The structure of bovine brain myelin proteolipid and its organization in myelin

(myelin basic protein/membrane proteins/protein folding/amino acid sequence/secondary structure prediction)

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ABSTRACT A model, based on amino acid sequence data, is proposed for the organization of the myelin proteolipid in myelin membrane. The model has three distinctive features: three trans-membrane segments that traverse the lipid bilayer, two cis-membrane domains that enter and exit the same side of the membrane, and a highly charged segment resembling myelin basic protein on the cytoplasmic side of the membrane. It is proposed that the cis-membrane domain(s) can promote the formation and stabilization of the multilamellar myelin structure by hydrophobic interaction with the apposite bilayer across the extracellular space.

In the central nervous system, myelin is formed as an extended, modified oligodendroglial plasma membrane that spirals around the axon to form a multilamellar structure (1). During maturation, the membranes become compacted with a close apposition of both the internal and external faces of the membrane. The relative thicknesses of the bilayer, cytoplasmic, and extracellular spaces are 47 Å, 30 Å, and 30 Å, respectively (2, 3).

Although the myelin sheath is characterized by a relatively high proportion of lipid (70–80%), it is the myelin proteins that provide specificity and perform an important, albeit ill-defined role in the maintenance of myelin structure. The predominant proteins are the water-soluble extrinsic myelin basic protein (M_r 18,000), characterized by a high proportion of basic and other polar amino acids (4, 5), and the hydrophobic, chloroform/methanol-soluble proteolipid (M_r 30,000) (4, 6). The basic protein is well-characterized (4, 5); however, the proteolipid has resisted structure elucidation, and only recently has its amino acid sequence been determined (7–9). In this article we propose a model (Fig. 1) for bovine brain myelin proteolipid, based on amino sequence data and analogy with other proteins, and suggest how the proteolipid may help to stabilize the myelin sheath.

RESULTS AND DISCUSSION

General Structural Features. The most notable characteristic of the proteolipid sequence (Fig. 2) is the clustering of hydrophobic amino acids into distinct domains. A plot of amino acid hydropathy (11) (Fig. 3) shows four hydrophobic domains of about 30 amino acids each, which alternate with polar segments containing most of the charged and neutral hydrophilic residues. The polar domains also contain all of the predicted β -turns (12), suggesting that these regions are extensively folded. These features suggest a model, such as has been proposed for bacteriorhodopsin (13) and other intrinsic membrane proteins (14), wherein the polypeptide chain passes repeatedly through the lipid bilayer, the hydrophobic regions being embedded in the lipid and the polar domains exposed on the external and internal faces of the membrane (Fig. 1). For purposes of discussion, various domains

in the proteolipid are defined in terms of polarity, proposed orientation in the membrane, and sequence homology. Thus, T1 (residues 59–90), T2 (residues 151–177) and T3 (residues 238–267) are hydrophobic trans-membrane segments that span the bilayer; C1 (residues 1–35) and C3 (residues 206–216) contain hydrophobic cis-membrane segments that enter and exit the same side of the bilayer; E1, E2, and E3 are intervening extra-membrane sequences that contain nearly all of the charged amino acids (Figs. 1 and 2). The segments C1', C2, and C3' are designated as cis-membrane segments based on homology (10) but probably are located outside of the lipid bilayer. As we have already reported (10), the proteolipid shows a striking degree of internal sequence homology, with various domains (cis, trans, or extra) mutually homologous within a domain type—e.g., T1, T2, and T3 are homologous.

Orientation of Polar Domains in the Membrane. Assuming that the proteolipid polypeptide chain is threaded through the membrane in a regular fashion (Fig. 1), we propose that the basic segments, E1 + C2 and E3, are located on the cytoplasmic face of the membrane and that the other polar segments, C1', E2, and C3', are on the extracellular side. Nearly all of the trans-membrane proteins studied so far have clusters of predominantly positively charged amino acids on the cytoplasmic side of the membrane (14). It has been proposed that these charged groups interact electrostatically with the negatively charged inner face of the membrane during insertion of the protein into the membrane, thereby anchoring the cluster and preventing it from passing through (14). A second argument is that E1 and E3 are remarkably similar, in terms of amino acid sequence (36% homology) and predicted secondary structure to the myelin basic protein, which appears to be located entirely on the cytoplasmic side of the membrane (5, 15, 16). Finally, there is evidence (see below) that C1 and the region E2 + C3 + C3' contain disulfide bonds and are therefore likely to be located in and on the outer leaflet of the cell membrane bilayer (17).

