Localization of Sites for Ionic Interaction with Lipid in the C-Terminal Third of the Bovine Myelin Basic Protein

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The myelin basic protein from bovine brain tissue was purified and the two peptides obtained by cleavage of the polypeptide chain at the single tryptophan residue were isolated. The interaction of these peptides and the intact basic protein with complex lipids was investigated by following the solubilization of lipid–protein complexes into chloroform in a biphasic solvent system. The C-terminal peptide fragment (residues 117–170) and the intact basic protein both formed chloroform-soluble complexes with acidic lipids, but not with neutral complex lipids. The N-terminal fragment (residues 1–115) did not form chloroform-soluble complexes with either acidic or neutral complex lipids. The molar ratio of lipid to protein that caused a 50% loss of protein from the upper phase to the lower chloroform phase was the same for the intact basic protein as for the smaller C-terminal peptide fragment. Phosphatidylserine and phosphatidylinositol were approximately twice as efficient as sulphatide at causing protein redistribution to the chloroform phase. The results are interpreted as indicating that the sites for ionic interactions between lipid and charged groups on the basic protein of myelin are located in the C-terminal region of the protein molecule.

Although the myelin basic protein has little or no classical α - or β - structure in aqueous media, except in the presence of lipids or detergents (Palmer & Dawson, 1969; Chao & Einstein, 1970; Eylar & Thompson, 1969; Kornguth & Perrin, 1971; Anthony & Moscarello, 1971), there appears, nonetheless, to be a specific and folded conformation (Erand *et al.*, 1974; Whitaker *et al.*, 1975). Proton-magnetic-resonance studies with the basic protein indicate that there is little, if any, restriction of the motion of the residue side chains (Liebes *et al.*, 1975), whereas fluorescence studies (Jones & Rumsby, 1975) indicate that the tyrosine residues and the single tryptophan residue are largely exposed to the aqueous environment surrounding the protein molecule. The physical and chemical properties of the myelin basic protein have been comprehensively reviewed (Carnegie & Dunkley, 1975).

The myelin basic protein interacts specifically with acidic lipids (Banik & Davison, 1974; Palmer & Dawson, 1969). Further detailed studies indicate that the N-terminal region of the basic protein (residues 20–113) interacts in a different manner compared with the rest of the protein molecule (Gould & London, 1972; Demel *et al.*, 1973; London & Vossenberg, 1973; London *et al.*, 1973). The interaction of the basic protein with lipids has been reviewed by Rumsby & Crang (1977) in relation to the molecular architecture of the myelin sheath.

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To define further how sites for ionic interaction with lipid are distributed on the basic-protein molecule we have purified the two peptide fragments liberated by BNPS-skatole [a bromine adduct of 2-(2-nitrobenzenesulphenyl)-3-methylindole] cleavage of the basic protein at the single tryptophan residue. The interaction of these two peptide fragments with complex lipids of defined fatty acid composition has been compared with that of the basic protein in a two-phase solvent system.

Materials and Methods

Myelin, purified from bovine brain white matter (Rumsby *et al.*, 1970), was used for the isolation of the basic protein by the method of Banik & Davison (1973). Further purification of the basic-protein preparation by exclusion chromatography on Sephadex G-75 [superfine grade; Pharmacia (G.B.) Ltd., London W5 5SS, U.K.] eluted with 0.01 M-HCl (column dimensions 2.5 cm $\times$ 90 cm) removed high-molecular-weight contamination as assessed by the elution profile and polyacrylamide-gel electrophoresis. Cleavage of the purified basic protein at the tryptophan residue was accomplished by using BNPS-skatole (Pierce Chemical Co., Rockford, IL, U.S.A.) under conditions described by Martenson *et al.* (1975). Reaction products were separated on the same Sephadex G-75 exclusion column. Fractions containing the peptide fragments were pooled and freeze-dried. Regeneration of the methionine side

chains was accomplished by using 10% (w/v) thioglycollic acid (Martenson *et al.*, 1975). The individual peptide fragments from the myelin basic protein were further purified by repeated gel-exclusion chromatography on the same Sephadex G-75 (superfine grade) column. Fractions containing the peptides were pooled and concentrated in an Amicon ultrafiltration cell by using a PM-2 membrane. The peptide fragments were stored in 10 mM-Tris/HCl, pH 7.0 at 4°C.

