Topographical Arrangement of Membrane Proteins in the Intact Myelin Sheath

LACTOPEROXIDASE INCORPORATION OF IODINE INTO MYELIN SURFACE PROTEINS*

JOSEPH F. PODUSLO‡ AND PETER E. BRAUN§

From the Department of Biochemistry and Institute of Neurological Sciences, Medical School, University of Pennsylvania, Philadelphia, Pennsylvania 19174

SUMMARY

The lactoperoxidase-catalyzed iodination technique was utilized to incorporate radioactive iodine into membrane proteins which lie on the outer surface of the myelin sheath. An intact, myelinated nerve bundle, the dorsal column of the cat spinal cord, was employed. The enzymatically iodinated proteins were identified by polyacrylamide gel electrophoresis, and the specific radioactivity was determined. Results indicated that several high molecular weight proteins were predominantly labeled by the nonpenetrating lactoperoxidase. Proteolipid protein was also labeled, although to a lesser extent; basic protein was not labeled under these conditions. The data suggest that several high molecular weight proteins are exposed on the outer surface of the myelin sheath. Proteolipid protein is at least partially exposed on the outer surface, although it could be present at both membrane surfaces. Evidence is presented which suggests that the basic protein is located at the inner surface of the membrane, corresponding to the major dense line of myelin.

*A recent approach to an investigation of the molecular organization of membranes involves the chemical or enzymatic modification of membrane surface constituents and their subsequent identification. Such experimental approaches, however, must meet certain stringent criteria as emphasized by Maddy (1) and Wallach (2). Briefly, the chemical or enzymatic probe must (a) react under physiological conditions of pH, tonicity, and temperature; (b) be impermanent to the membrane; (c) show a high degree of specificity for particular amino acid residues; and (d) be detectable in trace amounts. The utilization of lactoperoxidase to incorporate radioactive iodine into the tyrosine and histidine residues of proteins which lie on the outer membrane surface not only fulfills these criteria but provides significant insight into the protein topography of biological membranes.

This lactoperoxidase-iodination method for covalent labeling of surface membrane proteins was first described by Phillips and Morrison (3, 4) for the human erythrocyte, by Marchalonis et al. (5) for normal and neoplastic lymphocytes, and by Hubbard and Cohn (6) for the human erythrocyte. This technique for investigating the vectorial arrangement of membrane proteins has also been applied to other biological membranes. Poduslo et al. (7) have examined the protein arrangement on the surface membrane of mouse fibroblasts (L cells) and normal and virus-transformed baby hamster kidney fibroblasts (BHK). Phillips (8) investigated the human platelet membrane; Vitetta et al. (9) isolated and characterized cell surface immunoglobulin from mouse splenic lymphocytes, and Salton et al. (10) identified membrane ATPase of Micrococcus lysodeikticus on the inner face of the membrane by this method.

In the present communication, this technique is extended to an investigation of the arrangement of the membrane proteins on the outer surface of the intact myelin sheath. A primary consideration in such a study is that the myelin preparation used must be intact and reflect the in vivo structural state. Isolated myelin cannot be used because of structural distortions that take place during the isolation procedure and because the inner and outer membrane surfaces can no longer be distinguished. A unique preparation is described which circumvents these problems, namely, an intact myelinated nerve bundle, the dorsal column of the cat spinal cord.

EXPERIMENTAL PROCEDURES

Dorsal Column Preparation—Twenty-nine adult cats ranging in weight from 2.0 to 3.2 kg were used. The animals were anesthetized by an intraperitoneal injection of 35 mg per kg body weight of sodium penicillinat. A laminectomy of the spinous processes and laminae of the mid- to upper thoracic level was performed. This was followed by a rhizotomy and removal of the spinal cord from the animal. The excised cord was then stripped of the meninges including the pia mater, and the dorsal column was dissected away from the remaining spinal cord. Fig. 1 illustrates a cross-section of the cat spinal cord with the dorsal columns or posterior white columns (posterior funiculus) projecting above the remaining portion of the spinal cord. The column was...
per 5 ml by sonication in an ultrasonic cleaning bath, and the
suspended in the solvent at a concentration of 1 mg of myelin proteins
acrylic plastic chamber which is equipped with a well to accom-
modate a stirring bar. A 2 to 3 cm segment of the dorsal column
was placed in the chamber; the ends of the preparation were
sealed with petroleum jelly, and the enzymatic reaction was
was performed directly in the l-ml capacity chamber.

