Biochimica et Biophysica Acta, 509 (1978) 397—409

BBA 78036

PREPARATION OF MEMBRANE VESICLES FROM ISOLATED MYELIN

STUDIES ON FUNCTIONAL AND STRUCTURAL PROPERTIES

ANDREAS J. STECK [a,*], PETER SIEGRIST [a], PETER ZAHLER [a], NORBERT N. HERSCHKOWITZ [a] and ROLF SCHAEFER [b]

[a] *Departments of Pediatrics and Biochemistry, University of Bern, Bern, and* [b] *Department of Structure Biology, Biozentrum, University of Basel, Basel (Switzerland)*

(Received November 11th, 1977)

Summary

Myelin membranes purified from bovine brain are shown to form membrane vesicles when incubated in hypotonic buffer. Following restoration of isotonicity a resealing of the membrane occurs as judged by a significant decrease in $^{22}Na^{+}$ permeability. Electron spin resonance measurements using stearic acid spin label I indicate a small decrease in membrane fluidity with increasing ionic strength between 50 and 80 mM NaCl. Iodination of myelin membrane vesicles by lactoperoxidase shows a four-fold increase in the amount of iodine incorporation into the myelin basic protein from 0—150 mM NaCl, while the iodination of the proteolipid protein remains essentially unaffected by the change in ionic strength. This dependence of the iodination of the myelin basic protein on the ionic strength can be explained by the electrostatic interactions of this protein with membrane lipids. In view of striking analogies with studies on model membranes correlating protein binding with membrane permeability changes, we suggest a similar structure-function relationship for the myelin basic protein.

Introduction

The recent discovery in purified myelin of a protein kinase [1—3] phosphorylating one of the major myelin proteins, the myelin basic protein, has challenged the traditional view that myelin is an inert membrane assuming only the function of a passive insulator. Though the functional role of this enzyme in myelin is still unknown, the level of phosphorylation of specific membrane

* Present address: Department of Neurology, CHUV, 1011, Lausanne, Switzerland.
Abbreviation: SDS, sodium dodecyl sulfate.

proteins has been implicated in the regulation of various membrane functions, including water and ion permeability ([4—6], for review, see ref. 7). The idea of a direct participation of the myelin membrane in the regulation of the ionic composition around the axon is not new. Physiological [8,9] and, more recently, morphological [10] studies have provided suggestive evidence for the presence of ionic channels in the myelin membrane. Unfortunately, as yet there is no simple model system for the study of these properties in myelin. Isolated myelin cannot be used because such preparation yields a heterogeneous population of mostly loose membrane fragments, where the membrane barrier is lost. There is no longer an intracellular and an extracellular environment as these spaces become continuous. Many of these difficulties can be overcome by the use of membrane vesicles [11]. Vesicles generated from erythrocyte membrane have been extensively utilised to investigate structural and functional properties of the membrane [12,13]. The vesiculation of plasma membranes appears, however, to be a general phenomenon and membrane vesicles from a variety of origins have proved good models in membrane research [5,14,15].

In the present communication we describe the preparation and characterization of vesicles from isolated myelin. Data are presented which show that these myelin membrane vesicles are capable of maintaining transmembrane ionic gradients and can be used to measure the diffusion of ions through the membrane. A remarkable feature of these myelin membrane vesicles is that changes in ionic strength modify their permeability to sodium ions. In order to investigate further the behaviour of the myelin membrane vesicles with respect to their ion permeability, we sought additional information on the membrane molecular organization, using two different techniques, electron spin resonance (ESR) and lactoperoxydase iodination. The results obtained are discussed in the light of recent data on model membranes, relating permeability changes to specific alterations of lipid-protein interactions.

Materials and Methods

Myelin isolation

Myelin was prepared according to the method of Norton [16] from bovine brain (white matter). Bovine brains were obtained from a local slaughterhouse, frozen immediately with liquid N_2 and stored at —70°C until use.

Myelin membrane vesicles preparation

The final pellet of purified myelin was suspended in 5 vols. 1 mM phosphate buffer, pH 8.0.

After gentle homogenization the membranes were diluted with additional buffer (0.2 mg protein/ml final concentration) and incubated at 2—4°C for 18 h. This was followed by centrifugation (4°C) at 24 000 × *g* for 30 min. The pellet was resuspended in the incubation buffer (5 mg protein/ml final concentration), vortexed and passed through a 25 G needle. This preparation was kept at 4°C and used within 12 h.

