I and PAS-II corresponded to the band BR and band BF, respectively. As Fig. 1C shows, the acid extracts of the myelin contained one major protein band (BF), which was not stained by periodic acid-Shiff reagent. On the other hand, the acid-insoluble residue contained three periodic acid-Shiff reagent-positive bands; two of which corresponded to band BR and BF on Coomassie blue-stained gel, respectively, as shown in Fig. 1B. A fast migrating band (PAS-III) observed in Figs. 1A and B was considered to be glycolipids because no protein band was observed at this position in Coomassie blue-stained gel.

The acid-insoluble residue, which contained both of the glycoproteins, was used for further purification of the glycoproteins in the myelin.

In preliminary experiments, when the acid-insoluble material was dissolved directly by a 10 % sodium dodecyl sulfate solution without removing the lipids, a

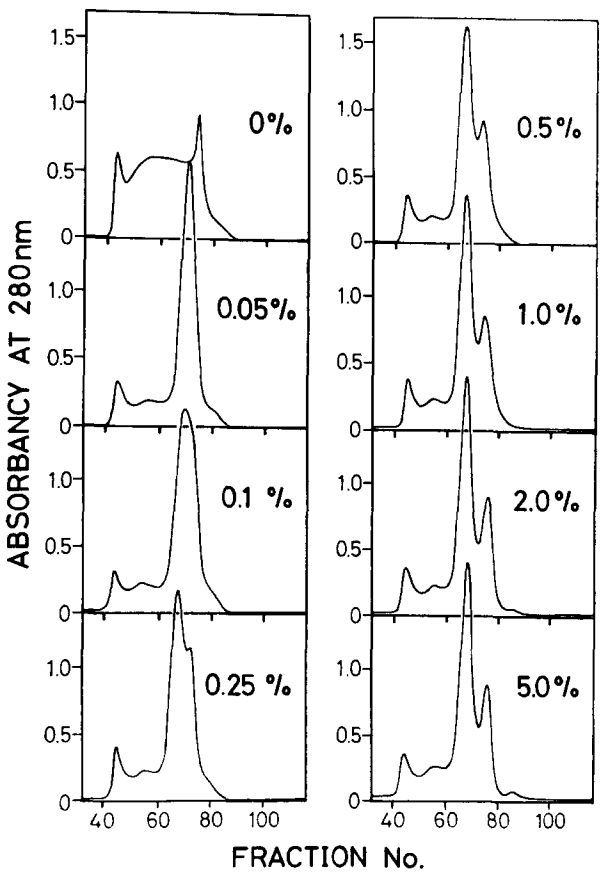

Fig. 2. Gel chromatographic profiles of acid-insoluble proteins of myelin on Sephadex G-200 column. 70 mg of the proteins were dissolved in 3 ml of 0.1 M sodium phosphate buffer, pH 7.2, containing 10 % dodecyl sulfate and applied to a column (2.64×94 cm) equilibrated and eluted with 0.1 M phosphate buffer containing various percentages of dodecyl sulfate concentration indicated in the figure. Fractions of 3.9 ml were collected at a flow rate of 15 ml/h.

large amount of lipids was also dissolved with the proteins and interfered with the separation of the proteins on gel filtration chromatography. In order to get an effective gel filtration, the step of removing lipids from the acid-insoluble residue was essential.

As Fig. 2 shows, the separation patterns of the proteins on Sephadex G-200 column chromatography were affected considerably by the dodecyl sulfate concentration in the elution buffer. At any concentration, there was an excluded peak of high molecular weight or aggregated materials. At low dodecyl sulfate concentrations, such as 0.05 and 0.1 %, one major peak was observed. However, this major peak showed several protein bands instead of a single band when the gel was stained by Coomassie blue after dodecyl sulfate-polyacrylamide gel electrophoresis. By increasing the concentration of dodecyl sulfate from 0.25 to 2 %, a much better separation of two major peaks was observed. The first and the second peaks contained mainly PAS-I and PAS-II, respectively, on dodecyl sulfate-gel electrophoresis. No obvious difference in patterns was observed between 2 and 5 % dodecyl sulfate concentration. Therefore, 2 % dodecyl sulfate was used routinely in the elution buffer.