incubation Procedure-The cat dorsal column was incubated in
1.0 ml of isotonic sodium phosphate buffer (pH 7.4, 0.109 M).
Lactoperoxidase was added to the reaction mixture to give a
final concentration in the range of 0.6 to 0.9 μM. A fresh solution
of lactoperoxidase was used for each experiment. In each case
1.0 mCi of 1\textsuperscript{31}I was added as Na\textsuperscript{131}I along with 50 μM carrier NaI
(see “Results”). The reaction was initiated by the addition of
10 μl of 1.0 mM H\textsubscript{2}O\textsubscript{2} to give a concentration of 10 μM in the reaction
mixture. Twenty similar additions of hydrogen peroxide were made at 15-s intervals to maintain the reaction. Controlled
additions of low concentrations of peroxide minimized membrane
oxidation. After the reaction a 10-fold excess of NaI was added
in the absence of the preparation was removed from the chamber and rinsed with
Locke’s solution.

Isolation of Myelin-Myelin was isolated according to the
method of Poduslo and Norton as described by Norton (11). A
2-cm segment of the cat dorsal column with a wet weight of about
125 mg was homogenized in 2.5 ml of 0.32 M sucrose (5% homoge-
nate) and layered over 2.5 ml of 0.9 M sucrose. The serine value was corrected for phosphatidylserine. The total
protein was collected by centrifugation at 12,000 × g for 10 min.

Myelin was then suspended in a known volume of solvent from
which aliquots were taken for protein determination. Following
centrifugation, the pellet was solubilized to give a final concen-
tration of 1 mg per ml of protein in the following: 0.1 ml of 10%
(w/v) sodium dodecyl sulfate-0.1 ml of 0.3 m Tris buffer (pH 8.0),
0.01 ml of β-mercaptoethanol, 0.1 ml of glycerol, 0.01 ml of 0.4% bac-
thrombophelate, and 0.08 ml of H\textsubscript{2}O. The myelin proteins were
extensively sonicated after each addition. A 2-cm segment of a
hemidisection cat dorsal column provides approximately 1 mg of
myelin protein. As little as 0.5 mg of total protein can be con-
veniently handled.

Polyacrylamide Gel Electrophoresis—A discontinuous gel elec-
Figure 1. Cross-section of the cat spinal cord showing the location of the dorsal or posterior column, and a diagram of the in-
cubation chamber.

then split down the posterior medium septum; thus, one-half of
the preparation serves as control for the other half. Throughout
the dissection, the preparation was continuously bathed with
Locke’s solution.

Incubation Chamber—A specially designed chamber was em-
oployment for incubation of the dorsal column. Fig. 1 illustrates the
acrylic plastic chamber which is equipped with a well to accom-
modate a stirring bar. A 2 to 3 cm segment of the dorsal column
was placed in the chamber; the ends of the preparation were
sealed with petroleum jelly, and the enzymatic reaction was
was performed directly in the l-ml capacity chamber.

Isolation Procedure-The cat dorsal column was incubated in
1.0 ml of isotonic sodium phosphate buffer (pH 7.4, 0.109 M).
Lactoperoxidase was added to the reaction mixture to give a
final concentration in the range of 0.6 to 0.9 μM. A fresh solution
of lactoperoxidase was used for each experiment. In each case
1.0 mCi of 1\textsuperscript{31}I was added as Na\textsuperscript{131}I along with 50 μM carrier NaI
(see “Results”). The reaction was initiated by the addition of
10 μl of 1.0 mM H\textsubscript{2}O\textsubscript{2} to give a concentration of 10 μM in the reaction
mixture. Twenty similar additions of hydrogen peroxide were made at 15-s intervals to maintain the reaction. Controlled
additions of low concentrations of peroxide minimized membrane
oxidation. After the reaction a 10-fold excess of NaI was added
in the absence of the preparation was removed from the chamber and rinsed with
Locke’s solution.