Phosphatidylserine and sphingomyelin from ox brain and phosphatidylinositol from wheat germ were Grade 1 from Lipid Products (Redhill, Surrey, U.K.). Phosphatidylcholine, cerebroside and sulphatide were prepared from an acetone-dried powder of bovine brain tissue by extraction with chloroform/methanol (2:1, v/v), followed by separation of the lipids on silicic acid and DEAE-cellulose (acetate form) as described by Marinetti (1967). The purity of each lipid fraction was checked by t.l.c. in Kieselgel H (E. Merck, Darmstadt, W. Germany) in two different solvent systems. Phospholipids were better than 99% pure, whereas the sulphatide contained about 1% cerebroside. The cerebroside sample contained an estimated 4% contamination, chiefly cholesterol. Fatty acid methyl esters from each lipid class were prepared as described by Morrison & Smith (1964) and were analysed by g.l.c. in a Hewlett-Packard 5750 chromatograph on a 2.1 m × 6 mm (6 ft × ¼ in external diam.) glass column packed with 5% (w/v) EGSS-X coated on Gas-Chrom Z (Applied Science

Laboratories, State College, PA, U.S.A.) operated at 170°C.

Amino acid analyses and fluorescence measurements were performed as described elsewhere (Jones & Rumsby, 1975). The tryptophan content of the basic protein was determined spectrophotometrically (Beaven & Holiday, 1952). Phospholipid analysis was by the method of Bartlett (1959). Glycolipids were assayed by the method of Hess & Lewin (1965) or gravimetrically. Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin [crystalline; Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K.] as standard.

For a two-phase system to study lipid interaction with protein (Palmer & Dawson, 1969; Banik & Davison, 1974) samples of lipid (up to 400 µg) were evaporated under N₂ in stoppered test tubes. Chloroform/methanol (2:1, v/v; 1.0 ml) was added to each tube, followed by 0.25 ml of 10 mM-Tris/HCl buffer, pH 7.0, which contained about 100 µg of protein. The contents were emulsified on a Vortex mixer. Tubes were then left at room temperature (20°C) for 15 min, then remixed and further left for 45 min. After a final mixing the phases were separated by centrifugation (1000g for 10 min). The volumetric reproducibility of the system was ±1%. For each lipid/protein ratio duplicate tubes were taken through the experiment together with blanks with (1) no protein and (2) no lipid. Upper and lower phases were analysed in duplicate for lipid and protein.

Table 1. *Amino acid composition of the bovine myelin basic protein and of BNPS-skatole-cleavage products peptides II and III*

The myelin basic protein and its BNPS-skatole-cleavage products were isolated and purified as described in the Materials and Methods section. Amino acid analyses were performed as described elsewhere (Jones & Rumsby, 1975). Tryptophan was assayed spectrophotometrically (Beaven & Holiday, 1952). Amino acid data for the myelin basic protein are compared with results reported by Carnegie & Dunkley (1975). All results are expressed as mol% and are means from two separate analyses.

Amino acid	Myelin basic protein		Peptide II		Peptide III	
	This study	Carnegie & Dunkley (1975)	Found	Predicted	Found	Predicted
Aspartic acid	6.5	6.5	7.98	7.0	7.16	5.6
Threonine	4.7	4.1	5.21	5.2	2.58	1.9
Serine	10.0	10.6	12.17	10.4	11.90	11.1
Glutamic acid	6.5	5.9	6.78	6.1	5.98	5.6
Proline	7.6	7.1	9.59	8.7	5.16	3.7
Glycine	14.0	14.7	12.80	11.3	20.82	22.2
Alanine	8.2	8.2	7.64	7.8	9.42	9.3
Cysteine	0.0	0.0	0.0	0.0	0.0	0.0
Valine	1.8	1.8	2.94	2.6	0.0	0.0
Methionine	1.2	1.2	0.89	0.9	2.05	1.9
Isoleucine	1.8	1.8	1.10	1.7	1.95	1.9
Leucine	5.9	5.9	4.74	6.1	5.60	5.6
Tyrosine	2.4	2.4	1.42	1.7	3.42	3.7
Phenylalanine	4.7	4.7	4.09	5.2	2.66	3.7
Histidine	5.9	5.9	7.14	7.0	3.76	3.7
Lysine	7.6	7.6	5.70	6.1	9.49	11.1
Arginine	10.6	10.6	9.84	11.3	8.05	9.3
Tryptophan	0.6	0.6	0.0	0.0	0.0	0.0

For lipid analysis samples were dried down *in vacuo*. For protein analysis of the lower phase, samples were dried down *in vacuo* and were then reconstituted with synthetic upper phase. For such measurements the protein standard was albumin in synthetic upper phase.