Chemical characterization

Membrane preparations to be subjected to electrophoresis were dissolved in

0.052 M Tris buffer, pH 8.7, (Merck), containing 4% sodium dodecyl sulfate (SDS) (Serva), 5% β-mercaptoethanol (Merck), 8% glycerol and 0.02% Bromphenol Blue as tracking dye and heated at 100°C for 5 min to complete solubilization. Gel electrophoresis was performed on slab gels according to the discontinuous SDS gel system described by Laemmli [17] with the following modifications. For the separating gel a 10—15% linear acrylamide gradient was poured at 4°C from a standard two-chambered gradient mixer containing a 15% and a 10% acrylamide (Serva) solution in a 1.3 mm thick slab-gel apparatus fitted with a comb with 12 teeth, each 0.7 mm wide. We also obtained a better resistance against breakage of the slab gels by substituting glycerol, as used by Laemmli, with a 3% solution of polyacrylamide ($M_r > 5 \cdot 10^6$, BDH chemicals) to give a final solution of 0.48—0.72% (Schaefer, R., unpublished). Polymerization was carried out at room temperature and started by exposing the slab to a fluorescent light. The slabs were fixed in 40% trichloroacetic acid stained with Coomassie Blue (2.5 g/l) for 30 min and destained in 7.5% acetic acid/5% methanol. Gel densitometry was carried out with a Joyce-Loebel Chromoscan Densitometer at 575 nm. All protein determinations were performed by the method of Lowry et al. [19] with bovine serum albumin (Sigma) as the standard. Lipids were extracted according to the method of Folch-Pi et al. [20] and separated by one-dimensional chromatography (see Results). The identity of the lipids was established by co-chromatography of pure lipids and staining was by iodine vapor. Phospho- and glycolipids were further characterized by a molybdate- or orcinol-containing spray.

Negative staining and electron microscopy

For negative stains, lightly carbon-coated 200 mesh copper grids (Pioloform) were used. Samples were diluted in their own buffer (0.5 mg protein/ml final concentration) and were applied as drops to a grid and excess drawn off with filter paper. One drop of a 2% solution of phosphotungstic acid (pH 7.0) was then added and excess removed. Grids were examined immediately after preparation in a Philips 200 electron microscope.

Measurements of sodium permeability

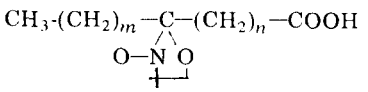
7—8 μCi of carrier-free $^{22}Na^+$ (EIR, Würenlingen) were added to 1.7-ml aliquots (5 mg protein/ml) of freshly prepared myelin membrane vesicles in 1 mM phosphate buffer, pH 8.0, and preincubated at 4°C for 1 h. After addition of different amounts of NaCl the volume was adjusted to 4.0 ml and the suspension was incubated for an additional 40 min at 37°C (in some cases 4°) in a shaking bath to equilibrate. To remove non-incorporated $^{22}Na^+$ the preparation was centrifuged (4°C) at 40 000 × *g* for 12 min and washed four times with 4.0-ml aliquots of the appropriate buffer. Counts in the last wash were not over background for myelin membrane vesicles in 150 mM NaCl, while they were on the average 1.4 times higher than the background for myelin membrane vesicles in NaCl-free buffer.

To measure $^{22}Na^+$ self-diffusion the pellet was resuspended in 4.0 ml of prewarmed buffer of corresponding ionic strength and incubated in a shaking bath at 37°C. 1 ml samples were collected at 10, 30, 50 and 70 min and immediately centrifuged (4°C) at 24 000 × *g* for 15 min. Aliquots of the resulting super-

natant and pellet were counted in a gamma counter (Packard 3003 Auto Gamma Spectrometer) to measure extra- and intravesicular $^{22}Na^+$. The 10 min values of the supernatant were taken as zero incubation time and the amount of $^{22}Na^+$ found in this initial determination was subtracted from the subsequent determinations. The $^{22}Na^+$ self-diffusion is expressed as a percentage of the $^{22}Na^+$ present in the pellet.

Electron spin resonance

Spin label experiments were performed with the stearic acid spin label I (m,n) $m = 12$, $n = 3$ [21]



kindly supplied by Dr. J. Seelig, Biozentrum, University of Basel, Switzerland. Spin label incorporated into myelin membrane vesicles was prepared as described by Landsberger et al. [22]. Spin label-loaded bovine serum albumin complex (2 ml) was added to 1 ml of myelin membrane vesicles (25 mg protein/ml) in 1 mM phosphate buffer, pH 8.0. The mixture was gently stirred for 18 h at 4°C. Bovine serum albumin and excess spin label were removed by centrifugation on sucrose step gradients (0.15 M, 0.23 M and 0.85 M sucrose in 1 mM phosphate buffer, pH 8.0). The myelin membrane vesicles banding on the 0.85 M sucrose cushion was collected, diluted 5-fold with phosphate buffer and concentrated by centrifugation (25 mg protein/ml final concentration). After the addition of different NaCl amounts and equilibration at these ionic strengths for 1 h, the material was transferred to 50 μl capillary tubes for ESR measurements. Spectra were recorded at 9.3 GHz and 20°C on a Varian E-9 spectrometer equipped with a temperature control accessory.