Isolation of Myelin-Myelin was isolated according to the
method of Poduslo and Norton as described by Norton (11). A
2-cm segment of the cat dorsal column with a wet weight of about
125 mg was homogenized in 2.5 ml of 0.32 M sucrose (5% homoge-
nate) and layered over 2.5 ml of 0.9 M sucrose. The serine value was corrected for phosphatidylserine. The total
protein was collected by centrifugation at 12,000 × g for 10 min.

Myelin was then suspended in a known volume of solvent from
which aliquots were taken for protein determination. Following
centrifugation, the pellet was solubilized to give a final concen-
tration of 1 mg per ml of protein in the following: 0.1 ml of 10%
(w/v) sodium dodecyl sulfate-0.1 ml of 0.3 m Tris buffer (pH 8.0),
0.01 ml of β-mercaptoethanol, 0.1 ml of glycerol, 0.01 ml of 0.4% bac-
thrombophelate, and 0.08 ml of H\textsubscript{2}O. The myelin proteins were
extensively sonicated after each addition. A 2-cm segment of a
hemidisection cat dorsal column provides approximately 1 mg of
myelin protein. As little as 0.5 mg of total protein can be con-
veniently handled.

Polyacrylamide Gel Electrophoresis—A discontinuous gel elec-
tronic system for separation of myelin proteins on 15% acryl-
}
ing this relationship we then determined the number of absorbance units of each of the proteins on the gel. The actual amount of either protein was estimated by dividing absorbance units of these proteins by their extinction coefficients, namely 5.9 for basic protein (17) and 14.5 for proteolipid protein (18) (see also "Discussion"). The protein on the gel other than these two major proteins was determined by difference, and designated cumulatively as the "remaining proteins" (Fig. 2). The cumulative \( E^{\%} \) for this protein was calculated to be 7.6. Specific radioactivities in the labeling experiments were then readily determined from the percentage composition of these three classes of proteins in a given quantity of myelin applied to the gels.

**Lipid Extraction of Isolated Myelin**—In one experiment, isolated myelin from an iodinated dorsal column preparation was treated with chloroform-methanol (2:1, v/v) and partitioned into an upper and lower phase with 0.2x volume of \( H_2O \) (19). The two phases were separated; the lower phase was washed twice with theoretical upper phase (chloroform-methanol-water, 3:48:47, v/v/v). The upper phases were combined, dried under \( N_2 \), and counted for radioactivity in iodine. The lower phase was dried under \( N_2 \) as a chloroform-enriched fraction to prevent denaturation of the proteolipid protein. The dried residue was then extracted three times with ether-ethanol (3:2, v/v). The ether-ethanol supernatants were combined, concentrated under \( N_2 \), and counted. The ether-ethanol precipitate was solubilized in sodium dodecyl sulfate according to the standard procedure. Aliquots were removed for counting of the radioactive iodine.

**Other Procedures and Methods**—Hydrogen peroxide concentrations were determined spectrophotometrically using a molar extinction coefficient of 72.4 at 250 nm according to George (20). Lactoperoxidase was obtained from Calbiochem, and the concentration was determined at 412 nm using a millimolar extinction coefficient of 112 according to Morrison et al. (21). Radioactive \( ^{131}I \) was obtained from New England Nuclear as carrier-free sodium iodide.

**RESULTS**

**Quantitation of Myelin Proteins**—In the 250-nm absorbance scan shown in Fig. 2, the numerous proteins larger than proteolipid protein are not readily resolved at this particular full scale absorbance setting. The artificial noise in the spectrophoto- metric scan at the end of the gel below the basic protein is presumably due to light scatter and probably represents sodium dodecyl sulfate or lipid complexes that have not been eluted from the gel during the fixation process. The protein composition as a percentage of total protein in myelin is stated in Fig. 2.