Oxidation State of Cysteine Residues. Myelin proteolipid contains 14 half-cystine residues, the oxidation states of which have not been completely established. Half of the cysteine residues are found in the polar and half in the hydrophobic domains: 6 in C1, 1 in T2, 3 in E1 + C2, and 4 in E2 + C3 + C3'. We have proposed that E1 + C2 is located on the cytoplasmic side of the membrane C1', E2, and C3' are on the exterior. Since intracellular proteins containing cysteine generally exist in the thiol form because of the reducing environment of the cell (17), we predict that the three cysteine residues in E1 + C2, as well as the single cysteine in T2 (which presumably is isolated in the bilayer), are in the reduced state. Conversely, the 4 cysteine residues in E2 + C3 + C3' on the external face probably exist as disulfides, in analogy with the majority of extracellular proteins (17). We further propose (see below) that the 6 cysteine residues in C1 occur as disulfides. Only 25–30% of the cysteine residues in proteolipid can be carboxymethylated, even under forcing conditions (18). This suggests that 4 of the 14 cysteine residues are in the thiol form. The sequence studies of Jolles *et*

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FIG. 1. Hypothetical model for myelin proteolipid in a membrane lipid bilayer. T1, T2, and T3 are homologous, α -helical trans-membrane segments; C1 and C3 are homologous cis-membrane segments; and E1, E2, E3, C1', and C3' are located outside of the bilayer. See text and ref. 10 for further description. Charged residues are indicated by + or -, and cysteine/cysteine is indicated by •. E2 contains a covalently linked fatty acid chain, indicated by a zigzag line.

al. (19) suggest that at least 2 of the cysteine residues (at positions 6 and 9) in C1 are in the oxidized form and, in our own sequence studies (20), we found that the NH₂-terminal tryptic peptide (Gly-1 to Arg-8) could only be isolated after cleavage of the disulfide bonds, indicating that Cys-5 and/or Cys-6 are crosslinked to other portions of the polypeptide chain. In addition, our studies (7) showed that cysteine residues 183, 200, 219, and 227 are crosslinked within the cleavage fragment comprising residues 181–276, indicating that the cysteines in the segment E2 + C3 + C3' are not crosslinked to other domains.

Hydrophobic Trans-Membrane Domains T1, T2, and T3. The hydrophobic segments T1, T2, and T3 comprise, more or less, residues 59–90 (32 residues), 151–177 (27 residues),

FIG. 3. Predicted location of hydrophobic domains and of β -turns in myelin proteolipid. Upper curve: the hydrophobic constants of Kyte and Doolittle (11) were averaged over nine amino acids; hydrophobic domains are located above the dashed line. Lower curve: relative probability of any four adjacent residues being a turn is plotted on the first residue.

and 238–267 (30 residues). They contain no charged amino acids (except possibly His-65 in T1) and only one cysteine residue (in T2). Although secondary structure calculations tend to predict β -sheet structure, we propose that T1, T2, and T3 are α -helical segments that span the lipid bilayer, in analogy with bacteriorhodopsin (13) and other membrane proteins (21). The failure of hydrophobic domains in membrane proteins to follow secondary structure prediction rules has also been noted by Argos *et al.* (21). Consideration of geometry also favors the helical structure. Given the standard α -helix parameter of 1.5 Å pitch height per residue, one can calculate that 31 residues would be needed to span the 47-Å thick myelin membrane bilayer. This is close to the segment lengths of T1, T2, and T3. Finally, thermodynamic considerations favor α -helical structures, wherein all potential hydrogen-bonding sites on the polypeptide backbone are internally bonded, in hydrophobic environments such as lipids (22). In a β -sheet, most of these sites remain exposed, resulting in a relatively high energy state.