Iodination procedure

Lactoperoxidase-catalysed iodination of membrane proteins was performed essentially according to the procedure described for myelin by Poduslo et al. [23]. After the addition of different NaCl amounts, freshly prepared myelin membrane vesicles were equilibrated at these ionic strengths for 1 h at room temperature in 1 mM phosphate buffer, pH 8.0. The iodination reaction was conducted in 1.0 ml final volume containing 1.25 mg of membrane proteins, 0.6 μM lactoperoxydase (Boehringer), 0.13 mCi of carrier-free ^{125}I (IER, Würenlingen) and 50 μM NaI. The reaction was initiated by the addition of 10 μl of 1.0 mM H_2O_2 with similar additions at 15-s intervals and the reaction terminated after 5 min by the addition of a 10-fold excess of NaI. The suspension was then washed twice with phosphate buffer to remove excess radioactivity and aliquots were removed for analysis. The proteins were solubilized and separated by electrophoresis as indicated above. The distribution of radioactive iodide in the gel was determined by slicing the gels into 5-mm segments and counting the gamma emission of each slice. Control incubations performed under identical conditions, but detecting the lactoperoxidase, showed a negligable amount of nonspecific iodination.

Results

Electron microscopy

Our initial attention was directed towards establishing the vesicular character of our preparation. Examination of negatively-stained preparations by electron microscopy revealed a population of free or aggregated vesicles of varying sizes, mostly within the 200—300 nm range (Fig. 1). These myelin membrane vesicles are not permeated by the negative stain and appear to be bounded by a unilamellate structure, though a concentric multilamellar pattern was occasionally observed. No differences in morphology were noted between myelin membrane vesicles incubated in a low or a high ionic strength buffer. Negatively-stained preparations of purified myelin failed to show this typical vesicular structure but formed masses of amorphous electron dense material or entangled membrane-like structures.

Chemical characterization

In comparing the protein distribution for the original myelin with that for myelin membrane vesicles incubated in low or high ionic strength buffer as determined by Coomassie Blue staining of slab gels in SDS, no relevant difference in protein composition was observed.

The lipid composition of these three membrane preparations was analyzed by thin layer chromatography. There was no difference in the major phospho- and glycolipids visualized after separation in a chloroform/methanol/water

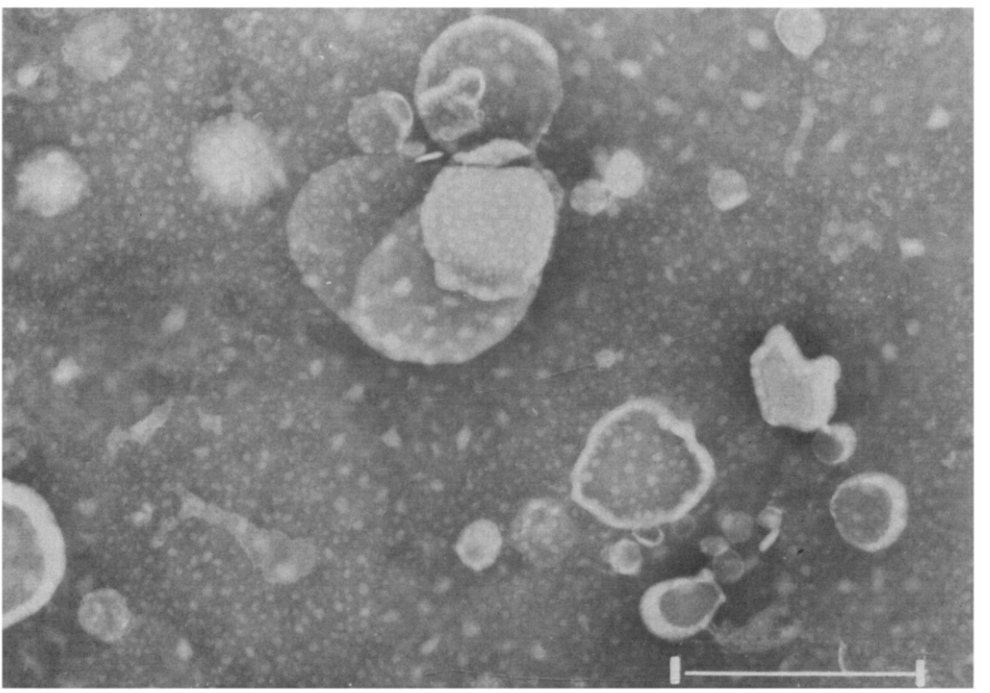


Fig. 1. Electron micrograph of myelin membrane vesicles incubated in 150 mM NaCl, 1 mM phosphate, pH 8.0, after negative staining with phosphotungstic acid. Bar is 0.5 μm.