**Lactoperoxidase-catalyzed Incorporation of Iodide into Myelin Surface Proteins**—A densitometric scan at 555 nm of a Coomassie blue-stained acrylamide gel after fixation is shown in Fig. 3. The basic protein is off-scale with this particular absorbance setting in order to demonstrate at least 10 to 12 proteins of higher molecular weight than proteolipid protein. Slice 1 to 3 represents the 6% acrylamide spacer gel, whereas the remaining separating gel is 15% acrylamide. The interface between the two gels is recorded as a very sharp peak due to optical distortion. At the end of the gel another sharp peak is seen which is not a result of Coomassie blue staining. This is a refractive front which moves close behind the bromphenol blue dye front (not shown). This is believed to be unpolymerized or uncross-linked acrylamide which also results in optical distortion in the densitometric scan at 250 nm. The interface between the gels and the various refractive bands is also present on acrylamide gels that have been subjected to electrophoresis in the absence of protein. The Coomassie blue-stained band which precedes basic protein in the gel is presumably a lipid-detergent complex. Two distinct radioactive peaks associated with at least two high molecular weight proteins are clearly evident near the top of the 15% acrylamide gel. Proteolipid protein is also being labeled, although to a lesser degree. Basic protein is not being labeled by this technique; the low counts reflect gel background.

**Effects of Varying the Concentration of Carrier NaI**—Maximal incorporation of radioactive iodine into the myelin surface proteins was achieved by adding an appropriate amount of carrier NaI to the incubation mixture. Dorsal column preparations were incubated with varying amounts of carrier NaI (0, 25, 75, 100, and 200 \( \mu \)M) in addition to the standard reaction mixture. The specific activities (counts per min per \( \mu \)g of protein) for basic protein, proteolipid protein, and the remaining protein were then determined. In each case, the specific activity of the remaining proteins reflected the two predominant radioactive peaks corresponding to the labeling of at least two high molecular weight proteins. Label was also associated with proteolipid protein; however, basic protein was not labeled regardless of the amount of carrier present. Maximal incorporation of the isotope into the myelin proteins occurred with 50 \( \mu \)M carrier; as a result, this amount of NaI was included in all subsequent experiments.

**Comparison of Intact Dorsal Column Preparation and Isolated Myelin**—To assure the reactivity of the individual myelin proteins to iodination by lactoperoxidase, a comparison was made between the levels of iodination for the isolated cat dorsal column myelin and the intact preparation. Isolated myelin
containing 1.25 mg of myelin proteins was suspended in isotonic phosphate buffer and iodinated according to the standard procedure. Fig. 4 compares specific activities of basic protein, proteolipid protein, and the remaining proteins in the intact preparation and in isolated myelin. At least a 7-fold increase is seen in the specific activity of the remaining proteins of the isolated myelin. This reflects not only an increased iodination of the same high molecular weight proteins of the intact preparation, but also labeling of additional high molecular weight proteins. Iodination of basic protein showed a 390-fold increase above the background of the intact preparation. At least a 7-fold increase in labeling of proteolipid protein was seen. Essentially no difference is observed in the specific activity of the labeled high molecular weight proteins of the two preparations.

Comparison of Intact Dorsal Column Preparation and the Total Homogenate—The inner, cytoplasmic surfaces of myelin can, to a limited extent, be made accessible to lactoperoxidase by simple homogenization of the dorsal column under isotonic conditions. This, therefore, allows a comparison of the labeling of the outer membrane surface to the labeling of both membrane surfaces. A segment of the dorsal column containing 1.0 mg of myelin protein was homogenized in isotonic phosphate buffer and iodinated by the standard procedure. A comparison of the distribution of radioactive iodine in the myelin proteins of the two different preparations is illustrated in Fig. 5. Although the basic protein is essentially unlabeled in the intact myelin sheath, it becomes heavily labeled in the disrupted (homogenized)

preparation. The proteolipid protein, on the other hand, is labeled only by a 3.5-fold increase. Essentially no difference is observed in the specific activity of the labeled high molecular weight proteins of the two preparations.