FIG. 2. Amino acid sequence of bovine brain myelin proteolipid (from ref. 7). Homologous domains (see Fig. 1 and ref. 10) are indicated as C1, C1', T1, etc.; hydrophobic segments are indicated by heavy lines; and charges are indicated by + or -. It is proposed that all cysteine residues, except those at positions 108, 138, 140, and 168, are involved in disulfide linkages.

Hydrophobic Cis-Membrane Domain C1. Except for residues Glu-4 and Arg-8, the first 35 amino acids in the proteolipid are all hydrophobic or neutral. As in the case of the other hydrophobic domains, the Chou and Fasman method (12) predicts at least a partial β -sheet structure. However, utilizing the same arguments as for T1, T2, and T3, we postulate that C1 is also predominantly α -helical and is imbedded in the bilayer. As discussed above, C1 contains six cysteine residues at least some of which are involved in disulfide bonds. Since the NH_2 -terminal tryptic peptide (Gly-1 to Arg-8) could be isolated only after cleavage of the disulfide bonds (20), the cysteine residue(s) in this peptide must be crosslinked with other parts of the proteolipid, and Cys-5 and Cys-6 must not be crosslinked to each other. We further postulate that all disulfide bonds in C1 are located within this domain, since the cysteines in E2 + C3 + C3' seem to be internally crosslinked and since the remaining cysteine residues are predicted to be in the reduced form. Internal crosslinkage places constraints on the structure of C1, since any disulfide bonding arrangement would cause it to have a bent structure that could not traverse the bilayer. Therefore, assuming that C1 is imbedded in the membrane, it must enter and exit the same side of the bilayer. We define such a structure as a cis-membrane domain.

Our model for C1 (Fig. 4) consists of two α -helical segments (residues 1–13 and 18–31 with turn regions at residues 13–17 and 32–35). The first turn contains the helix breaker Pro-14. Ser-17 is so located that its side-chain hydroxyl can hydrogen bond to an exposed backbone NH at the beginning of the second α -helix, an arrangement that has been noted for several other proteins (23, 24). Such a bonding would also reduce the polarity of the serine hydroxyl; similarly, the hydroxyl of Thr-21 might hydrogen bond to other sites in the turn region. It is noteworthy that neither the β -turn prediction method of Rose (25), which takes into account the hydrophilic nature of turns, nor that of Chou and Fasman (12) predicts a turn at residues 13–17. This is not surprising, however, since both methods are based on data for water-soluble globular proteins, in which turn regions are usually exposed to solvent water and not lipid.

Although crystallographic data on polypeptide turns in a lipid environment are lacking, Rose *et al.* (26) have described turns similar to that postulated here, within the hydrophobic interiors of globular proteins. Two of these turn sequences, Trp-Pro-Trp-Gln in chymotrypsinogen and Val-Pro-Tyr-Gln in trypsin, are similar to the C1 sequence Ala-Pro-Phe-Ala in that all have a proline in position 2, followed

FIG. 4. Representation of the proposed cis-membrane domain C1 based on a model constructed from Nicholson molecular models. Shaded areas indicate hydrophobic side chains and hatched areas indicate disulfide crosslinks. See text for details.

by an aromatic amino acid. A model similar to C1 has been proposed (27) for the COOH terminus of cytochrome b_5 , which loops into and out of the same side of the bilayer. The cytochrome b_5 sequence, Ile-Pro-Ala-Ile-Ser (compared with the proteolipid putative turn sequence Ala-Pro-Phe-Ala-Ser), also has a serine residue positioned where it could hydrogen bond to the backbone of an incipient α -helix.

Another noteworthy feature of the C1 model is that all of the hydrophobic residues are located on the exterior surfaces where they can interact with lipid. The α -helical structure of the NH_2 -terminal segment results in Glu-4 and Arg-8 being located on the same side of the helix, where they could form an ion pair, as in bacteriorhodopsin (13), thus neutralizing the charge. Further, the first three cysteine residues are located on one side of the helix, where they are positioned to crosslink with the COOH-terminal region of C1 and thus to stabilize the hairpin structure. Although cysteine crosslinks are more likely to occur in turns, they have been found in α -helices (23)—e.g., in insulin (28), phospholipase A_2 (29), and crambin (30). Finally, four of the five glycine residues in the C1 model are located in the interior, thus minimizing side chain interactions and permitting a closer approach of the helical segments to one another.