Analytical polyacrylamide-gel electrophoresis at pH 4.5 was performed essentially as described by Reisfeld *et al.* (1964). Phenol/acetic acid/urea/polyacrylamide-gel electrophoresis was performed by using the system of Takayama *et al.* (1964). Chloroform was freshly distilled from glass, and all other chemicals, except where indicated, were of analytical grade.

Fig. 1. Purification of cleavage products liberated from the myelin basic protein by treatment with BNPS-skatole. Myelin basic protein was treated with BNPS-skatole as described in the Materials and Methods section. Hydrolysis products were applied to a Sephadex G-75 (superfine grade) gel-exclusion column (2.5 cm $\times$ 90 cm) eluted with 0.01 M-HCl at 9 ml/h; 4 ml fractions were collected. Peaks shown as I, II and III are unhydrolysed basic protein, the N-terminal and C-terminal cleavage fragments respectively. Peaks II and III account for about 74% of the material applied to the column. Material eluted before the intact basic protein is considered to be oligomeric aggregation material, perhaps formed during freeze-drying or the cleavage reaction. Lines under peaks indicate the range of fractions pooled for freeze-drying and for subsequent electrophoresis and, for peaks II and III, repurification.

Results

Gel-electrophoretic analysis of the basic-protein preparation prepared from myelin by the procedure of Banik & Davison (1973) revealed the presence of some minor high-molecular-weight components as contamination. These were resolved from the main basic-protein fraction by gel-exclusion chromatography on Sephadex G-75 (superfine grade) to yield a preparation of the basic protein, which showed only a single band on gel electrophoresis. The corrected amino acid composition of the basic protein prepared in this way is given in Table 1, and results for the same protein reported by Carnegie & Dunkley (1975) are included for comparison.

Products from the BNPS-skatole cleavage of the myelin basic protein were initially resolved on Sephadex G-75 (superfine grade), giving the elution profile shown in Fig. 1. Gel electrophoresis of the peak fractions simultaneously with hydrolysis mixture and intact basic protein (Fig. 2) indicated that peak I was unhydrolysed basic protein (Fig. 1), and peaks II and III, of lower molecular weight than the intact basic protein, were likely to be the N-terminal and the C-terminal peptide fragments derived from the basic protein by BNPS-skatole cleavage of the polypeptide chain at the single tryptophan residue, 116. Material eluted before the basic protein (Fig. 1) possibly represents oligomers of fragments produced during freeze-drying (Martenson *et al.*, 1975).

Fig. 2. Electrophoretic analysis of fractions from the gel-exclusion-column separation of BNPS-skatole-cleavage products of the myelin basic protein.

Electrophoresis was on 7.5% polyacrylamide gels at pH 4.5 as described in the Materials and Methods section. Fractions 34 (a), 41 (b), 56 (c), 69 (d) and 83 (e) from the gel-exclusion-column profile shown in Fig. 1 were analysed together with the total hydrolysis mixture (f and g, 10 and 20 μ l respectively) and the initial purified intact myelin basic protein (h). ■, Strong staining; ▨, weak staining.

Fig. 3. Repurification of peptide fragments II and III by gel-exclusion chromatography

Peptide fragments II and III from the exclusion-column separation shown in Fig. 1 were repurified on Sephadex G-75 (superfine grade) as described in the Materials and Methods section in Fig. 1. Rechromatography of peptide II is shown in (a) and of peptide fragment III in (b). The material eluted immediately after the peptide peaks is the thioglycollic acid used to regenerate methionine residues. Bar lines under each peak indicate the range of fractions pooled to obtain the peptide for subsequent use. Column details and fraction size are as in Fig. 1.