The purified proteins obtained by rechromatography of the first peak and of the second peak were designated the BR protein and the PAS-II protein, respectively, each of which showed a single band on dodecyl sulfate-gel electrophoresis (Fig. 3). On 5–15 % polyacrylamide gel electrophoresis, the relative mobility of the PAS-II protein was identical with that of the BF protein which was purified previously from the acid extracts of the myelin [6]. However, the BF protein was not stained by periodic acid-Shiff reagent. In this respect, the PAS-II protein seemed to be different from the BF

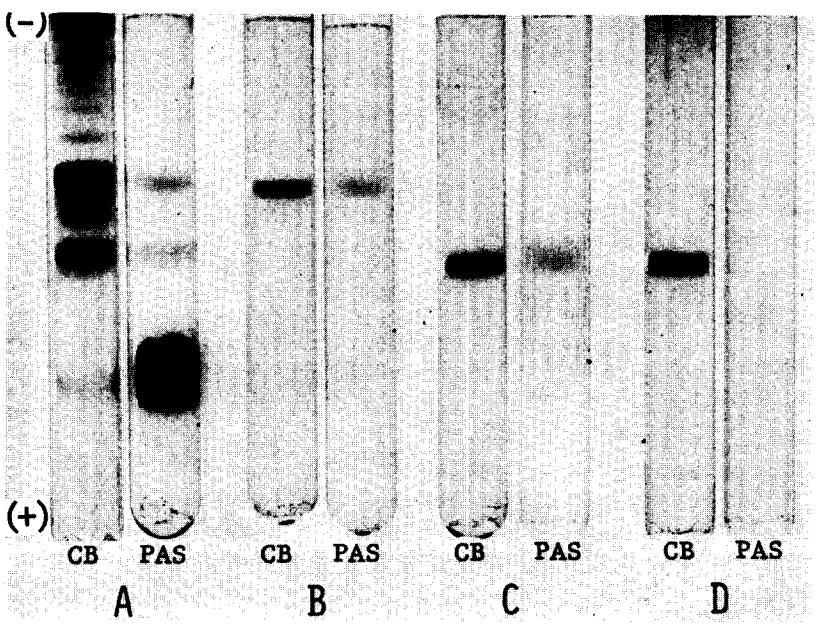

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole myelin proteins, (A); the purified BR protein, (B); the PAS-II protein, (C); the BF protein (D). From left to right, the quantities of protein applied to the gels were 30, 90, 15, 30, 15, 45, 8 and 40 μg, respectively. The experimental conditions are the same as that given in Fig. 1.

protein. And it was considered to be important to compare this new glycoprotein (PAS-II protein) with the BF protein.

Amino acid analysis

As shown in Table I, the amino acid compositions of both the BR and the PAS-II proteins are different not only from the Folch-Lees type proteolipid apoprotein but also from the encephalitogenic protein in the central nervous system myelin. In comparison with the BF protein, the BR protein showed significantly higher glycine, tyrosine and histidine and lower threonine, glutamic acid and lysine contents. In the PAS-II protein higher serine, leucine and histidine and lower aspartic acid and lysine contents were observed than in the BF protein. In conclusion, the amino acid composition of the BR, the PAS-II and the BF proteins were quite different from one another.