(65 : 28 : 45, v/v) system. Furthermore, there was no evidence for the presence of cholesterol esters or free fatty acids in any of these membrane fractions analyzed by chromatography in a petroleum benzine/diethylether/acetic acid (180 : 20 : 2, v/v) system. The accumulation of these compounds in myelin has been reported to occur in conditions leading to a break-down of the membrane [24].

Permeability studies

The sodium permeability was studied by following the $^{22}Na^{+}$ leakage from washed myelin membrane vesicles. Experiments were performed in the presence of 0, 20, 50, 100 and 150 mM NaCl at 37°C (Fig. 2). There was a three times higher apparent leakage rate of $^{22}Na^{+}$ from myelin membrane vesicles incubated in a medium of low ionic strength, where about 25% of the $^{22}Na^{+}$ had diffused after 1 h, as compared to myelin membrane vesicles incubated in a medium of high strength, where only 8% of the $^{22}Na^{+}$ had diffused after 1 h. In all cases described in Fig. 2 the myelin membrane vesicles were preincubated for 1 h at 4°C with $^{22}Na^{+}$, then incubated for 40 min with various NaCl concentrations at 37°C, after which time the concentration of NaCl inside and outside

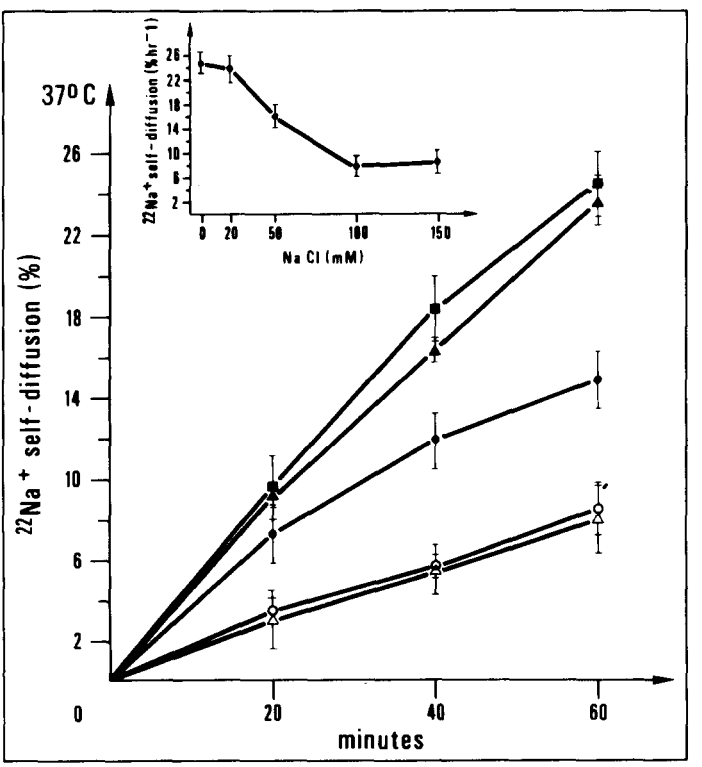


Fig. 2. Effect of increasing ionic strength on permeability of myelin membrane vesicles. The concentration of NaCl is as follows (mM): ■———■, 0; ▲———▲, 20; ●———●, 50; △———△, 100; ○———○, 150 mM. The data shown are the mean ±S.D. for 4 separate experiments. Measurements were performed as described in the text. The inset shows the NaCl concentration dependence of $^{22}Na^{+}$ permeability for 1 h values.

should be equilibrated. Under these conditions and assuming that $^{22}Na^+$ efflux follows simple diffusion laws, the leakage rate should not be affected by changes in the concentration of the NaCl buffer [25]. The exceptional behaviour of $^{22}Na^+$ efflux, with respect to increasing ionic strength, suggests a change in membrane properties resulting in a decrease in permeability.

Fig. 3 compares the effect of the incubation temperature on the $^{22}Na^+$ leakage. Increasing the incubation temperature from 4 to 37°C does not affect the permeability for myelin membrane vesicles incubated in a high ionic strength medium. A slight decrease in permeability is only observed for myelin membrane vesicles incubated in a low ionic strength medium. The $^{22}Na^+$ capture (a value representing the amount of $^{22}Na^+$ retained by the myelin membrane vesicles after successive washings) is almost identical for myelin membrane vesicles in low or high ionic strength medium incubated at 37°C, while this value is lower for both preparations at 4°C (data not shown). This fact raises the question of whether these membranes form incompletely sealed particles at low temperature.