Lipid Extraction of Isolated Myelin—Radioactive iodine was found associated with lipid extracted from the isolated myelin of the iodinated dorsal column. To determine the extent of this labeling of myelin lipids, chloroform-methanol-extracted myelin was partitioned as described under “Experimental Procedures,” and the amount of label associated with the upper and lower phases was determined. The upper phase had 26% of the total counts of the isolated myelin. Of the remaining 74% in the lower phase, 34% of the counts was extractable with ether-ethanol while the remaining 40% was associated with the insoluble precipitate. Preliminary investigation of the ether-ethanol extract showed labeling of most of the major lipid classes.

As a measure of nonspecific iodination, the dorsal column preparation was incubated in the absence of lactoperoxidase. When the solubilized myelin proteins were separated by electrophoresis, no radioactive label was found associated with the membrane proteins. Membrane lipids, on the other hand, were labeled to the same extent as in the presence of the enzyme. Recovery of counts from the acrylamide gel under the electrophoretic conditions described was on the order of 60%. This recovery approached 100% if the electrophoretic run was terminated when the marker dye (bromphenol blue) was 2 to 3 cm from the bottom of the gel. This again suggested labeling of lipids which migrated near the marker dye.

Comparison of Specific Activities of Myelin Proteins before and after an Additional Myelin Purification Step—The possibility of a non-myelin contaminant or a labeled lipid which co-migrates on electrophoresis with a given myelin protein was investigated. Myelin isolated by the normal procedure was subjected to a final CsCl purification step as described under “Experimental Procedures,” and the dense lower layer was investigated. No
FIG. 6. Effect of an antioxidant on the iodination of myelin surface proteins. Dorsal column preparations were incubated for 45 min with lactoperoxidase in the presence (hatched bars) or absence (solid bars) of 0.001% butylated hydroxytoluene. PLP, proteolipid protein; BP, basic protein.

change was seen in the specific activities of any of the myelin proteins.

Effects of Butylated Hydroxytoluene on the Iodination of Myelin Surface Proteins—In view of the recent report of Welton and Aust (22) that lipid peroxidation affects the level of incorporation of iodide into membrane surface proteins, we enzymatically iodinated myelin surface proteins in the presence of the antioxidant butylated hydroxytoluene. Fig. 6 shows the changes in specific activities for the myelin proteins with the inclusion of a low concentration (0.001%) of this antioxidant. There was a small increase in the specific activity of the proteolipid protein from 21 to 34 cpm per μg, while the basic protein remained at the same, low background level. The remaining proteins had a larger increase in the specific activity (255 to 436 cpm per μg). This increase occurred only in the two predominant radioactive peaks.

Time Course of Diffusion of Lactoperoxidase into Dorsal Column—The extent of incorporation of iodine depended on the length of time during which the lactoperoxidase was allowed to diffuse around individual nerve fibers in the dorsal column. Fig. 7 illustrates a time course study in which the lactoperoxidase was preincubated with the dorsal column for varying time periods, from 5 to 90 min. In the last 5 min of each preincubation, radioactive iodine, carrier NaI, and H₂O₂ were added to initiate the reaction and label the myelin surface proteins. For the 5-min incubation the reaction was initiated at the same time as the addition of the lactoperoxidase. Extension of the reaction beyond the 5-min period for a given preincubation time did not alter the specific activities for any of the myelin proteins, other than what was expected due to enzyme diffusion. Thus, the iodine incorporation is not limited by the reaction itself.

A linear increase is seen in the specific activity of proteins exclusive of proteolipid and basic proteins. This 2.7-fold increase is the result of enhanced labeling seen in the two predominant radioactive peaks corresponding to at least two high molecular weight proteins. A 5.6-fold increase is also seen in the specific activity of the proteolipid protein which occurs after an apparent time lag. These specific activities, however, are at a much lower level compared to the high molecular weight proteins. The specific activity of the basic protein does not change significantly.

DISCUSSION

It is becoming increasingly apparent that the task of assigning functional roles to particular membrane components can be facilitated by a knowledge of their precise localization within the membrane. The use of lactoperoxidase to catalyze the iodination of proteins is one method by which it is possible to obtain at least a partial identification of those proteins which are located on the surface of the membranes.