TABLE I

AMINO ACID COMPOSITIONS AND NH_2-TERMINAL AMINO ACIDS OF PURIFIED GLYCOPROTEINS AND OTHER BOVINE MYELIN PROTEINS

Values are given as mean ±S.D. for several determinations. The numbers of determinations are shown in parentheses

Amino acid*	Amino acid composition (mol/100 mol)				
	BR protein (11)	PAS-II protein (4)	BF protein** (6)	Encephlito-genic protein** (12)	Folch-Lees type proteolipid apoprotein***
Aspartic acid	8.4±0.6	6.6±0.6	9.8±0.2	6.9±0.2	4.2
Threonine	6.2±0.4	6.8±0.2	9.7±0.4	4.3±0.1	8.5
Serine	6.8±0.4	10.2±0.7	6.1±0.2	9.7±0.2	5.4
Glutamic acid	8.4±0.4	8.5±0.6	10.3±0.1	6.5±0.2	6.0
Proline	3.7±0.3	2.0±0.1	2.0±0.1	7.4±0.3	2.9
Glycine	10.1±0.2	8.4±0.2	7.5±0.1	15.0±0.4	10.3
Alanine	6.4±0.2	6.8±0.2	4.2±0.1	8.6±0.2	12.5
Cysteine	0.9±0.4	1.0±0.2	0.3±0.4	0	4.2
Valine	8.6±0.6	8.0±0.9	7.9±0.2	1.4±0.2	6.9
Methionine	1.2±0.2	2.4±0.1	2.4±0.1	1.3±0.1	1.7
Isoleucine	4.3±0.3	4.8±0.6	5.2±0.1	1.6±0.1	4.9
Leucine	7.5±0.4	11.2±0.9	8.0±0.2	6.2±0.1	11.1
Tyrosine	5.5±0.5	3.7±0.2	1.5±0.1	2.5±0.1	4.7
Phenylalanine	3.6±0.3	6.3±0.5	4.0±0.1	4.9±0.1	7.9
Histidine	3.1±0.3	2.8±0.2	0****	6.0±0.3	1.9
Lysine	8.7±0.9	6.6±0.1	14.7±0.6	8.0±0.6	4.3
Arginine	6.8±0.1	4.0±0.3	5.6±0.2	9.8±0.7	2.6
Ammonia	8.6±1.4	13.3±1.8	13.9±3.4	8.4±0.7	–
NH_2-terminal amino acid	Isoleucine	Methionine	Blocked	–	–

* Tryptophan was not determined.
** Data of Kitamura et al. [6].
*** Data of Tenebaum and Folch-Pi [33].
**** Data of Deibler et al. [34].

NH_2-Terminal amino acid analysis

On the polyamide layer, each phenylthiohydantoin-(NH_2-terminal amino acid) of the purified proteins migrated to a position identical with authentic phenylthiohydantoin-isoleucine/leucine for the BR protein and phenylthiohydantoin-methionine for the PAS-II protein. No trace of any other NH_2-terminal amino acid could be detected. Analysis of the constituent amino acids of the phenylthiohydantoin-amino acids on an amino acid analyzer disclosed the NH_2-terminal amino acid of the BR protein and of the PAS-II protein to be isoleucine and methionine, respectively.

The NH_2-terminal amino acid of the BF protein was concluded to be blocked because it did not show any spots on polyamide layer by the same analysis. These results are listed in Table I.

Carbohydrate determinations

The purified BR, PAS-II and BF proteins were subjected to analysis for various carbohydrates. The BR protein contained 2.6 mol glucosamine, 2.7 mol mannose, 0.8 mol fucose, 1.0 mol galactose, 0.8 mol sialic acids and a trace of glucose per molecule, assuming a molecular weight of 28 000 [6]. The PAS-II protein contained 2.1 mol glucosamine, 1.5 mol mannose, 0.3 mol fucose, 0.8 mol galactose, 0.8 mol glucose and a trace of sialic acids per molecule, assuming a molecular weight of 13 000 [6]. Neither galactosamine nor xylose were found in either of the proteins. On the other hand, no carbohydrate was found in the BF protein.