The Basic Polar Domain E1 + C2. The segment E1 + C2, comprising residues 92–150, is characterized by a large number of charged and polar amino acids residues, which give E1 + C2 a net charge of about +8 assuming that histidine is half-ionized at physiological pH. Secondary structure calculations (12, 25) predict a number of β -turns (Fig. 3) and several possible short β -sheet regions. We have postulated that E1 + C2 is located on the interior or cytoplasmic face of the membrane.

We propose that the polypeptide chain, after it exits the bilayer, forms an antiparallel β -sheet structure consisting of three short β -strands linked by β -turns (Fig. 5). Before returning to the bilayer the peptide chain forms a fourth β -strand hydrogen bonded to the first or, as shown in Fig. 5, to the third β -strand. Few, if any, other combinations are possible because of the predicted (Fig. 3) tight β -turns beginning at residues 100 and 108. The antiparallel β -sheet arrangement seems more likely than parallel, because the latter always has five or more strands and is buried in the interior of proteins (23). The domain E1 + C2 is not large enough to enclose such a structure. Furthermore, antiparallel β -sheets are often polarized, as in our model, where 9 of the 11 hydrophobic side chains are on the same side. The remainder of the E1 + C2 seems to consist of turns and irregular struc-

FIG. 5. Hypothetical structure of the extra-membrane segment E1 + C2. Arrows represent antiparallel strands in a β -pleated sheet structure. Hydrophobic residues are circled. Amino acids on the lower edge of the arrows are located on one side of the sheet structure; those on the upper edge are on the opposite side.

ture, with the possible exception of a short helical segment between Leu-133 and Val-136. It is impossible to predict a unique structure for this part of the molecule, but we suggest that the charged, hydrophilic coil region is off to the side of the β -sheet.

This arrangement suggests a flattened structure for E1 + C2, one side being hydrophobic and the other polar and positively charged. The charged face of E1 + C2 could be oriented parallel to the lipid bilayer and stabilized by the negatively charged phospholipids found on the cytoplasmic face. The hydrophobic face, which could not remain exposed in an aqueous environment, might serve as a site of interaction with another protein—e.g., another proteolipid molecule or myelin basic protein. We have also constructed a model for a portion of myelin basic protein, based on secondary structure calculations of Martenson (15), that is remarkably similar to the E1 + C2 model (31). Since the cytoplasmic space between lamellae is only 30 Å thick (3), the structure of any protein occupying this space must be severely constrained. A β -sheet structure, including side chains, is 10–15 Å thick, nearly half the width of the space. Thus, an interaction between two β -sheet structures is reasonable.

Other Structural Features of the Proteolipid. Secondary structure calculations on C1' predict an α -helical stretch approximately between residues 37 and 47, just as the polypeptide chain emerges from the bilayer, and one or two β -turns between residues 51 and 57 (see Fig. 1). Thus, nearly half of the C1' segment may have a fairly rigid structure. In the region E2 + C3 + C3', we propose, in analogy with C1, that the segment comprising residues 198–209 is also α -helical, about half being imbedded in the bilayer. Turns are predicted between residues 210 and 222 (Fig. 3). If a disulfide bond links Cys-200 and Cys-219, then we obtain a structure for C3 that is not only homologous (10) with C1 but is also analogous—i.e., it is a crosslink-stabilized cis-membrane domain (Fig. 1).

Mechanism of Assembly in the Membrane. Weinstein *et al.* (14) have recently proposed that clusters of positively charged amino acids can act as anchor points in the assembly of proteins into membranes. Since most or all cells have an inside negative potential, the cluster hypothesis predicts that polypeptide segments with a high positive charge will reside on the cytoplasmic side of the membrane. Using this idea as a starting point, we propose a mechanism for assembly of the proteolipid in the membrane. The positively charged domain E1 + C2 would first bind to the negatively charged membrane (Fig. 6A). The hydrophobic segments then enter the membrane, possibly as bent helical structures, leaving all of the polar segments in the cytoplasm (Fig. 6B). The other polar domains (C1', E2, and C3') are then pulled through the bilayer to give an arrangement in which they are now on the outside of the membrane, with the hydrophobic helices (T1–3) spanning the bilayer (Fig. 6C). C1 is shown as retaining its bent shape because its interior turn disfavors an extended helix. At this point, all of the sulfhydryls in C1 and E2 + C3 + C3' are either in the extracellular space or are close to the bilayer outer surface, where they can undergo oxidation to disulfides, and thus stabilize the bent hydrophobic structures in C1 and C3 (Fig. 6D).