Catalysis of iodination by lactoperoxidase has been shown by Morrison and Bayse (23, 24) to involve an enzyme-substrate complex of peroxide, iodide, and tyrosine. Consequently, only those membrane proteins can be iodinated which are oriented in such a way that tyrosine residues, and to some extent histidine residues, are accessible to the enzyme. Thus, the incorporation of iodine into some, but not all, proteins of intact myelin demonstrates differences in the location or orientation of constituent proteins within the membrane.

Specifically, the distribution of radioactive iodine in the proteins of the intact myelin sheath reveals extensive labeling of several high molecular weight proteins, whereas the characteristic proteolipid protein of myelin is labeled only to a low degree. In contrast, the basic protein, often thought of as a peripheral membrane protein according to the criteria of Singer and Nicolson (25), remains essentially unlabeled.

Interpretation of these data with respect to the molecular organization of the myelin sheath are predicated on several observations. First, the parallel increase in specific activity of the labeled proteins with increased reaction time is convincing evidence that the differences in susceptibility to iodination are not merely differences in the rate of reactivity of the individual proteins. Secondly, the fact that the basic protein can be extensively labeled (9 times more than proteolipid protein, on a weight basis) in myelin isolated from the dorsal column emphasizes that the apparent differences of protein labeling in the in-
tact myelin are real. This observation also demonstrates the inability of the lactoperoxidase with a molecular weight of 78,000 to diffuse across the membrane and confirms the intactness of the dorsal column preparation.

These data, therefore, allow the conclusion that the basic protein of myelin is absent from the outer membrane surface (and presumably the intraperiod line of myelin) or that it is associated with the outer surface in such a way that the tyrosine and histidine residues are sterically inaccessible to lactoperoxidase or are not exposed to the external aqueous environment. We favor the first interpretation on the grounds that simple mechanical disruption of the dorsal column preparation to expose inner surfaces under isotonic conditions permits the labeling of basic protein at a level comparable to that of proteolipid protein. Such isotonic homogenization would be expected to result in minimal molecular rearrangement of the basic protein such that previously sterically masked residues become exposed. This is supported by the finding of comparatively similar levels of iodination for the remaining proteins.

In support of our contention that the basic protein is present only on the inner cytoplasmic surface of the membrane (and hence the major dense line of myelin) are the histochemical studies of Adams et al. (28). In contrast, however, Dickinson et al. (27) suggest that this protein is a component of the intraperiod line of myelin.

The location of the proteolipid protein in the membrane is less clear. The labeling of this protein in the intact myelin indicates that at least part of the molecule is exposed to the external aqueous environment. These data, however, do not preclude its additional presence on the cytoplasmic side of the membrane. By comparison to these two characteristic myelin proteins, several high molecular weight proteins were labeled to a much greater extent in all experiments. Such a finding is consistent with recent observations that a limited number of high molecular weight proteins of other biological membranes are accessible to lactoperoxidase (6-8, 28, 29). It is premature to speculate whether or not these labeled myelin high molecular weight proteins are also present on the inner cytoplasmic surface of the membrane or if they span the full thickness of the membrane.

It is emphasized that studies of this type require that the membrane be structurally intact. The dorsal column of the cat spinal cord obviates the ambiguities inherent in investigating the surface protein arrangement in isolated myelin. The column is composed of large numbers of myelinated nerve fibers and their supportive oligodendrogial cells and astrocytes. Other non-myelin proteins of the column are undoubtedly labeled; however, the extensive myelin isolation procedure would be expected to remove such proteins. Control experiments clearly showed that additional purification steps did not alter the specific activity of the myelin proteins.