Together peaks II and III accounted for some 74% of the hydrolysis mixture applied to the column. Peaks II and III were separately pooled and rechromatographed on the same column (Figs. 3a and 3b). In each separation the main peak was pooled as indicated and concentrated in 10mM-Tris/HCl, pH7.0, by ultrafiltration. Absorption spectra of the purified peptides II and III confirmed the absence of tryptophan residues. Phenylalanine-structured absorption superimposed on the tyrosine absorption band was detected and was more pronounced in spectra from peptide II, which has six phenylalanine residues per molecule, compared with two in peptide III. Both peptide fragments have two tyrosine residues per molecule, and fluorescence-emission spectra of the two peptides showed single peaks at 302nm which were indistinguishable from that of free tyrosine. The amino acid composition of peptides II and III is recorded in Table 1 compared with predicted values obtained from sequence data.

Recovery of protein in the two-phase solvent system after equilibration averaged 100% (93–103%). Results of protein interaction with lipid are pre-

sented (Fig. 4) as percentage of total recovered protein remaining in the upper phase against the lipid/protein molar ratio. Recovery of phospholipid was 100% within experimental error and was always in the lower phase, as was glycolipid.

With sphingomyelin and cerebroside in the two-phase system there was no detectable redistribution of intact basic protein or either peptide fragment II or III to the chloroform phase at any lipid/protein molar ratio studied. For sphingomyelin these were basic protein 47:1, peptide II 58:1 and peptide III 30:1. For cerebroside the ratios were basic protein 114:1, peptide II 89:1 and peptide III 51:1. With phosphatidylcholine there was a slight redistribution of the intact basic protein and peptide III to the chloroform phase (Fig. 4a), but this was only at high molar ratios of lipid/protein. Peptide II showed no redistribution with this lipid.

There was a marked redistribution of protein to the chloroform phase with the acidic lipids phosphatidylinositol, phosphatidylserine and cerebroside sulphate (Fig. 4). The most significant finding was that only the intact basic protein and peptide fragment III

Fig. 4. Interaction of the myelin basic protein and peptide fragments II and III with different complex lipids

Conditions of reaction in the two-phase system are as described in the Materials and Methods section. Results shown are for the interaction of the intact myelin basic protein (▲), peptide II, the N-terminal fragment (●), and peptide III, the C-terminal fragment (■), with phosphatidylcholine (a), phosphatidylinositol (b), phosphatidylserine (c) and sulphatide (d) over a range of lipid/protein molar ratios. Points shown are the means of results from two separate experiments.

were carried into the chloroform phase by interaction with these acidic lipids. Peptide fragment II was not solubilized into the lower layer at any lipid/protein molar ratio with cerebroside sulphate, phosphatidylserine or phosphatidylinositol. Peptide fragment III was solubilized into the chloroform phase to the same extent as the intact basic protein molecule at a given lipid/protein ratio with all the acidic lipids used in the experiments. For the two glycerophosphatides phosphatidylserine and phosphatidylinositol a 50% loss of protein from the upper phase occurred at approx. 10–11 mol of lipid/mol of basic protein or peptide fragment III. For cerebroside sulphate the corresponding value was approximately twice this, being 22 for the intact basic protein and 20 for fragment III.

Discussion

Martenson *et al.* (1975) resolved the components of BNPS-skatole cleavage of the myelin basic protein

by passage through Sephadex G-100 and repurified the peptide fragments by repeated chromatography on Sephadex G-50 (fine grade). In the present study a single column of Sephadex G-75 (superfine grade) was found to be more satisfactory and served for both resolution of the initial hydrolysis mixture and repurification of peptides. A finer resolution of unhydrolysed basic protein and peptide fragments in the hydrolysis mixture was achieved on Sephadex G-75, and this meant that repurification of the peptides was achieved in a single rechromatography step on the same column to give peptide products which were homogeneous by electrophoresis and which, from amino acid, electrophoretic, chromatographic and spectroscopic criteria, were identified as residues 1–115 (N-terminal peptide fragment II) and 117–170 (C-terminal peptide fragment III) of the myelin basic protein. Methionine residues were successfully regenerated by treatment with thiolcolic acid.

The specific interaction of the myelin basic protein with acidic lipids reported by Palmer & Dawson (1969) and Banik & Davison (1974) has been confirmed in the present study. The observation that neither peptide fragment showed any interaction with complex lipids such as cerebroside, sphingomyelin and phosphatidylcholine is not surprising, since this is also the case for the intact basic protein (Demel *et al.*, 1973; the present work). However, the contrasting interaction of the two peptide fragments with acidic lipids is of considerable interest in relation to the structuring of lipid-interacting groups along the intact basic-protein molecule. The finding that of the peptide fragments, only peptide III (C-terminal region, residues 117–170) interacted with the acidic lipids and that its 50% solubilization ratio was essentially the same as that of the intact basic-protein molecule leads us to conclude that the sites for ionic interaction with lipid reside chiefly in the C-terminal section of the protein.