Electron spin resonance studies

A representative ESR spectrum of spin label I [12,3] in myelin membrane

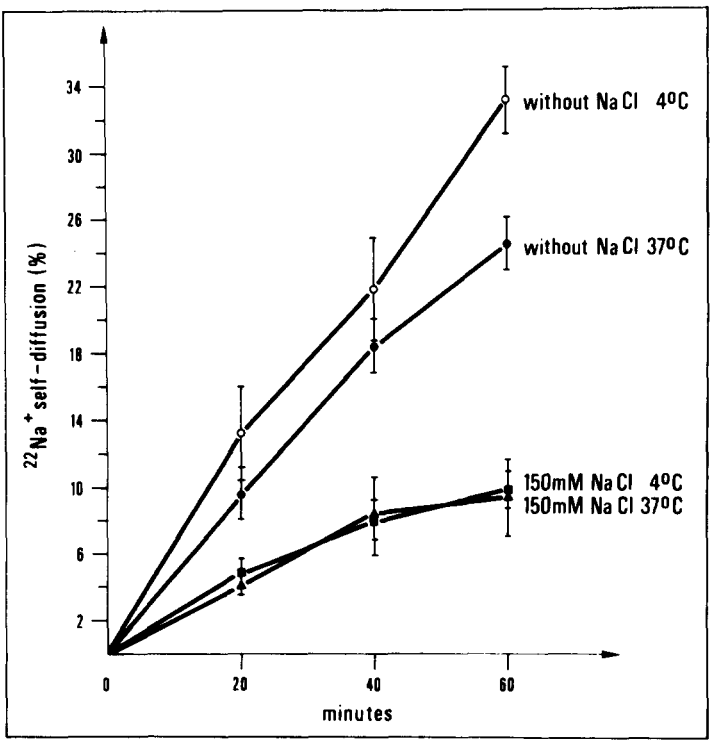


Fig. 3. Effect of loading conditions on permeability of myelin membrane vesicles. Following preincubation with $^{22}Na^+$ for 1 h at 4°C, myelin membrane vesicles were incubated in 1 mM phosphate, pH 8.0, without NaCl or with 150 mM NaCl for 40 min at 37 or 4°C. $^{22}Na^+$ self-diffusion was then measured according to the standard procedure at 37°C. The data shown are the mean ± S.D. for 4 separate experiments.

vesicles is shown in Fig. 4. Similar spectra have been obtained from biological membranes of various origins [22,26]. In all cases, the ESR spectra are characteristic of a rapid anisotropic motion of the spin-labeled fatty acid oriented perpendicularly to the membrane surfaces. It is, therefore, safe to assume that stearic acid spin label is incorporated into the lipid region and thus probes the physical structure of the bilayer. Based on this assumption this spin label was used to investigate possible perturbations in the bilayer structure of these myelin membrane vesicles as a function of changes in the ionic strength of the medium. ESR measurements of membrane vesicles with the spin label I [12,3] were carried out in the presence of 0, 20, 50, 80, 100, 150 and 300 mM NaCl at constant temperature (20°C) (Table I). The splitting between the extreme high- and low-field peaks ($\Delta(G)$ in Fig. 4 and Table I) is reproducibly increased by 5% from an ionic strength higher than 50 mM NaCl. An increase in the $\Delta(G)$ value indicates that the spin labels are located in a more immobilized (that is more rigid) environment in the myelin membrane.

Lactoperoxidase-catalyzed incorporation of iodide into surface proteins

The lactoperoxidase-iodination method involves the enzymatic modification of membrane proteins and catalyzes the iodination of tyrosine residues. Because the lactoperoxidase, a protein of molecular weight 78 000, is impermeant to the membrane, this reagent provides information on the surface accessibility of these specific amino acid residues. The distribution of radioactive iodine in the surface proteins of myelin membrane vesicles is shown in Fig. 5. The densitometric scan at 575 nm of a Coomassie Blue-stained polyacrylamide gel reveals the typical polypeptide chain pattern of myelin with the DM-20 protein showing as a fine band between the proteolipid protein and the basic pro-