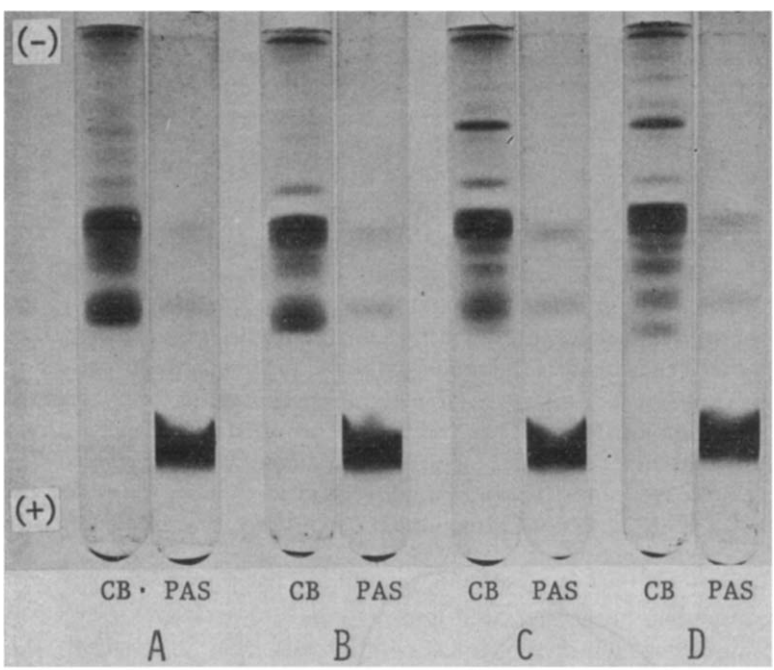

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the peripheral nerve myelins obtained from various animals. A, bovine; B, pig; C, rabbit; D, guinea pig. The quantity of protein applied to each gel was 25 μg for Coomassie blue stain and 50 μg for periodic acid-Shiff reagent stain. The experimental conditions are the same as given in Fig. 1.

Distribution of proteins and glycoproteins in the peripheral nerve myelin of various species

Bands BR and BF were commonly observed in the peripheral nerve myelin of cows, pigs, rabbits and guinea pigs, although the distribution of the proteins varied among species tested on dodecyl sulfate-polyacrylamide gel electrophoresis as shown in Fig. 4. The protein staining profiles of bovine and pig myelin were quite similar. Some additional fast migrating bands on these profiles were observed in rabbit and guinea pig myelin.

On the other hand, the periodic acid-Shiff reagent-staining profiles were quite similar to one another. There were two glycoprotein bands corresponding to PAS-I and PAS-II bands. From these results, it was suggested that these two glycoproteins are common components of peripheral nerve myelin in mammalian species.

DISCUSSION

Considerable amounts of the proteins of the myelin were not extracted by repeated acid extractions, but remained insoluble as previously reported [5]. In order to purify these acid-insoluble proteins, it was necessary to dissolve them with detergents such as sodium dodecyl sulfate after removal of the lipids. The solubilized proteins could be separated on Sephadex G-200 gel chromatography eluted with 0.1 M phosphate buffer containing 2 % dodecyl sulfate. 0.1 to 1.0 % of dodecyl sulfate solution have been commonly used as an elution buffer on gel chromatography [25–29]. As shown in the results, however, better separation was obtained by increasing the dodecyl sulfate concentration from 0.1 to 2.0 % in the elution buffer of the gel column. Although the reason for the effect of the high dodecyl sulfate concentration was not clear, one possibility was that pore sizes of the gel matrix were modified by the high concentration of dodecyl sulfate micells, and another that a high dodecyl sulfate concentration was necessary to dissociate tight protein to protein or protein to lipid interactions in the acid-insoluble residue. The following observations which suggest the latter possibility have been reported. Nicot et al. noted that the Folch-Lees type proteolipid complex was not dissociated to protein and lipids at a dodecyl sulfate concentration of less than 1.5 % on a gel chromatography [30]. Katzman also reported that proteins were not dissociated during gel chromatography eluted with 0.1 % dodecyl sulfate and 0.01 % mercaptoethanol although the fat-free brain proteins were completely dissolved with a 5 % dodecyl sulfate solution containing 1 % mercaptoethanol [25]. On the basis of this evidence, we decided to use a high concentration, 2 %, of dodecyl sulfate as an elution buffer, and we obtained satisfactory results.

