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Central Nervous System Myelin: Structure, Function, and Pathology

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Multiple sclerosis (MS) and a number of related distinctive diseases are characterized by the active degradation of central nervous system (CNS) myelin, an axonal sheath comprised essentially of proteins and lipids. These demyelinating diseases appear to arise from complex interactions of genetic, immunological, infective, and biochemical mechanisms. While circumstances of MS etiology remain hypothetical, one persistent theme involves recognition by the immune system of myelin-specific antigens derived from myelin basic protein (MBP), the most abundant extrinsic myelin membrane protein, and/or another equally susceptible myelin protein or lipid component. Knowledge of the biochemical and physical-chemical properties of myelin proteins and lipids, particularly their composition, organization, structure, and accessibility with respect to the compacted myelin multilayers, thus becomes central to the understanding of how and why these antigens become selected during the development of MS. This review focuses on current understanding of the molecular basis underlying demyelinating disease as it may relate to the impact of the various protein and lipid components on myelin morphology; the precise molecular architecture of this membrane as dictated by protein-lipid and lipid-lipid interactions; and the relationship, if any, between the protein/lipid components and the destruction of myelin in pathological situations.

KEY WORDS: central nervous system; encephalitogenic basic proteins; membrane proteins; multiple sclerosis; myelin proteins; nuclear magnetic resonance; protein conformation.

Introduction

The central nervous system (CNS) is susceptible to a number of demyelinating diseases which are characterized by selective foci of myelin breakdown (that vary both in their distribution and severity) with no apparent effects on the underlying axon, and subsequent fibrous gliosis or astrocyte scarring (1,2). Within this broad classification, there exist a number of distinctive diseases such as acute disseminated encephalomyelitis, diffuse sclerosis, central pontine myelinolysis and multiple sclerosis (MS), which for the most part are restricted to the white matter, and are classed according to the type

and number of lesions, and the particular course of the disease. In distinction to those involving hypomyelination (termed leucodystrophies), these conditions apparently result from the active degradation of myelin by the affected individual's lymphocytic or phagocytic population. These demyelination diseases appear to arise from complex interactions of genetic, immunological, infective and biochemical mechanisms. This review focuses on current theory and understanding of the etiological origin(s) of demyelinating diseases, particularly on the underlying molecular basis for disease as it may relate to the specific proteins and lipids which comprise CNS myelin.

Demyelinating diseases – multiple sclerosis (MS)

PATHOLOGY

MS has been the subject of intense scrutiny over the past number of years due principally to its high incidence and its continuing elusive and enigmatic nature (3-5). Clinically, MS can be subdivided into two groups described by: 1) the chronic phase comprised of episodic events coupled with a continuous remitting/relapsing cycle; and 2) an acute phase which involves a rapid and often fatal progression of massive demyelinating foci. Early MS lesions (6) develop primarily within the vicinity of small blood vessels (*i.e.*, perivascular cuffing) which is correlated with a breaching of the blood-brain barrier as detected by computer-assisted tomography (CT) (7). These evolving regions of demyelination or plaques are distinguished by the infiltration of such cells as macrophages, neutrophils and lymphocytes in addition to the localization and proliferation of the CNS endogenous phagocytic cells, the microglia (8-11). Eventually these cell types become so numerous that they are readily detectable in the cerebrospinal fluid (CSF) (12,13). Of all the invading cell types, the phagocytic macrophages constitute the primary agents employed by the body in the seemingly inadvertent destruction of the myelin sheath. Histological studies (10) have shown that the macrophages repeatedly "peel off" and ingest the outer

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Manuscript received March 31, 1988; revised April 3, 1990; accepted May 2, 1990.

layers of the myelin until the axon is completely stripped of its protective membrane. By comparison, myelin within the periplaque region (area immediately adjacent to the lesion) has a grossly normal-appearing structure (14–16). Thus, based on ultrastructural and biochemical criteria, there is no immediately apparent rationale as to why a particular region of myelin is selected for destruction.

As the lesion begins to progress in size, the plaque area becomes crowded with additional T cells, phagocytes containing myelin debris, and eventually with hypertrophic astrocytes. This process of demyelination continues within the white matter in all directions radially from where the original plaque was formed until, for some as yet undefined reason, the cellular attack is halted. Under normal circumstances most tissues within the body would begin to repair this damage, but when myelin is destroyed, the situation is instead exacerbated by the inability of the CNS to effectively replace or repair the lost membranes (17,18). Attempts are made by the surviving oligodendroglial cells to remyelinate the denuded axons, but at a much slower rate than normal and with a considerably reduced efficiency. The factors that control or suppress remyelination remain virtually unknown (19,20) and offer a potentially important area of research in MS therapy.

EPIDEMIOLOGY

MS is not truly a worldwide phenomenon, as its distribution is biased towards temperate climates and more economically developed countries (21). Perhaps most striking is the fact that the incidence of MS increases as one moves further from equatorial regions both in a southward and particularly in a northward direction. These are not inviolate rules, as exceptions do occur (*e.g.*, the high incidence in Israel and the relatively low incidence in temperate Japan).

Investigations into populations migrating from high-risk to low-risk regions have suggested an age dependence where, for instance, adult immigrants from the United Kingdom (high risk) to South Africa (low risk) had an MS occurrence similar to that of the UK population (22). In contrast, South African descendants of UK immigrants had a much lower incidence. Similar surveys of the movements of children aged 0–14 revealed that the risk of contracting MS in later life was much lower than that of the corresponding adult population. This observation led to the suggestion that if migration occurred prior to adolescence, the child assumed the risk of the new geographical location.

Further epidemiologic evidence is the observation of several putative "epidemics" of MS in certain island populations — most notably in the Faroe Islands (23,24), Iceland (25) and the Orkney/Shetland Islands of Northern Scotland (26). In the Faroe Islands, for example, MS was completely unknown until a number of cases were confirmed between the

years 1943 and 1960, but virtually no cases were reported either prior to or following this particular time span. In correlating these observations with the history of the Faroe Islands, this outbreak of MS was seen to coincide with the occupation of the islands by British troops during the Second World War (1940–45). In addition, the garrisoning of the British forces was accompanied by an outbreak of canine distemper of epidemic proportions. Similar observations were made concerning the number of reported MS cases, troop occupation and canine distemper in Iceland and the Orkney/Shetland Islands. It has long been known that by virtue of their small populations and isolated nature, islands are unable to support a perpetual infection and are prone to the reintroduction and epidemic spread of such viral diseases as measles and canine distemper (27). While this does not necessarily imply that canine distemper or measles are the causal agents of MS, the data do point towards the possibility that some MS-causing infectious agent(s) were carried to these islands by the occupation forces, and that they have a counterpart in the epidemic of canine distemper.