Other mechanisms—e.g., one employing the helical hairpin hypothesis of Engelman and Steitz (22)—can also be envisioned. Before insertion, the hydrophobic segments C1 and T1–3 probably do not exist as isolated, extended α -helices, since these structures would not be thermodynamically favored in an aqueous environment. More likely, the helices would aggregate or dimerize (22), or they might fold back upon themselves to reduce the amount of exposed hydrophobic surface (see Fig. 6). The extended helical segments are not completely hydrophobic, in that 28 of the 93 amino acids comprising T1–3 have side-chain functional groups ca-

FIG. 6. Proposed mechanism for insertion of proteolipid into the lipid bilayer. See text for description.

pable of forming hydrogen bonds. Thus, these segments may be regarded as amphiphilic. Folding would also expose peptide hydrogen bonding sites that could be solvated by water. However, once inside the lipid bilayer, the polypeptide units would prefer to be in the extended α -helical form (22) and could straighten out only by pulling some of the polar segments through the bilayer. Conceivably, the energy gained by reforming hydrogen bonds in the α -helix would help in this process. In addition, the helical segments of one or more proteolipid molecules may aggregate in the lipid bilayer to minimize exposure of polar side chains to the lipid. Models of the helical domains in the proteolipid show that the hydrophilic side chains tend to be clustered on one side of the helix, suggesting that a regular association of the helices within the bilayer may occur.

The Role of Proteolipid in Myelin. It is of interest to understand the factors that promote the formation and stabilization of the unique myelin structure. Boggs and Moscarello (4) have postulated that the proteolipid causes adhesion of the bilayers through hydrophobic interactions with adjacent lamellae. Our model provides a ready explanation for how such an interaction could occur. Assuming that the cis-membrane domain C1 can dissociate from the membrane and enter the extracellular space, if only for a small fraction of the time, then there should be a nearly equal likelihood that it would re-imbed itself in the apposite lamella. The cooperative effect of a large number of such interactions may be the force that promotes the compaction of myelin (Fig. 7). In

FIG. 7. Possible mechanism of compaction of myelin. Proteolipid molecules are shown as structures traversing the membrane with their "feet" in the cytoplasmic space and their "hands" (cis-domains) imbedded in the bilayer. As lamellae approach one another, the cis-domain, C1 (and possibly C3), is inserted in the apposite bilayer, stabilizing the compact form. Within the cytoplasmic space, possible interactions include association of hydrophobic β -sheet faces of proteolipid with proteolipid, proteolipid with basic protein (hatched), and basic protein with basic protein.

principle, C3 could also interact with the apposite membrane lamella. However, because of the shorter length of the extracellular segments E2 and C3', and possible restrictions due to disulfide bonds, it is difficult to predict whether E2 and C3' are long enough to span the 30-Å extracellular space. It may be significant that Thr-198 at the end of C3 is esterified with a fatty acid (9), which might interact with the apposite membrane. On the other hand, the E2 and C3' segments may not be stretched across the extracellular apposition but may act as a spacer or strut to maintain the 30-Å separation between lamellae (2).

Generalizations. The proteolipid model that we propose is speculative but is consistent with what is known about the proteolipid and provides a working hypothesis for future studies. Stoffel *et al.* (9) have also postulated a less detailed proteolipid model (of which we became aware while this paper was in revision), but it differs significantly from ours. The models will have to be tested, in particular by unambiguous location of disulfide bonds. Our model, besides providing a rational picture of the organization of the proteolipid in the bilayer, explains how the proteolipid may help to stabilize myelin. Furthermore, the concept of the cis-membrane domain may have validity for other membrane proteins. Finally, since we have demonstrated strong sequence homology between myelin proteolipid and the small proteolipid of the ATP synthase F_0 complex (10), it seems likely that what is learned about myelin proteolipid will also be applicable to other intrinsic membrane proteins.

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