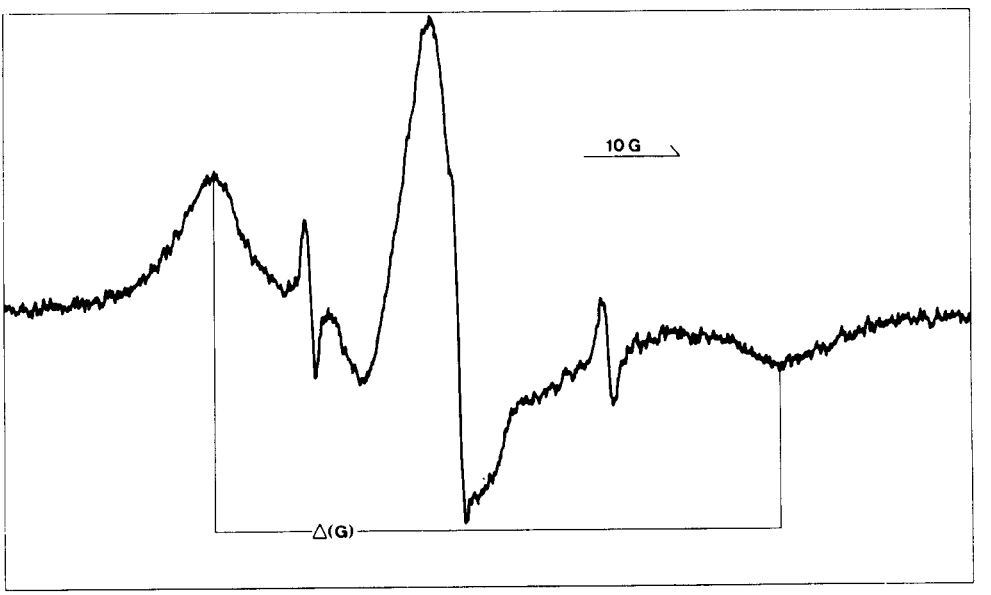


Fig. 4. Typical ESR spectrum of myelin membrane vesicles in 1 mM phosphate, pH 8.0, spin labelled with I [12,13]. The measurement of the $\Delta(G)$ value is indicated. G, Gauss.

TABLE I

Δ(G) VALUES OF SPIN LABEL I [12,13] INCORPORATED INTO MYELIN MEMBRANE VESICLES AS A FUNCTION OF NaCl CONCENTRATION

Values are the mean of 3 measurements ± S.E.

NaCl (mM)	Δ(G)
0	58.2 ± 0.8
20	58.3 ± 0.8
50	58.2 ± 0.8
80	61.8 ± 0.8
100	61.9 ± 0.7
150	62.1 ± 0.7
300	61.8 ± 0.7