NEUROIMMUNOLOGY

While the primary cause of MS remains speculative, it is suspected that MS is precipitated by an autoimmune response (for extensive reviews, see 28, 29). Immune regulation is a mixture of complicated interactions between the T lymphocytes, antigen and the cell surface major histocompatibility complex (MHC) antigens. As a consequence of this intricate system and considering the unwarranted attack of the immune elements on myelin, it is highly probable that one or more of these regulatory pathways is circumvented during the development of MS. Under such circumstances, the immune system becomes sensitized to a particular organ or tissue and consequently stimulates the recruitment of cytotoxic elements that eventually leads to the destruction of seemingly normal cells. Some of the better understood autoimmune diseases are Hashimoto's disease (30), Grave's disease (31), pernicious anemia (32) and the most relevant to MS, myasthenia gravis, which results from the production of autoantibodies to acetylcholine receptors located at the neuromuscular junction (33,34). A similar series of events may induce the recognition of myelin antigens and the onset of MS.

AUTOIMMUNE AND VIRAL ETIOLOGY OF MS

How the immune system could become sensitized to myelin antigens is unknown, but in accordance with the epidemiological data it has long been suspected that this is a result of some infectious agent — possibly a systemic virus (35–37). Viral models of demyelinating disease, such as the visna virus (38,39) in sheep, have been well documented

TABLE 1
Possible Events Related to CNS Demyelination
Observed in Multiple Sclerosis

1. Systemic virus infection, either measles, varicella, influenza, etc.
2. Viral cross-reactivity with MBP (or another myelin protein, e.g., proteolipid protein) leading to immunization against myelin.
3. Production of circulating T_H cells specific for MBP.
4. Systemic interleukin-2 and/or γ -interferon response.
5. Activation of vascular endothelium, astrocytes and/or microglia.
6. Expression of MHC Class 2 (Ia) antigens and presentation of MBP.
7. Recognition of Ia-MBP complex by MBP-specific T_H cells.
8. T_H cell activation coupled with a lack of immunosuppression by T_S cells (reasons unknown).
9. Invasion of CNS by phagocytic cells, T and B cells and active demyelination enhanced by local antibody production, circulatory complement, free radical formation, etc.
10. Remission instituted by reactivation of T_S cell suppression; relapses induced by localized IL-2 or γ -interferon production and recurrence of inflammatory response caused by Ia-MBP presentation.

and appear to follow an identical course to MS, *i.e.*, phagocytosis of myelin by cellular infiltration and a clinical appearance that can be either relapsing, remitting or acute. The human equivalent of a visna virus has been much more difficult to isolate. A number of viruses have been implicated such as herpes simplex (40), coronavirus (41,42), measles (43,44), and Theiler's virus (45,46). More recently, the human lymphotropic viruses (HLTV-I and HLTV-II) (47-49) and immunodeficiency virus (HIV) associated with acquired immune deficiency syndrome (AIDS) (37,50,51) have become the latest in a long line of potential MS-causing candidates. Recent studies using ³⁵S-labelled RNA probes prepared against genomic RNA sequences of measles virus, canine distemper virus, rubella virus and simian virus S have failed to provide conclusive evidence to support the theory that MS has a viral origin (52). This does not, however, preclude the involvement of viruses in MS as they may perform a "catalytic" role by presenting a myelin cross-reactive antigen.

Several myelin-specific antigens, both proteins and lipids, have been implicated in MS etiology, the most thoroughly investigated being the major extrinsic membrane protein of myelin, the so-called myelin basic protein (MBP) (29). As reviewed by Oldstone (53), a small percentage of antibodies (*ca.* 4%) raised to viral proteins cross-react with host determinants from uninfected tissues. Attempts to evaluate this viral "mimicry" of host antigens in MS have shown that MBP contains a number of potential sites with primary sequences similar to those of viral proteins (54,55). One specific observation (55) in support of this type of mechanism is

demonstrated by the response of animals to hepatitis B polymerase (HBpol). Inoculation with HBpol produces an antigenic response and production of sera that recognize MBP, presumably due to similarities in primary sequence and secondary structure. Moreover, animals immunized with HBpol show an accumulation of inflammatory cells within CNS-localized endothelium similar to the early stages of MS lesion development. Therefore, although hepatitis B may not be the "MS virus," these observations do support a molecular mimicry model for the development of demyelinating foci. Further support for a central role of MBP is provided by the observation that immunization with MBP leads directly to the clinical and pathological condition of experimental allergic encephalomyelitis (EAE), an *in vivo* model of MS (56-60). MBP is not, however, the only potential myelin antigen, as similar arguments have been made for the proteolipid protein (61) and lipid components (*e.g.*, gangliosides) (62) of myelin.

Circumstances of MS etiology thus remain hypothetical. However, the cellular responses summarized in Table 1 (adapted from Waksman (63)) represent the current thinking on the possible course of events connected with the CNS demyelination observed in MS. One central and persistent theme of these postulations is the presentation and recognition of myelin antigens derived either from MBP or some other equally suitable myelin-specific determinant. Knowledge concerning the physical-chemical properties, organization and accessibility of the proteins and lipids within the compacted myelin multilayers thus becomes central to the understanding of how and why these antigens become selected during the development of MS. The unusual structural characteristics and functional requirements of the myelin sheath raise a number of pertinent biochemical questions concerning: 1) the properties of the various lipid and protein components and their impact on the overall myelin morphology; 2) the precise molecular architecture of the membrane as dictated by protein-lipid, protein-protein and lipid-lipid interactions; and 3) the connection, if any, between the lipid/protein components and the destruction of myelin in pathological situations such as MS.