In the two-phase system protein becomes solubilized into the chloroform layer after ionic interaction with lipid, which results in the formation of a lipid-protein complex of increased hydrophobic nature. The converse, i.e. hydrophobic interaction leading to the production of water-soluble lipid, was never detected. Such interactions may not be stable in the upper phase of the system, which contains approx. 50% (v/v) methanol. It may be therefore that the two-phase system only detects strong ionic interactions between lipid and protein. However, if this were the case we would expect that the number of positive charges on each peptide fragment and on the intact protein would determine the extent of lipid binding. The molar ratios of lipid to protein required for solubilization would then decrease in the order basic protein > fragment II > fragment III, since the number of basic amino acid residues

(lysine, arginine and histidine) per molecule decreases in the same order. The results, however, show that the actual order is basic protein $\approx$ fragment III $\ll$ fragment II. Peptide fragment III, with less basic residues, interacts with acidic lipids comparably with the whole basic-protein molecule and much better than peptide II, which has more basic residues. Thus the interaction between protein and lipids in the two-phase system cannot be solely ascribed to ionic effects operating between lipid and protein. Other factors, such as the fatty acid composition of the lipids (Table 2), the nature and orientation of lipid head groups, and especially the conformation of the basic-protein molecule and the two peptides, must be involved in bringing about redistribution in the two-phase system.

The various lipids used in the present investigation differed markedly in their fatty acid composition (Table 2). Yet, even though such differences will affect the fluidity characteristics of the individual lipids in water, they clearly do not play a significant role in protein redistribution to the chloroform layer. The different fatty acyl chains will all be highly soluble in the solvent phase, as is shown by the fact that lipid was never detected in the upper layer of the two-phase system. Similarities in fatty acid composition between comparable acidic and neutral complex lipids (e.g. sulphatide and cerebroside) show that ionic interactions between lipid and protein are at the basis of the redistribution of peptide III and the intact basic protein to the chloroform layer.

The conformation of the proteins will be a principal factor influencing interaction with lipid. Current views suggest that the myelin basic protein has an open but ordered structure (Epand *et al.*, 1974; Whitaker *et al.*, 1975) and that the protein can interact with lipids in two distinct ways, the regions on the protein molecule responsible for such inter-

Table 2. *Fatty acid composition of complex lipids interacted with the myelin basic protein*

Lipids were obtained as stated in the Materials and Methods section. Methyl esters of fatty acids were prepared by using BF_3 /methanol (Morrison & Smith, 1964) and were separated by g.l.c. on a 5% EGSS-X column operated at 170°C. Individual methyl esters are quoted as percentage of the total fatty acids between $\text{C}_{16:0}$ and $\text{C}_{26:1}$. Only major fatty acid components are given.

Fatty acid	Phosphatidyl-serine	Phosphatidyl-choline	Phosphatidyl-inositol	Sphingomyelin	Cerebroside	Sulphatide
$\text{C}_{16:0}$	2.8	47.5	38.7	3.4	4.9	5.5
$\text{C}_{18:0}$	34.2	12.3	—	42.6	12.2	8.5
$\text{C}_{18:1}$	35.1	32.5	7.5	—	5.2	6.2
$\text{C}_{18:2}$	—	—	47.5	—	—	—
$\text{C}_{20:0}$	—	—	5.7	2.7	—	—
$\text{C}_{22:0}$	1.5	—	—	6.2	3.1	3.0
$\text{C}_{22:1}$	1.4	—	—	2.5	1.5	—
$\text{C}_{24:0}$	3.9	—	—	3.2	12.7	16.9
$\text{C}_{24:1}$	11.4	—	—	34.2	43.7	40.1
$\text{C}_{26:0}$	—	—	—	—	—	1.0
$\text{C}_{26:1}$	—	—	—	—	4.8	5.4
Other	9.7	7.7	1.2	5.2	11.9	13.4

actions being separated (Demel *et al.*, 1973; London *et al.*, 1973; London & Vossenberg, 1973). The absence of detectable ionic interaction with lipid observed for the *N*-terminal peptide fragment in the

present study suggests that the conformation of this part of the basic protein may be ordered so that ionic interactions with lipid are specifically hindered. This could arise by intramolecular interactions

