ASSOCIATION OF PROTEOLIPID APOPROTEINS FROM BOVINE MYELIN WITH PHOSPHOLIPID IN BILAYER VESICLES

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Received 11 October 1977

1. Introduction

Myelin is a plasma membrane that possesses a limited range of functions. The major function of myelin is as an insulator around nerve fibres. Although once considered to be atypical, the structural organisation of myelin now seems to be more in keeping with the fluid-mosaic model of membrane structure [1,2].

Myelin contains two major protein fractions: the basic proteins and the proteolipid proteins. Proteolipids are lipoprotein complexes that may be extracted from a variety of tissues with mixtures of chloroform and methanol [3]; these proteins are particularly abundant in central nervous system myelin and may account for more than 50% of the total membrane protein. Chemical labelling studies on the intramembraneous localisation of myelin proteolipid proteins have indicated that they are embedded in the lipid matrix of the bilayer and partially exposed at the membrane surface [4,5]. Taken together with their hydrophobicity, it seems probable that these proteins are integral membrane proteins as defined by Singer and Nicolson [1]. Support for this view has come from the work of Moscarello's group [6]. They incorporated the proteolipid protein from human myelin, lipophilin, into lipid vesicles; upon freeze-fracturing, these vesicles showed particulate fracture faces. This is further evidence for the presence of the protein in the hydrocarbon core of the lipid bilayer.

As a preliminary to studies on lipid–protein interactions between isolated components of myelin and their relevance to myelin biogenesis, this communication describes the purification of three delipidated proteolipid apoproteins from bovine central nervous system myelin. The proteins were subsequently incorporated into phospholipid bilayer vesicles and they are shown, by freeze-fracture microscopy, to be fully embedded in the lipid bilayer.

2. Materials and methods

Myelin was prepared from the white matter of bovine brains by the procedure of Benjamins et al. [7]. After extensive washing with distilled water, the myelin was lyophilized and stored at −70°C. Myelin was extracted with chloroform–methanol (2:1, v/v) and the basic protein was sedimented from the extract as described by Greenfield et al. [8]. The basic protein was redissolved in 0.01 M HCl and purified further in the same medium by gel filtration on a Sephadex G-50 (Pharmacia Ltd) column (2.5 × 90 cm). The chloroform–methanol extract containing the proteolipid proteins was delipidated by gel filtration on a Sephadex LH-20 (Pharmacia Ltd) column (2.5 × 70 cm) in chloroform/methanol/0.01 M HCl (50:50:1, by vol.) [9]. This procedure was repeated to ensure complete delipidation of the proteolipid proteins as determined by thin-layer chromatography and phosphorus analysis [10]. After solubilisation in 0.5% sodium dodecyl sulphate (SDS), protein concentrations were measured by the method of Lowry [11]. Protein samples were prepared for electrophoresis by the method of Greenfield et al. [8]; molecular weights of the constituent polypeptides of all protein preparations were measured by SDS–polyacrylamide gel electrophoresis [12]. Amino acid analyses were performed on delipidated samples of proteolipid protein (2 mg) as described by Lumb et al. [13].

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Egg phosphatidylcholine (Makor Chemicals, Israel) and proteolipid apoproteins were dissolved in 2-chloroethanol–water (9:1, v/v) and mixed in the desired ratios; the mixtures were dialysed versus a buffer of pH 7.4 that contained 2 mM Hepes (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulphonic acid), 2 mM histidine, 10 mM NaCl and 0.1 mM EDTA as described by Boggs et al. [6]. After dialysis at 4°C overnight, the lipoprotein suspensions were purified by sucrose density-gradient centrifugation [6]. Lipoprotein suspensions that contained between 30% and 40% protein by weight formed a narrow, distinct band at the interface between 20% (w/v) and 40% (w/v) sucrose solutions. When brominated egg phosphatidylcholine [14] was substituted for egg phosphatidylcholine, the procedure was identical except that the lipoprotein suspension was collected as a pellet after sucrose density-gradient centrifugation. Freeze-fracturing and electron microscopy of samples was as described [15].

3. Results and discussion

3.1. *Characterization of proteolipid apoproteins*

Phosphorus which remained associated with the protein preparation amounted to 0.08% by weight. This value is within the range quoted by other authors [16,17] and shows that the proteolipid proteins were delipidated quite thoroughly by gel filtration. The amino acid composition of the preparation was substantially similar to those already reported [16,17]; a high proportion of nonpolar amino acids is characteristic of this class of proteins. Interestingly, a polarity index of 40%, which was calculated from the amino acid composition, is consistent with the view that proteolipid proteins are integral membrane proteins [18].