Central nervous system myelin

Myelin is a lipid-protein membrane construct contained within the CNS and peripheral nervous systems (PNS) of vertebrates (for comprehensive reviews, see 64, 65). Acting primarily as an electrical insulator, myelin controls and increases the speed of signal transmission along the axon from the nerve cell body to the synaptic junction. The membrane encircles the axon at a number of discontinuous points or internodes and forms the nonconducting counterpart to the Nodes of Ranvier. The resulting multilamellar membrane arrangement of myelin is also seen, for example, in the chloroplast (66,67), in the interior of the mitochondria (68,69)

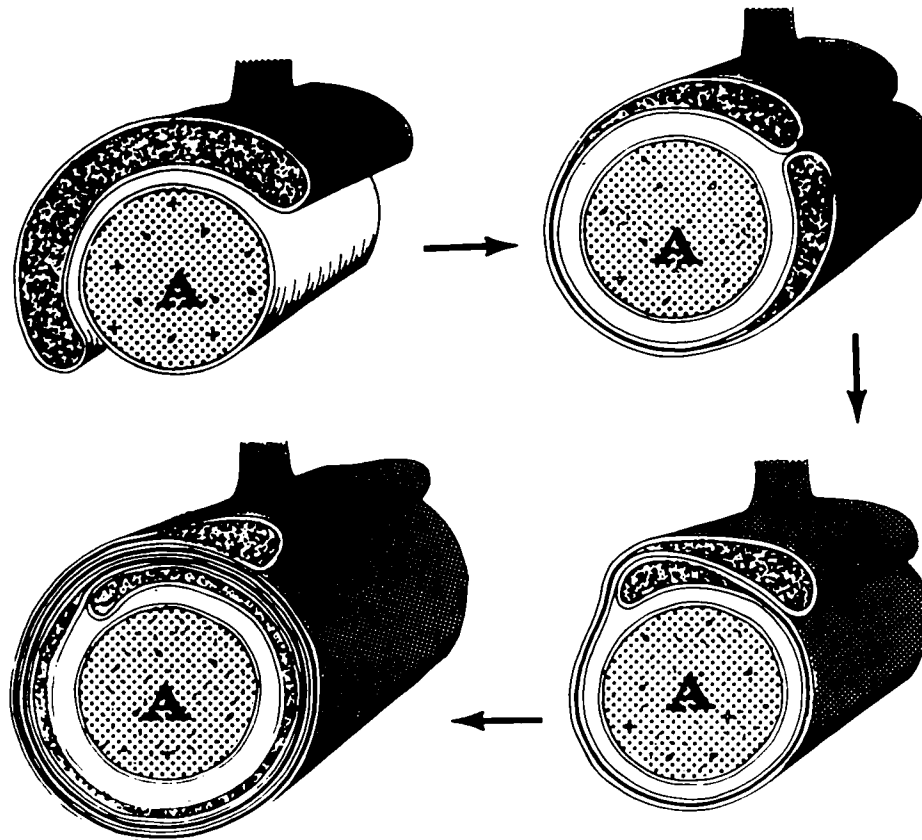


Figure 1—Schematic representation of the formation of the myelin sheath surrounding an axon (A) (from Ref. 74).

and in the disc membranes of the rod outer segments (70,71).

MYELIN FORMATION AND ULTRASTRUCTURE

The human central nervous system can be divided into the two general anatomical categories of gray and white matter composed, respectively, of nerve bodies and myelinated nerve fibers (72). Myelin is a product of the oligodendroglial cells which can direct processes of their plasma membranes often to distant target axons (73,74). These fragile-appearing extensions seek out an axon, establish a connection and proceed to envelop the axonal cylinder with a continuous wrapping of compacted membrane (Figure 1). Formation of myelin follows a set pattern (74,75). Once contact is made between the oligodendroglial and axonal membranes, the glial membrane begins to wrap around the axon until a complete circuit is accomplished and the extracellular faces adhere. At this point, a compaction process is initiated within the cytoplasmic space which results in the association of the two apposing bilayers and the creation of a major dense line (so-called because of its appearance in electron micrographs). Concurrent compaction of the outer leaflets of the oligodendroglial plasma membrane forms the intraperiod line which is the less-electron dense extracellular counterpart to the major dense line. The

resulting multilayers are thus composed of repeating units of [membrane]-cytoplasmic space-[membrane]-extracellular space-[membrane]. This highly ordered spiral array of membrane and aqueous compartments can, depending on the species, achieve an appreciable thickness of 5–20 multilayers (Figure 2).

As the process of myelin formation continues, the previously apparent connection between the main cell body of the oligodendrocyte and the compacting membranes becomes less and less distinct (74). In mature myelin, the apparent loss of this link may be due either to a complete severing of ties or the maintenance of a very tenuous connection that is difficult to visualize by electron microscopy. This isolation of the compacted myelin from the metabolic elements associated with the main portion of the cell may have profound consequences on the ability of the cell to augment membrane stability in the event of external environmental changes. It has also been observed that unlike the peripheral nervous system, where one myelinating Schwann cell envelops one axon, a single oligodendroglial cell may synthesize numerous myelin sheaths through independent processes (76,77). The evolution of these membrane extensions may explain the inability of the oligodendrocyte to reinitiate myelination following membrane breakdown due to the requirement of an enormous metabolic expenditure (20). In