As shown above, this report provides a convenient method for purifying two glycoproteins, the BR and the PAS-II proteins, on a relatively large scale from the myelin fraction of bovine peripheral nerve. Although it seems possible that the carbohydrate molecules in these glycoproteins might have been derived from glycolipids which were abundant in the myelin, it is very doubtful because, first, the acid-insoluble residue was completely free of lipids before application on a gel filtration column, and second, when a concentration of dodecyl sulfate solution greater than 1.5 % was used for the elution buffer, the protein components were clearly separated from the lipid components as shown by Nicot et al. [30].

Both glycoproteins were distinctly different not only from the encephalitogenic

basic protein but also the Folch-Lees type proteolipid apoprotein in the central nervous system. As characterized by Coomassie blue- and periodic acid-Shiff reagent-staining in dodecyl sulfate gel electrophoresis and amino acid analysis, the BR protein is likely to be identical to the so-called "PO" in rabbit [16], "J" in human and rabbit [9], "X" in rat [14] or "band IV" in rat [15] as the major protein in the peripheral nerve myelin. In this report, the presence of glycoproteins in peripheral nerve myelin, first reported by Everly et al. [13] and Wood and Dawson [14], has been confirmed by analysis of sugars in the purified proteins. The presence of fucose in the BR protein seems to prove that major protein in rat sciatic nerve myelin is labeled by [^{14}C]fucose described by Everly et al. [13]. Recently Wood and Dawson suggested that the protein moiety of the "X" protein, from amino acid analysis and its ability to induce demyelinating disease, may contain a similar amino acid sequence to that of rabbit P2 protein [31] which corresponds to the BF protein in this report. However, our results do not support their suggestions because the BR protein from bovine peripheral nerve myelin showed an amino acid composition quite different from the BF protein. Furthermore, both the BR and the BF proteins failed to produce any evidence of experimental allergic neuritis of encephalomyelitis when injected into guinea pigs and rabbits [32].

Recently, Brostoff et al. reported that rabbit PO protein contained less hexosamine (1.2 mol per molecule) than our result (2.6 mol per molecule) showed for bovine BR protein [16]. This discrepancy in hexosamine content in the proteins may be due to the difference of the animal species used or to the purity of the proteins.

Existence of the second glycoprotein, the PAS-II protein, has never been clearly demonstrated. One of the reasons for the difficulty in finding it was that this protein migrated to the same position as the BF protein on dodecyl sulfate-gel electrophoresis in 5–15 % polyacrylamide gels. In Everly's report [13], they admitted that on dodecyl sulfate-gel electrophoresis a fast migrating component besides a major glycoprotein also showed [^{14}C]fucose incorporation. It is possible that this component corresponds to the PAS-II protein in this report. The component VII, with a molecular weight of 14 000, which was periodic acid-Shiff positive in rat peripheral nerve myelin, reported by Singh and Spritz [15], also showed a mobility similar to that of the PAS-II protein on dodecyl sulfate-gel electrophoresis.

Studies on the distribution of glycoproteins in peripheral nerve myelin revealed that two glycoproteins, the BR and the PAS-II proteins, are observed commonly in all of the species of animals examined, cow, pig, rabbit and guinea pig. However, considerable differences among them in the relative amounts of the protein components stained by Coomassie blue were observed. The biological roles of these glycoproteins in the myelin membrane may be an interesting problem to be investigated further.