Analysis of the proteolipid apoproteins by SDS–polyacrylamide gel electrophoresis, as shown in fig.1, demonstrated three major polypeptides. Samples of total myelin proteins and purified basic proteins were electrophoresed at the same time for the purposes of comparison. The proteolipid apoprotein with highest mobility has mol. wt approx. 12 000. This protein (P-12 in fig.1) has been demonstrated before in myelin from bovine brain [19] but is believed to be dialysable and consequently lost if delipidation is accomplished

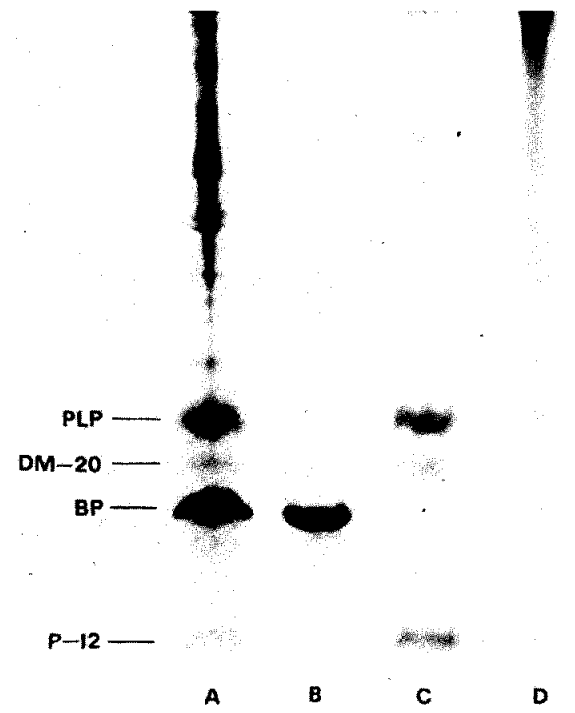
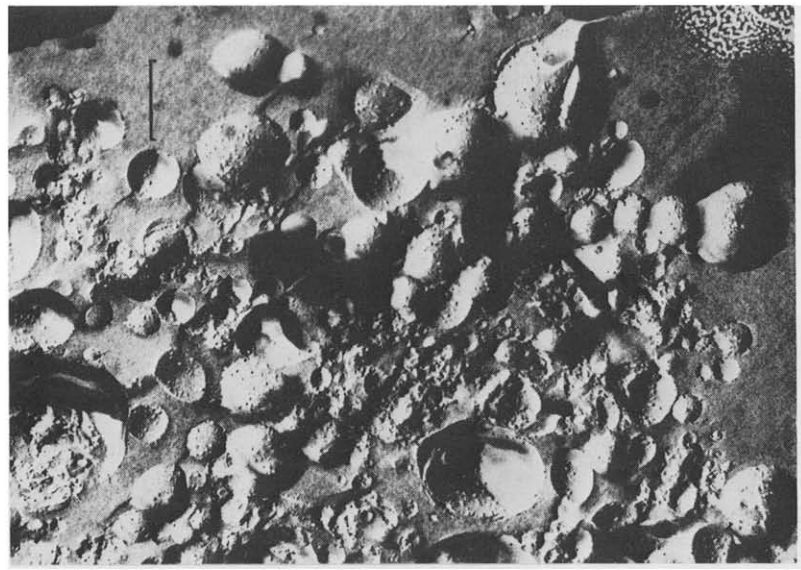


Fig.1. Polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS) of myelin proteins. Electrophoresis was carried out in a polyacrylamide slab gel 1.2 mm in thickness; the stacking gel (not shown) was 6% acrylamide and the running gel contained 15% acrylamide. After fixation in water/isopropanol/acetic acid (65:25:10, by vol.), the proteins were stained with 0.2% (w/v) Coomassie Brilliant Blue in water/isopropanol/acetic acid (80:10:10, by vol.). (A) Bovine myelin (20 μg protein); (B) basic protein (25 μg protein); (C) proteolipid apoproteins (12 μg of protein); (D) proteolipid apoproteins in association with phosphatidylcholine after purification of vesicles by density-gradient centrifugation (10 μg protein). *Abbreviations:* PLP, proteolipid protein; DM-20, [20]; BP, basic protein; P-12, proteolipid protein of 12 000 daltons.

by extensive dialysis. Agrawal's group have observed a protein of 20 540 daltons which they call the DM-20 protein in myelin from various sources, including beef brain [20]. This DM-20 protein can be seen in fig.1 both as a component of the total myelin proteins and as a constituent of the proteolipid apoprotein sample. The third apoprotein has mol. wt 23 500 and is the classical Folch-Lees proteolipid protein (PLP). Owing to their common solubility in organic solvent,