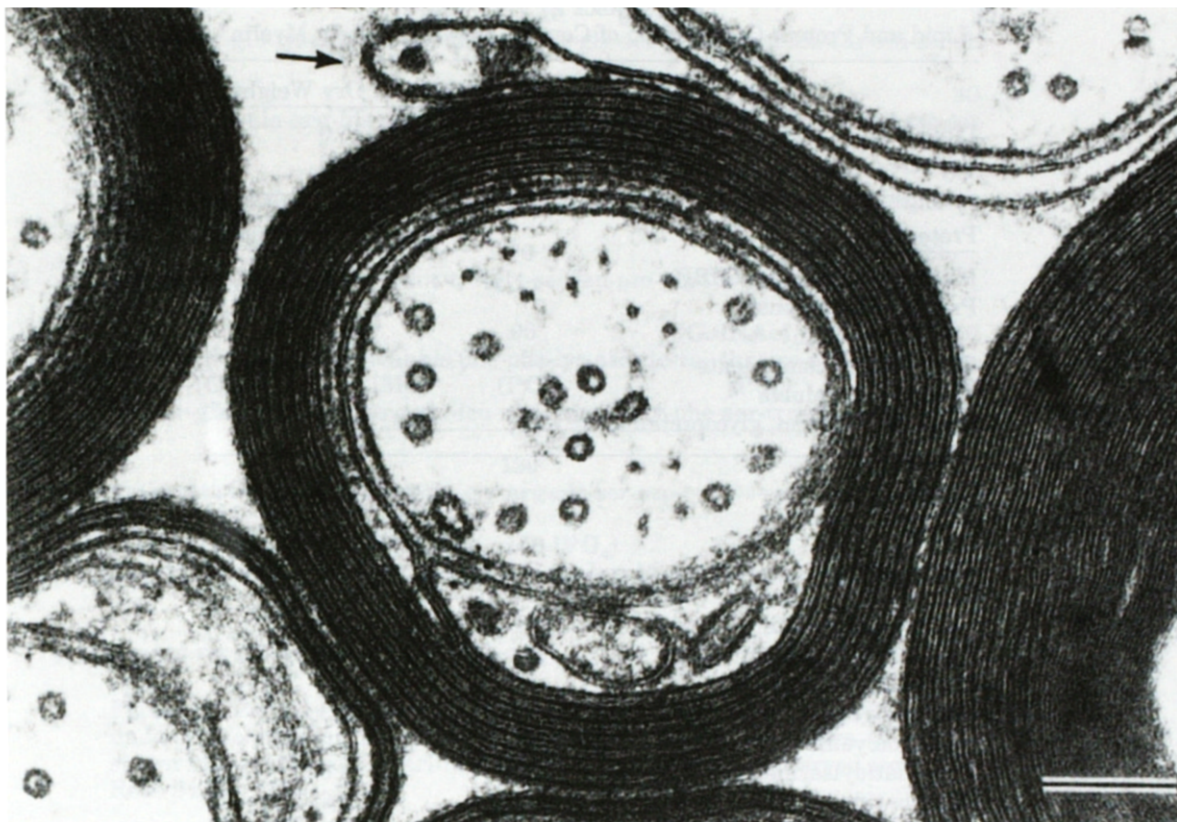


Figure 2—Electron micrograph of mature myelin sheath showing the repetitive multilayers of myelin membrane surrounding an axon (contained within the central core) (from Ref. 74).

addition, the employment of single cells to produce major portions of myelin lays the entire infrastructure open to extensive damage simply through the destruction of one cell. These factors may have some bearing on the pathological demyelination, as well as the inadequate replacement of the membrane, during the progression of MS (20,78).

The stimuli responsible for myelin formation must be under strict biochemical control. Initially, the axon attains some state conducive to myelination, possibly a critical axonal diameter (74), whereupon some factors initiate the compaction process. Myelin-associated glycoprotein (MAG) is a membrane protein of central and peripheral nervous system myelin sheaths localized in the periaxonal area. MAG may be implicated in axon-myelinating cell interactions because of its location, as well as the developmental regulation of MAG expression in the mouse brain (79). Of equal importance must be some termination step which retards the oligodendrocyte from encircling the nerve indefinitely. These events may be controlled, in part, by feedback mechanisms between the oligodendroglial cells and the axons and/or some undefined external input from surrounding cell types such as the astrocytes (80,81). Current investigations into these and other questions should further our understanding of the cellular events relating to myelination, and may eventually

lead to the development of therapeutic tools with which to combat demyelinating diseases.

LIPID-PROTEIN COMPOSITION

Dehydrated myelin is made up of approximately 75–80% lipid and 20–25% protein by dry weight (Table 2) (82–84). Within these two distinct groups, there exist a number of molecular species ranging from myelin basic protein to the major intrinsic hydrophobic proteolipid protein (PLP) among the proteins, and from cerebrosides to plasmalogens among the lipids. Each of these molecules has its own distinct chemical make-up and physical properties that contribute to the formation, stabilization and possibly the pathogenesis of the myelin membrane (*vide infra*).

Myelin basic protein (MBP)

SEQUENCE — MICROHETEROGENEITY

Myelin basic protein (MBP) is a water soluble extrinsic membrane protein with a molecular mass of 18.5 kDa (for reviews, see 84–86). MBP accounts for approximately 25–30% of the total myelin protein and is the second most abundant species after the proteolipid proteins. Labelling studies have shown that MBP is contained exclusively within the cytoplasmic spaces of myelin (87). MBP can be readily

TABLE 2
Lipid and Protein Composition of Central Nervous System Myelin^c

	<u>% Total Dry Weight</u>
Lipid	75.0
Protein	25.0
<hr/>	
<u>Protein</u>	<u>% Total Protein Weight</u>
Myelin basic protein (MBP)	22.5
Proteolipid proteins ^a	
PLP/lipophilin	30.0
Other LH-20 components	17.5
Thioethanol soluble	2.5
Others (Wolfgang, glycoproteins, etc.)	27.5
<hr/>	
<u>Lipid Weight</u>	<u>% Total Lipid</u>
Cholesterol	27.7
Cerebroside	22.7
Cerebroside sulfate	3.8
Ethanolamine phospholipids	15.6
Phosphatidylcholine	11.2
Plasmalogens ^b	12.3
Sphingomyelin	7.9
Phosphatidylserine	4.8
Phosphatidylinositol	0.6
Gangliosides (primarily GM ₁ and GM ₄)	<1.0

^aThe proteolipid fraction is a family of proteins that can be isolated on Sephadex LH-20 in acidified chloroform-methanol. The major homogeneous protein has been termed proteolipid protein (PLP) or lipophilin.

^bThe plasmalogens are ether-linked (as opposed to ester-linked) lipids composed primarily of ethanolamine phosphatides and, to a lesser extent, choline and serine phosphatides.

^cAdapted from Refs. 82-84.

isolated in its lipid-free form by acid extraction of chloroform/methanol-solubilized myelin. As shown in Figure 3, human MBP contains 170 amino acids including 12 lysine, 19 arginine, 2 glutamic acid and 9 aspartic acid residues. This gives the protein a net charge of +20 at physiological pH and, consequently, a high isoelectric point (>10). The majority of these basic and acidic amino acids are evenly distributed throughout the primary sequence and are interspersed with polar uncharged and hydrophobic residues. MBP contains one tryptophan residue [position 116], but no cysteine (and therefore no disulfide bonds). The amino acid sequences of several species (88-92) have been determined, and a great deal of sequence conservation (underlined regions in Figure 3) has been noted. One implication of this observation is that these portions of the molecule may have important functions in the MBP-directed myelin formation.