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REFERENCES

1 Laatsch, M., Kies, M. W., Gordon, S. and Alvord, E. C. (1962) J. Exp. Med. 115, 777–788
2 Waksman, B. H. and Adams, R. D. (1955) J. Exp. Med. 103, 213–235
3 Carnegie, P. R. and Dunkley, P. R. (1975) in Advances in Neurochemistry (Agranoff, B. W. and Aprison, M. H., eds.), Vol. I, pp. 95–135, Plenum Press, New York
4 Uyemura, K., Tobari, C. and Hirano, S. (1970) Biochim. Biophys. Acta 214, 190–197
5 Uyemura, K., Tobari, C., Hirano, S. and Tsukada, Y. (1972) J. Neurochem. 19, 2607–2614
6 Kitamura, K., Yamanaka, T. and Uyemura, K. (1975) Biochim. Biophys. Acta 379, 582–591
7 Eng, L. F., Chao, F. -C., Gestl, B., Pratt, D. and Tavaststjerna, M. G. (1968) Biochemistry 7, 4455–4465
8 Mehl, E. and Wolfgram, F. (1969) J. Neurochem. 16, 1091–1097
9 Csejtey, J., Hallpike, J. F., Adams, C. W. M. and Bayliss, O. B. (1972) J. Neurochem. 19, 1931–1935
10 Brostoff, S. W. and Eylar, E. H. (1972) Arch. Biochem. Biophys. 153, 590–598
11 Greenfield, S., Brostoff, S., Eylar, E. H. and Morell, P. (1973) J. Neurochem. 20, 1207–1216
12 Savolainen, H. (1972) Tower Int. Technomed. J. Life Sci. 2, 35–38
13 Everly, J. L., Brady, R. O. and Quarles, R. H. (1973) J. Neurochem. 21, 329–334
14 Wood, J. G. and Dawson, R. M. C. (1973) J. Neurochem. 21, 717–719
15 Singh, H. and Spritz, N. (1974) Biochim. Biophys. Acta 351, 379–386
16 Brostoff, S. W., Karkhanis, Y. D., Carlo, D. J., Reuter, W. and Eylar, E. H. (1975) Brain Res. 86, 449–458
17 Uyemura, K., Kitamura, K., Yamanaka, T. and Aizawa, K. (1974) J. Physiol. Soc. Japan 36, 348
18 Kitamura, K., Yamanaka, T. and Uyemura, K. (1975) Seikagaku Japan 47, 698
19 Wood, J. G. and Dawson, R. M. C. (1974) J. Neurochem. 22, 627–630
20 Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) Biochemistry 10, 2606–2617
21 Blombäck, B., Blombäck, M., Edman, P. and Hessel, B. (1966) Biochim. Biophys. Acta 115, 371–396
22 Summers, M. R., Smythers G. W. and Oroszlan, S. (1973) Anal. Biochem. 53, 624–628
23 Spiro, R. G. (1966) in Methods in Enzymology (Colowick, S. P. and Kaplan, N. O., eds.), Vol. III, pp. 3–26, Academic Press, New York
24 Warren, L. (1959) J. Biol. Chem. 234, 1971–1975
25 Katzman, R. L. (1972) Biochim. Biophys. Acta 266, 269–272
26 Jones, T. H. D. and Kennedy, E. D. (1969) J. Biol. Chem. 244, 5981–5987
27 Vacher-Lepretre, M., Nicot, C., Alfsen, A., Jolles, J. and Jolles, P. (1976) Biochim. Biophys. Acta 240, 323–331
28 Rosenberg, S. A. and Guidotti, G. (1969) J. Biol. Chem. 244, 5118–5124
29 Lenard, J. 1970 Biochemistry 9, 1129–1132
30 Nicot, C., Nguyen Le, T., Lepretre, M. and Alfsen, A. (1973) Biochim. Biophys. Acta 322, 109–123
31 Brostoff, S. W., Burnett, P., Lampert, P. and Eylar, E. H. (1972) Nat. New Biol. 235, 210–212
32 Uyemura, K., Kitamura, K., Ogawa, Y. and Matsuyama, H. (1973) Abstracts. International Symposium Aetiology and Pathogenesis of Demyelinating Disease, Kyoto, p. 21
33 Tenebaum, D. and Folch-Pi, J. (1966) Biochim. Biophys. Acta 115, 141–147
34 Deibler, G. E., Kies, M. W. and Uyemura, K. (1975) Trans. Am. Soc. Neurochem. 6, 85