(a)



(b)

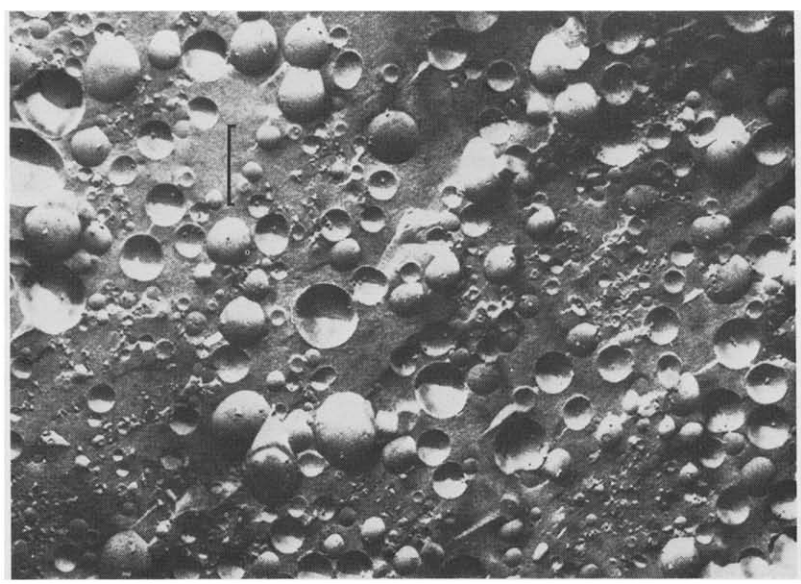


Fig.2. Freeze-fracture electron micrographs of (a) phosphatidylcholine–proteolipid apoprotein, and (b) brominated phosphatidylcholine–proteolipid apoprotein vesicles. In (a) the vesicles contained 37% protein by weight and in (b) they contained 32% proteir by weight. The bar represents 250 nm.

it seems probable that all three proteins have very similar physical properties.

3.2. *Characterization of lipoprotein vesicles*

In the absence of protein, dialysis of phosphatidylcholine from 2-chloro-ethanol resulted in the formation of multi-lamellar structures that precipitated in the dialysis bag. By contrast, when proteolipid apoproteins were included an opalescent lipoprotein suspension formed. Substitution of native egg phosphatidylcholine with brominated phosphatidylcholine caused the suspensions to appear somewhat clearer. Brominated phosphatidylcholine was used in reconstitution so that a population of very dense vesicles could be prepared. Studies with these vesicles will be reported elsewhere. After purification of the lipoprotein suspensions by sucrose density-gradient centrifugation the ratio of protein : lipid usually agreed quite well with that in the original mixture, and the recoveries of protein and lipid were in the range of 65–75%. Figure 2 shows that the association of phospholipid and protein during dialysis results in the formation of single-shelled vesicles. A comparison of figs 2(a) and 2(b) provides a clue as to why those suspensions containing brominated phosphatidylcholine should be clearer. These vesicles are of a generally smaller and more uniform diameter (100–200 nm) than those which contained native egg phosphatidylcholine (100–250 nm). This difference did not appear to be affected by changes in the phospholipid : protein ratio. A study of the influence of phospholipid composition on the morphology of the resulting vesicles is currently underway.

An examination of the fracture faces of the vesicles in fig.2(a) clearly shows the presence of many particles. This has also been observed for vesicles containing human myelin proteolipid protein [6]. The particles are considered to reflect the presence of protein in the hydrocarbon core of the lipid bilayer and support the notion that the bovine proteolipid apoproteins are fully incorporated into the lipid of these vesicles. SDS–Polyacrylamide gel electrophoresis of a sample of vesicles containing phosphatidylcholine and 40% by weight of proteolipid apoproteins is shown in fig.1, lane D. Although there is a trace of the PLP and P-12 proteins, it is evident that extensive aggregation of the proteolipid apoproteins has occurred.

In summary it appears that these vesicles may be valuable tools for a study of the types of lipid with which proteolipid apoproteins interact; furthermore it will be of interest to see how these lipids determine the morphology of the resulting vesicular structures.

Acknowledgements

I wish to thank Professor L. L. M. van Deenen for the hospitality shown during my stay in Utrecht. This work was done during the tenure of a Studentship from the Multiple Sclerosis Society of Canada in the Laboratory of Biochemistry, State University of Utrecht, The Netherlands. Dr A. J. Verkleij is thanked for performing the electron microscopy.

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