Further features of the primary structure of MBP include an acetylated N-terminal, a number of phosphorylation sites [for example: Ser-7; -12; -56; -110; -115; -151; -165 and Thr-34; -98] (93-99), two carbohydrate acceptor sites [Thr-95; -98] of N-acetylga-

lactosamine (GalNAc) (100,101 and references therein), a methylated Arg residue [107] which occurs in both the mono- and di-methylated form (102), two likely deamidation sites [Gln-103; -147] (103,104), a possible C-terminal modification (*i.e.*, loss of Arg) (105), an unusual tri-proline region [residues 99-101] which has been highlighted as an important structural feature (88) similar to that contained in immunoglobulin G (IgG) (106), and sequences homologous to cholera toxin A and B subunits [residues 102-118 and 67-77 in human MBP] which may possibly be involved in GM₁ ganglioside and GTP binding as well as ADP ribosylation of MBP (107).

A further aspect of MBP is that this 18.5 kDa species exists as a number of different chemical forms ("microheteromers") (103,108). Separation of the microheteromers by means of ion exchange chromatography (103) results in the elution of a number of MBP components (designated C1-C8) which vary in their cationic potential. The three most cationic species, components 1, 2 and 3 (*i.e.*, those components dissociated from the column at high salt concentration), tend to differ in their degree of phosphorylation (component 3) and the

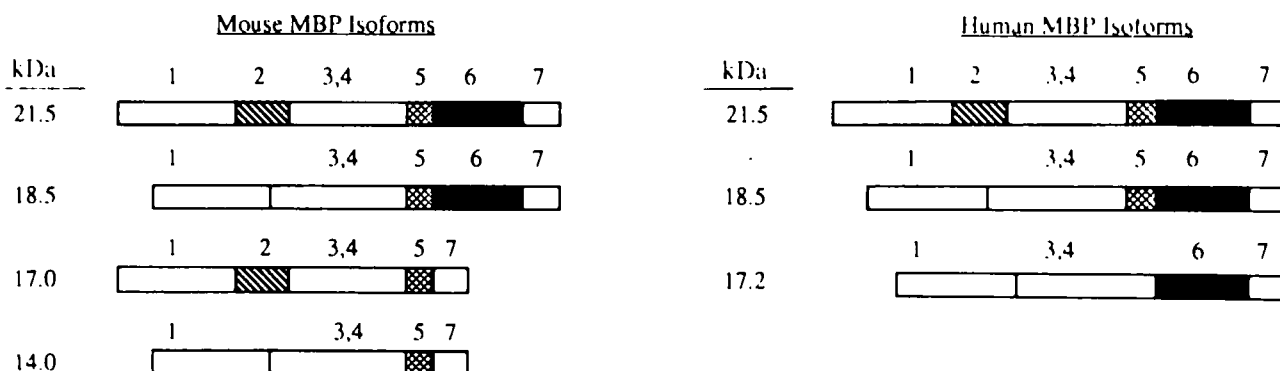


Figure 4—Primary transcripts (mRNA) of MBP derived from (A) mouse and (B) human sources. Exons coding for the various regions of the MBP primary sequence are labelled 1–7 (from Ref. 132).

peptide analogues of MBP are specific inhibitors of PKC (121); 2) the existence of a potential link between the phosphoinositol pathway and the phosphorylation of MBP (122); 3) the observation that MBP phosphorylation can be downregulated by cAMP — a known inhibitor of PKC (98).

Methylation

Isolation and analysis of MBP revealed that it contains a methylated Arg residue [position 107] that is present both in mono- and di-methylated forms (102) (mono, 0.4–0.8 mol/mol MBP; and di, 0.2 mol/mol MBP). Investigations into this feature of MBP (123) have suggested that methylation may represent an important step in the formation of myelin as seen primarily by the observation that, in cultured oligodendroglial cells, inhibitors to the methyl-transferase enzymes produce a significantly less compact myelin (124).

Developmental studies using radioactive tracers have shown that methylation of MBP occurs during the formative stages of myelination and, as with phosphorylation, this modification may constitute an initiation signal (123). The consequences of methylation may manifest themselves through an increase in the hydrophobicity of this region of MBP and, accordingly, increase protein–protein or protein–lipid interactions that may be necessary for MBP function. The *in vitro* investigations of Young *et al.* (125) have indeed demonstrated that the degree of methylation correlates with an increased interaction of the protein with lipid vesicles.

Glycosylation

Although MBP is not recovered from myelin as a glycosylated derivative, the protein is a natural acceptor of N-acetylgalactosamine (GalNAc) when incubated in the presence of a suitable galactosyl-transferase and UDP-GalNAc (101). The major sites of modification are Thr-95 and Thr-98 (O-glycosidic bond) which are in the vicinity of the unusual

tri-proline region 99–101. These proline residues have been found, through the use of synthetic peptides, to represent an essential sequence for the transfer of the GalNAc group and may possibly confer a preferential conformation upon this region of MBP (126–128). However, the function, if any, of this modification is as yet unknown and it remains to be determined if this glycosylation process occurs *in vivo*. Persaud *et al.* (129) have used *in vitro* glycosylated MBP spin-labelled with Tempoamine to study the effect of glycosylation on the secondary structure of MBP. These authors found that glycosylated MBP complexed with phospholipid vesicles was less susceptible to digestion by endoproteinase Lys-C than nonglycosylated MBP and suggested that glycosylation may affect MBP-membrane interactions.

Molecular Forms

Determination of MBP function within myelin is further complicated by the observation that, depending on the species, MBP is present in several molecular forms (*i.e.*, proteins of differing molecular weights) (130,131). Thus, human MBP occurs in three forms which have been cloned and their amino acid sequences determined (132). These include 21.5, 18.5 and 17.2 kDa forms which differ by the addition of a 26-residue peptide within the N-terminal region (21.5 kDa) and the loss of an 11-residue peptide within the C-terminal portion (17.2 kDa). The 18.5 kDa protein is the major native species expressed in myelin. The additions and deletions correspond to specific exons within the coding sequence (exons 2 and 5, respectively) and appear to result from alternative splicing of the primary transcripts (see Figure 4). A similar situation occurs in mouse MBP (133), with the additional occurrence of a 14 kDa species that may result from the splicing of exon 6. More recent investigations (134) have suggested a fifth molecular form of MBP expressed in the mouse. In general, the alternate forms (*i.e.*, those found in addition to the 18.5 kDa protein) are present in relatively minor concentrations and, al-

though their conservation indicates a degree of importance, there is at present no unique function known for these MBP isoforms.