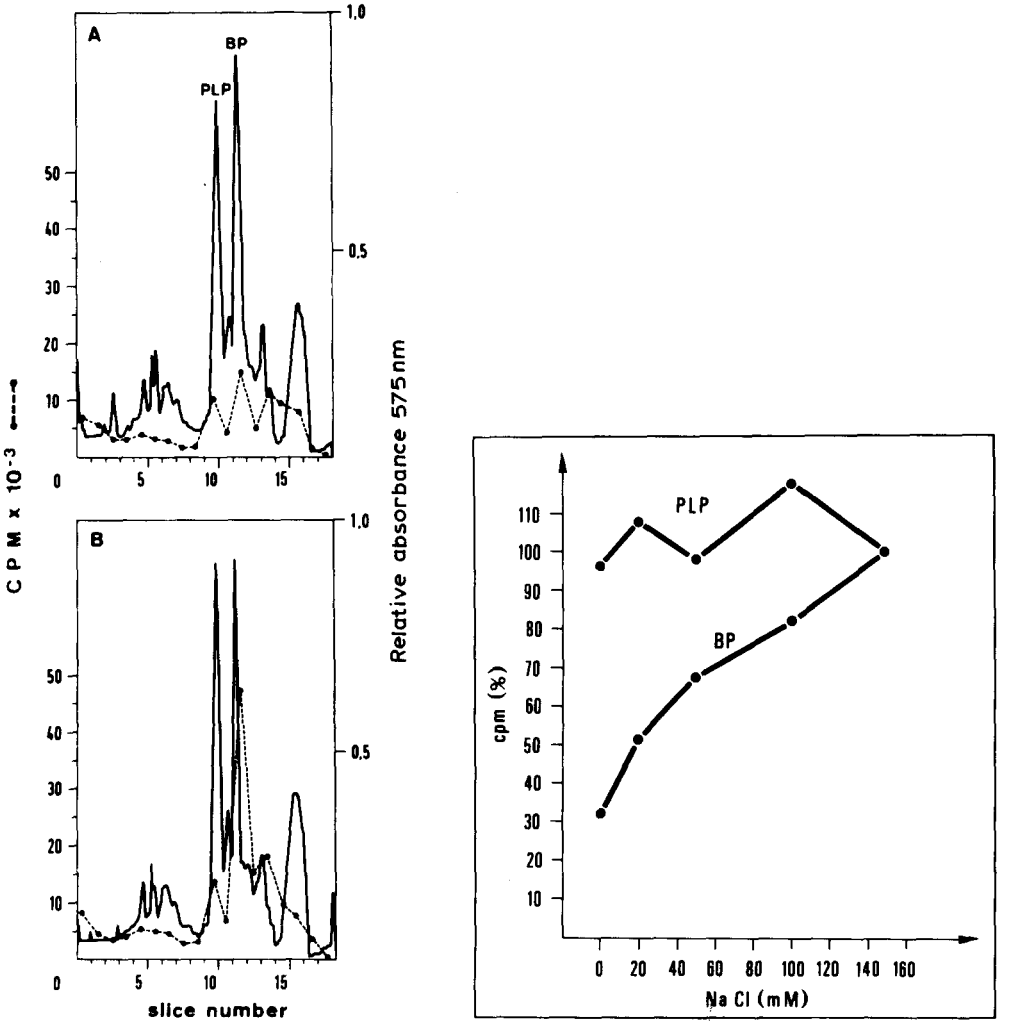


fig. 5. Comparison of labelling patterns (●·····●) of lactoperoxidase-iodinated myelin membrane vesicles incubated in 1 mM phosphate, pH 8.0, without (A) or with 150 mM NaCl (B). 100 μg of membrane proteins of each preparation were solubilized and subjected to electrophoresis according to the procedure described in Materials and Methods. PLP, proteolipid protein; BP, myelin basic protein.

Fig. 6. Comparison of the iodination of the myelin basic protein (BP) and the proteolipid protein (PLP) in myelin membrane vesicles incubated in a medium of increasing ionic strength. Experimental conditions are as described in Materials and Methods. Values expressed as percentage of activity in the presence of 100 mM NaCl. Results are the average of two separate experiments.

tein. We constantly observed a small additional peak (slice number 3 in Fig. 5) in the iodinated myelin membrane vesicles incubated in low ionic strength buffer. Separate experiments showed that this band corresponds to the lactoperoxidase which remains associated with the surface of the myelin membrane vesicles at low ionic strength, while this complex is dissociated when the ionic strength is increased. When considering the superimposed distribution profiles of iodine incorporation into the two different preparations, the obvious difference is an almost 4-fold increase in radioactivity in a peak which coincides with the myelin basic protein for myelin membrane vesicles incubated in high ionic strength buffer. The distribution of radioactivity of the remaining proteins, including the other major myelin protein, the proteolipid protein, is unchanged. Iodination was carried under conditions which have been shown to give maximal incorporation of the label into the myelin proteins [23]. Previous investigations have also shown that the incorporation of label was not affected by changes in the ionic strength of the iodinating medium [27]. As a measure of nonspecific iodination, these preparations were incubated in the absence of lactoperoxidase. When, in this case, the solubilized myelin proteins were separated by electrophoresis, almost no radioactive label was found associated with the membrane proteins, while on the other hand some label was found in a region of the gel preceding the myelin basic protein band, where it could represent iodination of lipids which are known to migrate as a lipid · SDS complex close to the gel front [23]. Myelin membrane vesicles were incubated in the presence of 0, 20, 50, 100 and 150 mM NaCl and the amount of iodine associated with the myelin basic protein and the proteolipid protein measured as the sum of the label incorporated into the corresponding protein peaks (Fig. 6). While there is an almost linear increase in the iodination of the myelin basic protein from 0 to 150 mM NaCl, the iodination of the proteolipid protein remains essentially unaffected by the change in the ionic strength.

Discussion

By adapting the techniques used for vesiculation of red cell membranes [11—13], it was possible to generate membrane vesicles from purified bovine brain myelin following a prolonged incubation in a low ionic strength alkaline buffer. This method takes advantage of the following well known facts: (a) in a medium of low ionic strength the myelin swells, yielding a population of mostly loose membranes [16]; (b) at this stage spontaneously formed vesicles are already observed [16,28]; (c) other isolated membranes also readily break down into vesicles under these ionic conditions [12,14,15]; (d) a slightly alkaline milieu (pH 8.0) appears to promote an inside-out reorientation of the membrane [12].

Vesicle formation would, therefore, first involve a breakdown of the membrane into small fragments which subsequently seal their torn edge or coalesce. We suspect that the prolonged incubation facilitates a splitting of the myelin membrane at the intraperiod line or the major dense line, but in the absence of mechanical forces (sonication) it is hard to imagine that the multilamellar myelin structure would lead uniformly to vesicular bilayers. Our data do not favour a mechanism of vesicle formation by budding or selective depletion of

membrane proteins, as has been shown for sheep erythrocyte ghosts [29], since we find no difference in the chemical composition between the myelin membrane vesicles and the parent myelin membrane. Furthermore these myelin membrane vesicles equilibrate as a single band in a continuous Ficoll gradient (data not shown), while membrane vesicles obtained from sheep erythrocyte ghosts separate into two bands in a density gradient as two different classes of membranes with a different protein to lipid ratio are being formed [29].