Quantitation of stained proteins on the acrylamide gel is quite often equivocal because of the differences in the dye-binding capacity of the various types of membrane proteins. In particular, myelin basic proteins have been shown to have anomalous staining characteristics which are presumably related to their high isoelectric point (31, 32). We have noticed a whitish turbidity in the basic protein region that occurs during fixation. Consequently, abnormal densitometric readings of the basic proteins on acrylamide gels will occur as a result of light scatter effects. A possible clue to the identity of this staining artifact is suggested by our finding that some myelin lipids, probably in complex with sodium dodecyl sulfate, are able to migrate with the basic protein. The identity of these lipids or the exact nature of their association with the protein, if any, is unknown. It seems likely, however, that some kind of interaction takes place between lipids and basic protein during fixation that gives rise to the observed turbidity. This emphasizes the necessity of running electrophoretic controls for lipids whenever their presence could affect experimental interpretations. The presence of lipid fronts which migrate near the marker dye indicates the electrophoretic behavior of the bulk of the lipids, but it does not exclude the possibility of small amounts of lipid with different electrophoretic mobilities characteristic of the gel system employed. In addition, it has been shown by others that anomalous staining of membrane lipids which behave as macromolecules can occur on these gels (33, 34).

These difficulties were circumvented by quantitating myelin proteins from the densitometric scan at 280 nm of the unfixed, unstained acrylamide gel. The quantitative determination of myelin proteins is facilitated by the fact that the protein composition is relatively simple, with about 90% of the protein consisting of two well characterized proteins with known extinction coefficients. This, therefore, allows the calculation of specific activities for the proteolipid protein and basic protein, and hence a more meaningful comparison of the labeling of these two proteins. With respect to this calculation, we have made several assumptions. The extinction coefficients we used were obtained from the literature and were for myelin proteins from the bovine central nervous system. Although no detailed phyleogenetic comparison of physical constants has been reported, amino acid compositions for each of these two proteins from the central nervous system of a variety of animals are remarkably similar, implying that the extinction coefficients are also very similar. In this regard, the amino acid composition of total myelin protein from the cat spinal cord is nearly identical to that from the bovine spinal cord. The second assumption we made is that the published extinction coefficients of these proteins are the same after the proteins have been denatured by sodium dodecyl sulfate. This is supported by our observation that the extinction coefficients of five commercially available crystalline proteins in buffered solutions did not change in the presence of sodium dodecyl sulfate.

A quantitative determination of the absolute specific activities of labeled proteins on the outer membrane surface would be valuable but was not attainable for the following reasons: (a) it was not possible to determine how many individual myelinated axons became iodinated in the reaction system which is limited by the diffusion rate of lactoperoxidase into the nerve bundle; (b) the surface area of the myelin sheath of the individual axons in the column cannot be ascertained, and the radioactivity associated with the individual myelin proteins does not correspond to their relative abundance within the myelin sheath; consequently, the absolute amount of protein which is iodinated cannot be determined; and (c) meaningful comparisons based on the number of potentially available tyrosine and histidine residues could not be made as the relative frequency and extent of iodination of these amino acids for all the myelin proteins are not known.

In light of the evidence attesting to the metabolic instability of myelin (35, 36) and the occurrence of proteolytic enzymes which play an important role in the early myelin breakdown, such as the postmortem proteolysis of myelin basic protein (31, 37, 38), we anticipated possible degradation of the dorsal column preparation after prolonged incubation periods. In fact, the basic protein is only significantly labeled after the 90-min incubation period, an observation we attribute to glial cell
deterioration. Consequently, our interpretation of the data is restricted to the shorter incubation times where the breakdown of myelin is minimized.

We are currently employing other chemical and enzymatic agents or probes in an ongoing effort to enlarge our understanding of the detailed structure of myelin.

Acknowledgments—We gratefully acknowledge the technical expertise of Mrs. Sylvia Chiang. We are indebted to Elaine Golds for valuable assistance in the quantitation of myelin proteins.

REFERENCES

30. Deleted in proof
38. Eammeck, R., and Brady, R. O. (1972) Brain Res. 46, 441-453
Topographical arrangement of membrane proteins in the intact myelin sheath.
Lactoperoxidase incorporation of iodine into myelin surface proteins.
J F Poduslo and P E Braun


Access the most updated version of this article at http://www.jbc.org/content/250/3/1099

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/3/1099.full.html#ref-list-1