SECONDARY AND TERTIARY STRUCTURE

While the conformation adopted by MBP in aqueous solution does not appear to have any conventional secondary structure (*i.e.*, helix or beta), it also apparently does not form a completely "random coil." The protein appears to maintain some ordered tertiary structure, likely within the mid-sequence of the protein, *viz.*, the tri-proline region found at positions 99–101 (for reviews, see 84, 86). ^{13}C and ^1H nuclear magnetic resonance (NMR) studies revealed that in water, the resonances of MBP maintain distinct chemical shifts that are indicative of different chemical environments which could be produced by folded portions of the protein (135–137); these chemical shift disparities could be eliminated in 6 M guanidinium hydrochloride which would effectively unfold the protein and thus give rise to essentially average chemical environments for each residue. Some question has been raised as to the possibility that the method of purification of MBP using organic solvents and acid may produce MBP in a "denatured" state. However, when basic protein was purified using 0.2 M CaCl_2 instead of the traditional solvents, it was found to be predominantly a "random coil" structure with only limited segments of secondary structure, therefore agreeing with previous structural studies (138).

The notion that MBP forms a nonrandom structure is also supported by the immunological studies of Whitaker *et al.* (139) who employed an antibody raised to an internal peptide of MBP [residues 43–88]; in solution, this antibody specifically recognized the isolated peptide but was unable to produce a complex with the intact protein. Thus it was postulated that the inaccessibility of this peptide to the antibody was the result of internalization of this region within the ordered core of the protein. Additional investigations (140–142) into conformational aspects of MBP by calorimetric and fluorescence techniques determined that MBP undergoes a reversible enthalpic transition that is associated with changes in the hydrophobic nature of the protein and is therefore consistent with some type of folded structure.

As mentioned, a notable aspect of the MBP conformation in aqueous solution is the absence of a significant degree of secondary structure as measured by circular dichroism (CD) (143–146) and classically by optical rotatory dispersion (ORD) (147,148) (see also Stoner (149)). These results have recently been confirmed by Fourier-transform infrared (FT-IR) experimentation (150). In addition, Deber *et al.* (151) specifically enriched the two Met residues situated near opposite ends of the protein [positions 21 and 167 for human MBP] with ^{13}C -methyl groups and observed that the motion of these labelled

residues was virtually unrestricted in water. The free rotation of these groups similarly argues against interaction of the N- and C-terminals with other portions of the protein.

MBP-LIPID INTERACTIONS

Several diverse sources of information have indicated that MBP-lipid interactions play a critical role in the formation of myelin. These include: 1) the *in vitro* ability of MBP to bind small lipid vesicles and reform the lipid into stable multilamellar layers reminiscent of the *in vivo* myelin sheath ((84) and references therein); 2) the fact that MBP possesses greater bilayer-stabilizing properties in dioleoylphosphatidylethanolamine (DOPE) systems than other basic proteins (calf thymus histone, lysozyme, melittin) as determined by ^{31}P -NMR and X-ray diffraction techniques (152); 3) the demonstration by X-ray techniques that isolated myelin displayed infinite swelling on the extracellular space in the presence of excess water but that the intracellular space (*i.e.*, the location of MBP) remained tightly compacted (153); 4) the existence of several dysmyelinating mutants specific for MBP (*e.g.*, shiverer mouse) which are incapable of producing large quantities of stable CNS myelin (154,155), and any myelin that is formed does not contain the intracellular major dense line (156); 5) the fact that synthesis of MBP during development is an essential step in the construction of mature myelin membranes (157–159); 6) the fact that following demyelination by hepatitis virus, MBP transcripts are increased dramatically within the areas most greatly affected, a result which also correlated with the formation of new myelin sheaths (160). These and other observations have led to speculation that MBP is responsible for initiating the compaction process within the myelin cytoplasm as well as maintaining the closely apposed multilayers in the mature structure.

Physical-chemical studies of the interaction of MBP with phospholipids (146,161–163), and those examining binding of basic protein to phospholipid membranes as monitored by ^1H , ^{13}C and ^{31}P NMR spectroscopy (151,164–167), have confirmed that the attraction between MBP and lipid is largely electrostatic. However, the amino acid sequence of MBP shows that approximately 25% of the total amino acids have hydrophobic side chains. Complementary studies (84,145,166,168–171) have therefore suggested a hydrophobic component of MBP/lipid binding and accordingly, the penetration of some residues or "regions" of the protein into the hydrophobic membrane interior. An example of the hydrophobic aspect of MBP is its capacity to increase the permeability of lipid vesicles (163) while polylysine (a poly-cation that interacts exclusively by electrostatic interactions) is incapable of producing a similar result. From such results, it was suggested that MBP-related permeability arises from the hydrophobic interactions of protein side chains pene-

Some Encephalitogenic Peptides	Species
Ala ₆₇ -His-Tyr-Gly-Ser-Leu-Pro-Gln ₇₅	Rabbit
Gln ₇₄ -Lys-Ser-Gln-Arg-Ser-Gln-Asp-Glu-Asn-Pro ₈₄	Rat
Phe ₁₁₄ -Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg ₁₂₂	Guinea Pig
Phe ₁₅₄ -Lys-Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-Gly-Ser-Pro-Met ₁₆₇	Monkey

Figure 5—Encephalitogenic peptides of MBP and their species dependency (from Ref. 84).

trating the membrane and distorting the lipid packing. Support for MBP hydrophobic interactions is also provided by intrinsic fluorescence studies of the single Trp residue, which displayed an increased fluorescence in the presence of lipid, consistent with its movement from a polar to nonpolar environment (172). There is some debate as to the extent to which MBP can penetrate the lipid acyl chains of a bilayer, as demonstrated by unsuccessful attempts to label native MBP chemically using a hydrophobic probe (87). Further evaluation by X-ray diffraction (173) and NMR (174) have indicated that the uncharged residues of MBP interact primarily within the head group region of the lipid bilayer with no significant penetration of extensive segments of the protein into the hydrophobic core of the membrane.

Two general molecular models can explain the membrane-adhesive qualities of MBP (reviewed in 84, 85). These include 1) a unimolecular model where the N- and C-terminals of monomeric MBP bind on apposing sides of the myelin bilayers to bridge the intracellular space, and 2) a bimolecular (or perhaps multimolecular) situation in which MBP compacts myelin multilamellae by protein-protein as well as protein-lipid interactions. The latter model is supported by Golds and Braun (175,176) as a result of cross-linking studies of intact myelin which indicated that a large fraction of the protein was isolated in the dimeric form. In addition, sedimentation analysis has demonstrated that the protein-protein association of MBP is enhanced in the presence of lipid/detergents (177). These interactions are most probably dependent upon hydrophobic interactions, as shown by the pH investigations of Moskaitis *et al.* (178) which revealed that MBP self-association could be achieved only in the absence of protonated His residues.