Fig. 5. Detailed interactions of the myelin basic protein with acidic lipids in central nerve tissue

Regions of the *N*- and *C*-terminal chains of the myelin basic protein located at the cytoplasmic apposition region (main dense line) in the myelin sheath are shown. All ionic interactions with lipid are localized along the *C*-terminal section of the protein. Acidic glycerophospholipids such as phosphatidylserine (PS) and phosphatidylinositol (PI) from one lipid face at the cytoplasmic apposition interact through their negatively charged phosphate moieties with basic amino acid residues on the basic protein such as arginine-130 and lysine-139. Sulphatide (Su) from the other lipid face at the apposition is positioned with its hydrophilic/hydrophobic boundary at the *N*-terminal region of the basic protein, where the possibility for hydrogen-bonding with the protein chain would exist. In this position sulphatide head groups in an extended configuration reach across the width of the basic protein (some 15nm) to interact with basic amino acid residues such as lysine-135 and lysine-152, again along the *C*-terminal section of the basic protein. Orientation of sulphatide in this way allows for the penetration of discrete non-polar sections of the *N*-terminal chain of the basic protein into the lipid phase. The region shown penetrating in this way is one of several sections of the basic protein that are protected from proteolysis on interaction with acidic lipid (London & Vossenberg, 1973; London *et al.*, 1973). Dotted sections of the *C*-terminal region of the basic protein are where parts of the polypeptide chain have been omitted for clarity. ○, Oxygen atoms; ●, carbon atoms.

between acidic and basic residues in the polypeptide chain. Such an arrangement could lead to an emphasis on hydrophobic interactions with lipid in this region of the myelin basic protein, as has been suggested by the protection studies of London *et al.* (1973) and London & Vossenberg (1973). The finding that lipid did not redistribute to the upper phase, even with peptide fragment II, in which hydrophobic interactions are perhaps emphasized, may be explained by the fact that such interactions are not maintained in the methanol/water mixture in the upper phase or that such hydrophobic interactions with lipid are only maximized when the lipid is in a highly ordered monolayer or bilayer state.

It is noteworthy that sulphatide was only half as effective as acidic glycerophosphatides at causing the redistribution of intact myelin basic protein and peptide III to the chloroform phase, especially since the sulphate group in such lipids is fully ionized above pH 3.5 (Quinn & Sherman, 1971) and is more strongly acidic than phosphate or carboxyl groups on lipids such as phosphatidylserine or phosphatidylinositol (Abramson & Katzman, 1968). Sulphatide might thus be expected to interact most effectively with positively charged groups on the intact protein or the peptide fragments. The conformation and overall size of the polar head group of a lipid will be important in any lipid-protein interaction. It may be that acidic glycerophospholipids are more effective at causing redistribution because their head groups have more conformational flexibility (reviewed by Lee, 1975), which would enable maximum bonding with suitable groups on proteins to occur. Sulphatides are very different in their head-group characteristics. In a fully extended conformation the head group of sulphatides could span across the basic-protein molecule (suggested diameter 15 nm) in the prolate-ellipsoid form (Epand *et al.*, 1974). As indicated by the present study, sulphatide interacts principally with basic residues on the C-terminal region of the basic protein. For basic-protein molecules in the hairpin form (Brostoff & Eylar, 1971) the net effect would be that the hydrophilic/hydrophobic boundary of the sulphatide (the sphingosine hydroxyl and the acyl-group hydroxyl and amide bond; Clowes *et al.*, 1971) would be positioned at the N-terminal section of the basic protein, where non-polar amino acid residues at discrete sites of this part of the polypeptide chain could associate with sulphatide acyl chains by hydrophobic interactions. Such an arrangement is shown in Fig. 5. Acidic lipids such as phosphatidylserine and phosphatidylinositol do not have head groups that can arrange in the same way and are envisaged as interacting with the C-terminal section of the basic protein from the opposing lipid layer to where sulphatide is sited. It is possible that this spanning arrangement for sulphatide may be crucial in maintaining the basic protein in its correct

orientation for bridging between adjacent membrane layers at the cytoplasmic apposition face in compact myelin (Rumsby & Crang, 1977).

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