Our results suggest that the ionic strength is the critical variable affecting membrane permeability, while the rate of the resealing process, that is the closure of the myelin membrane vesicles, is strongly temperature dependent. This explanation is consistent with the fact that permeability measurements for myelin membrane vesicles incubated at 4 or 37°C are very similar (Fig. 3), while $^{22}Na^{+}$ capture, a value that is affected by the rate of vesicle closure, is much lower at 4°C. Such a temperature dependence of the resealing process has been demonstrated for erythrocyte membranes [30]. As additional evidence of the critical role of the ionic strength on the permeability characteristics of these myelin membrane vesicles are experiments where KCl was substituted for NaCl, causing a similar decrease in permeability of the membrane for $^{22}Na^{+}$ (data not shown). In view of the limited range of NaCl concentrations over which a dependence of the $^{22}Na^{+}$ efflux occurs (Fig. 2, inset), it is also rather unlikely that the observed changes in $^{22}Na^{+}$ diffusion result from an isotope dilution effect by NaCl.

Therefore, in an effort to correlate these permeability changes with membrane organizational alterations, we turned to additional techniques to probe the structure of these myelin membrane vesicles. ESR is a very sensitive method of measuring the physical properties of membranes [27,31]. The requirement of the correct membrane fluidity for different membrane functions has been amply demonstrated [32,33]. ESR measurements have shown characteristic difference in fluidity between various membranes; the most fluid was the mitochondrial membrane, the erythrocyte and synaptosomal membrane was of intermediate fluidity, while the myelin membrane had the most ordered structure [31]. Our results (Table I) show that this highly ordered structure of myelin remains unperturbated over a wide range of change in ionic strength, with a stepwise decrease in fluidity around 50 mM NaCl. Although these data show no evidence of a correlation between membrane fluidity and permeability changes, it is conceivable that the spin label could be insensitive to local changes in protein organization since it is a measure of the fluidity of the bulk of the lipids. Biological membranes are, however, separated into domains and it has been postulated that certain membrane proteins are surrounded by specific lipids and that such locales are responsive to changes in cations [34]. The strong dependence of the iodination of the myelin basic protein in membrane vesicles on the ionic environment (Fig. 6) suggests that additional tyrosine residues are exposed by increasing the ionic strength. This effect could result from an organizational change in the membrane or a conformational change in the protein. Recent experiments (Berger, J., Schaefer, R., Franklin, R.M., Steck, A.J. and Herschkowitz, N.N., manuscript submitted for publication) have demonstrated an unfolding of the myelin basic protein in the presence of increasing ionic strength.

Previous studies [5,6] as well as our own work [37] have demonstrated that myelin basic protein, a positively-charged protein with a p*I* of 10.8, shows a strong dependence on electrostatic binding for its interaction with myelin lipids. In a series of studies [38—40] on the interaction of basic proteins with phospholipid vesicles, Papahadjopoulos et al. have presented convincing evidence for a correlation between an increase in $^{22}Na^{+}$ permeability and the ability of the myelin basic protein to partly penetrate into the phospholipid bilayer. The permeability effects due to protein interaction, as well as its binding, were inhibited by using high ionic strength solutions. Because of the striking analogies between our data and those obtained by Papahadjopoulos et al. it is tempting to interpret the decrease in labelling of the myelin basic protein in membrane vesicles incubated in a medium of low ionic strength (Table I) to a partial penetration of the protein into the lipid bilayer, resulting in a local perturbation of the membrane and an increase in membrane permeability (Fig. 2). A recent study [41] on the localization of proteins in the myelin membrane using a non-penetrating radioactive label 4,4′-diisothiocyano-2,2′ditritiostilbene disulfonic acid also suggests a relatively small exposure of the myelin basic protein to the hydrophilic environment in a medium of low ionic strength.

The results presented in this paper extend earlier observations of the effect of basic proteins on the permeability properties of model membranes to a biological membrane. The idea that myelin basic protein, a phosphoprotein [1—3], serves as a physiological means of regulating membrane permeability at specific sites in the myelin membrane is a good possibility in view of the following considerations. There is both electrophysiological [8,9] and ultrastructural [10] evidence to suggest the existence in the paranodal region of myelin of ionic channels. The presence in myelin of Ca^{2+}-binding sites [42], as well as Ca^{2+} channels [43] has been reported. Furthermore, phosphoproteins have been implicated in the regulation of membrane permeability in different systems [4—7]. In this perspective the phosphorylation of the myelin basic protein by the myelin-associated protein kinase may be the critical mechanism by which membrane permeability is regulated in vivo. We are currently investigating this possibility.

Acknowledgments

The authors wish to thank Dr. C. Rothen for preparation of the electron micrographs and Dr. J. Seelig for helpful discussions. The technical assistance of Miss M. Bieri and the help of Miss I. Sutter for the preparation of the manuscipt are gratefully acknowledged. This investigation was supported in part by grants numbers 3.660-0.75 and 3.574.75 from the Swiss National Science Foundation.

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