The conformation that MBP adopts when associated with a membrane is perhaps more pertinent than its conformation in aqueous solution as described above. CD measurements of MBP bound to detergent micelles (143,179–181) or lipid vesicles (145) have indicated varying amounts of α -helix (15–20%), β -structure (10–15%) and random coil (40–50%). The more recent FT-IR investigations of Surewicz *et al.* (150) have indicated that the degree of β -sheet formation may in fact be greater than previously suspected (>50%). However, as the co-presence of large amounts of lipids tends to interfere with spectroscopic determinations, the conformational details of the lipid-bound MBP remain to be

resolved in detail.

Studies on the interactions of the various microheteromers (components C1–C8) by liquid X-ray diffraction (182,183), aggregation assays (110,183,184) and vesicle permeability (185) have shown that the cationic character (*i.e.*, extent of positive charge) is directly proportional to the ability of the protein to interact favourably with lipids. This was demonstrated both with the naturally occurring components (C1–C8), as well as for isomers of altered charge produced by *in vitro* phosphorylation.

In the case of MBP derived from brain tissue of MS subjects, isolation of the various components has indicated that in comparison to normal MBP, the MS protein is often less cationic (as determined by the relative NaCl concentrations required to elute the various MBP samples from the ion exchange column) and relatively less efficient (vs. normal MBP) in its ability to bind lipids (183). Further insight into the lipid binding characteristics of MS-MBP has been provided by the NMR studies of Deber *et al.* (174), which revealed that normal MBP, but not the MS protein, was capable of effectively altering the mobility of the head group region of phosphatidylglycerol vesicles by means of electrostatic interactions. Although these observations may imply some relationship between the behaviour of MBP in native myelin and disease activity, it is probably premature to surmise that alterations of MBP represent a primary event in the etiology of MS.

ENCEPHALITOGENIC ACTIVITY OF MBP

Innoculation of test animals (*e.g.*, guinea pigs) with MBP results in experimental allergic encephalomyelitis (EAE) which has clinical manifestations indistinguishable from those of MS (for a review, see 57). Sensitization of the immune system to MBP is followed by a recruitment of helper T-cells and eventually the activation of cytotoxic elements such as macrophages that produce demyelinating lesions within the CNS (186,187). This experimental “multiple sclerosis” exhibits a chronic relapsing/remitting type as well as an acute form. As with MS, recovery from EAE is related to the ability of the host to produce effective suppressor T-cells to down-regulate the cytotoxic side of the immune reaction (188). That the encephalogenic response can also be generated passively by the introduction of T-cell clones sensitized to MBP provides convincing evi-

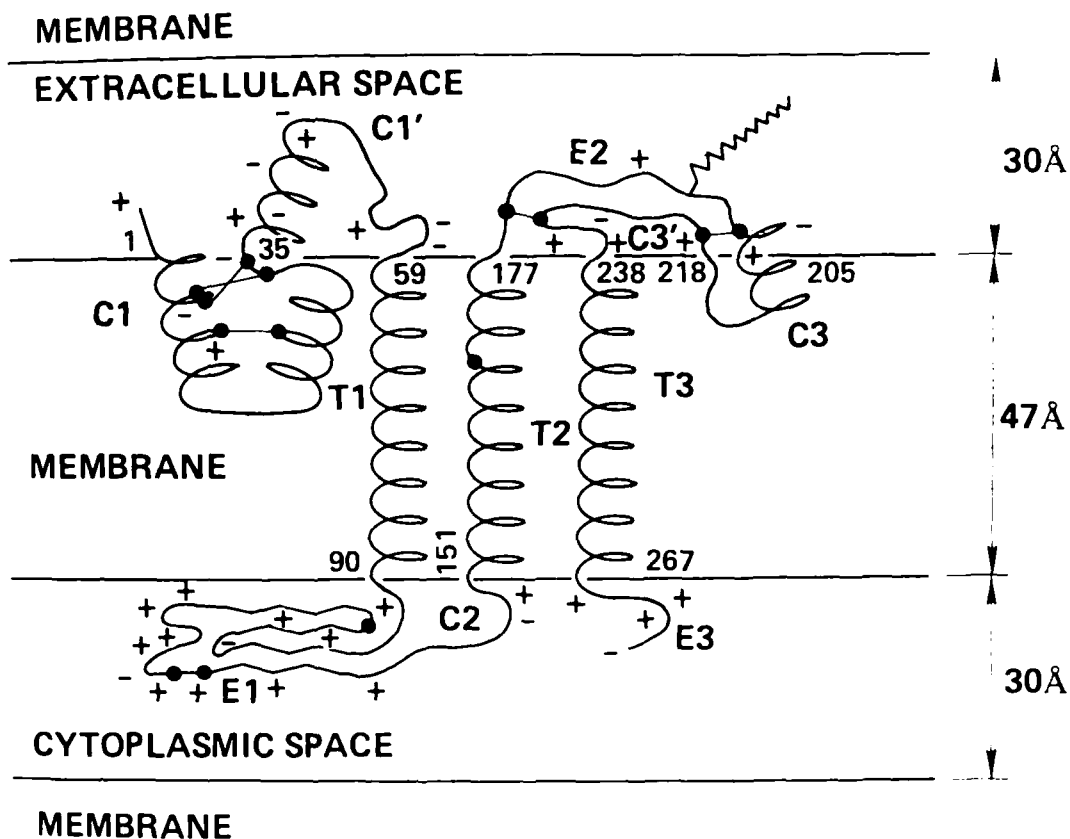


Figure 7—A proposed structure for PLP in the myelin membrane (from Ref. 200). T1, T2, and T3 are homologous, α -helical transmembrane segments; C1 and C3 are homologous cis-membrane segments; and E1, E2, E3, C1', and C3' are located outside of the bilayer. Charged residues are indicated by + or -, and cysteine/cystine is indicated by ●. E2 contains a covalently linked fatty acid chain, indicated by a zigzag line.

technique. These investigators found that MBP bound PLP in a saturable, reversible manner and that this binding was much greater for MBP than for other basic proteins.

The standard isolation procedure for PLP involves chromatography on a Sephadex LH-50 column in an acidified chloroform-methanol solvent. The elution profile indicates that the myelin proteolipid protein is actually a family of integral membrane proteins comprised principally of PLP (30 kDa) and DM20 (25 kDa), along with a collection of minor components of various molecular masses (84). These additional fractions have not been well characterized, but it has been proposed from cDNA studies that all of the integral proteins arise from a common gene and result from segmental deletions of the primary transcripts (195). Specific observations concerning the relationship between PLP and DM20 by Simons *et al.* (203) have confirmed this postulation. Using a PLP cDNA probe from a human retinal library, it was shown that DM20 contained the coding sequence of PLP with the exception of a deletion within a putative aqueous domain. These studies do not provide direct insight into the function of PLP

and its subsets but do open the possibility that the different integral protein components could have different functions during the development and maintenance of the cohesive myelin structure. Milner *et al.* (195) have argued against any functional necessity for the lower molecular weight PLP components, since mouse and monkey code only for the large PLP protein but are still capable of producing viable myelin.

Organization of PLP within the membrane on a molecular level has been investigated by a number of physical-chemical methods. Evidence from labeling experiments (87) has shown that, unlike MBP, regions of PLP are exposed on the extracellular side of the myelin membrane as well as being deeply intercalated within the hydrophobic core of the bilayer. Based on the amino acid sequence (see Figure 6) and other evidence, a membrane profile of PLP was constructed (200,204) that consists of three transmembrane or *trans* segments [residues 59-90; 151-177; and 238-267] which completely traverse the bilayer and two additional *cis* segments [residues 9-35 and 205-218] which are imbedded in the lipid but enter and exit on the same side of the

bilayer (Figure 7). A similar model has been proposed by Stoffel *et al.* (201) which these workers later confirmed using antipeptide antibodies (205). *In vitro* experimental details for the distribution of PLP between membrane and aqueous domains have been given by Kahan and Moscarello (206) through investigations using photolabelling reagents and selective proteolytic cleavage.

Based largely on comparisons with other membrane proteins (*e.g.*, bacteriorhodopsin) (207), it has been suggested that the PLP transmembrane segments maintain an α -helical conformation (200). This is a reasonable assumption considering that: 1) helical structure allows for the formation of intramolecular hydrogen bonds that can insulate the protein backbone from the low dielectric medium of the bilayer; and 2) the average length of PLP transmembrane regions is 30–31 residues which would be sufficient to span the 45 Å myelin bilayer (assuming a helix pitch of 1.5 Å/residue (208)). These hydrophobic regions would be connected by polar aqueous linker domains. Confirmation of a high helix content in PLP has been provided by FT-IR investigations which indicate approximately 55% α -helix, along with 36% β -structure (209), a measurement which did not appear to be dependent upon the transition state of the lipid. Alternatively, CD measurements have suggested an increased helix content of approximately 75% compared with a β -content of only 10% (210). It is difficult to discern which set of figures is the more accurate as each technique has unique error factors in estimating secondary structure (*e.g.*, light scattering, the choice of reference compounds, and integration errors).

PLP binds lipids primarily through interactions with its hydrophobic side chains (211–213) and it has been suggested that the positively charged aqueous domains are also capable of electrostatic interactions with the negatively charged head groups of phospholipids (162). In addition to its ability to bind lipid, the proteolipid fraction of myelin (PLP and DM20) coisolates with a small percentage of fatty acid (2–3%) that may be covalently bound (214,215). PLP-specific fatty acid acylation has since been demonstrated using a tritiated palmitic acid which was either incubated with a cell-free system or injected directly into the brain (216,217). Further investigations have determined that the acyl chain is linked through an O-ester bond to Thr-198 *in vivo* (see Figure 7) and is present primarily as palmitic acid, with some stearic and oleic acids (218). Isolation at various intervals has indicated that this is a relatively late post-translational modification that occurs at or near the myelin plasma membrane (217). In an interesting set of experiments, Bizzozero *et al.* (219) have demonstrated that incubation of isolated PLP with palmitoyl-CoA resulted in the formation of an acylated protein. These observations have led to the suggestion that PLP is an autocatalytic enzyme (*i.e.*, transfer of the acyl group

is due to properties inherent in PLP with no contribution from an additional enzyme); this property is a characteristic of several membrane proteins (220). However, other than added hydrophobicity, the specific function that the attached fatty acid may perform *in vivo* remains to be elucidated (see also 217).

The PLP gene has been isolated, cloned and sequenced. It appears to be comprised of seven exons and six introns where each exon (with the exception of the first and last) codes for one membrane domain (either *cis* or *trans*) plus the sequentially adjacent aqueous domain (221). Hybridization experiments have shown that the human and mouse PLP gene is located on the X chromosome (196). These investigations have also shown that the PLP sequence is highly conserved between species. For example, rat and human PLP were found to be essentially identical, differing at position 95 (Ser in rat, Ala in human), while only four substitutions appeared in the bovine protein. In genetic studies of actively myelinating animals, the transcriptional rate of PLP (as well as MBP) is coincident with the appearance of compact myelin within the CNS (195). This observation emphasizes the important relationship between myelin compaction and the appearance of the major myelin proteins.

The discovery of several hypomyelinating mutants also supports a role for PLP in the compaction or stabilization of myelin. The jimpy mouse, an X-linked mutant which is thought to result from incorrect construction of the primary PLP transcripts, displays a reduced degree of myelination and a less compact extracellular space (222,223). The human counterpart is described by an inherited X-linked myelin disorder known as Pelizaeus-Merzbacher disease (196). In addition, Duncan *et al.* (224) have described a similar X-chromosome mutant in the rat which has a reduced PLP mRNA content that correlates with an abnormally compacted myelin in which the extracellular spaces or interperiod lines are markedly expanded.

ENCEPHALITOGENIC ACTIVITY OF PLP

Innoculation with PLP appears to be associated with an encephalitogenic response similar to MBP-induced EAE. These observations have met with some opposition due to suspicions that the response was produced by a contaminating quantity of MBP (225), but these appear to have been discounted through the use of highly purified PLP preparations which produce a chronic progressive and relapsing/remitting disease when injected into guinea pigs (in its aqueous form consisting of an aggregate of protein molecules (199,226,227)). As with MS lesions, this condition corresponds to an accumulation of cellular infiltrates within the CNS and active demyelination. Conclusive evidence of a PLP-mediated demyelination has come from the immunological studies of Yamamura *et al.* (198), who demonstrated

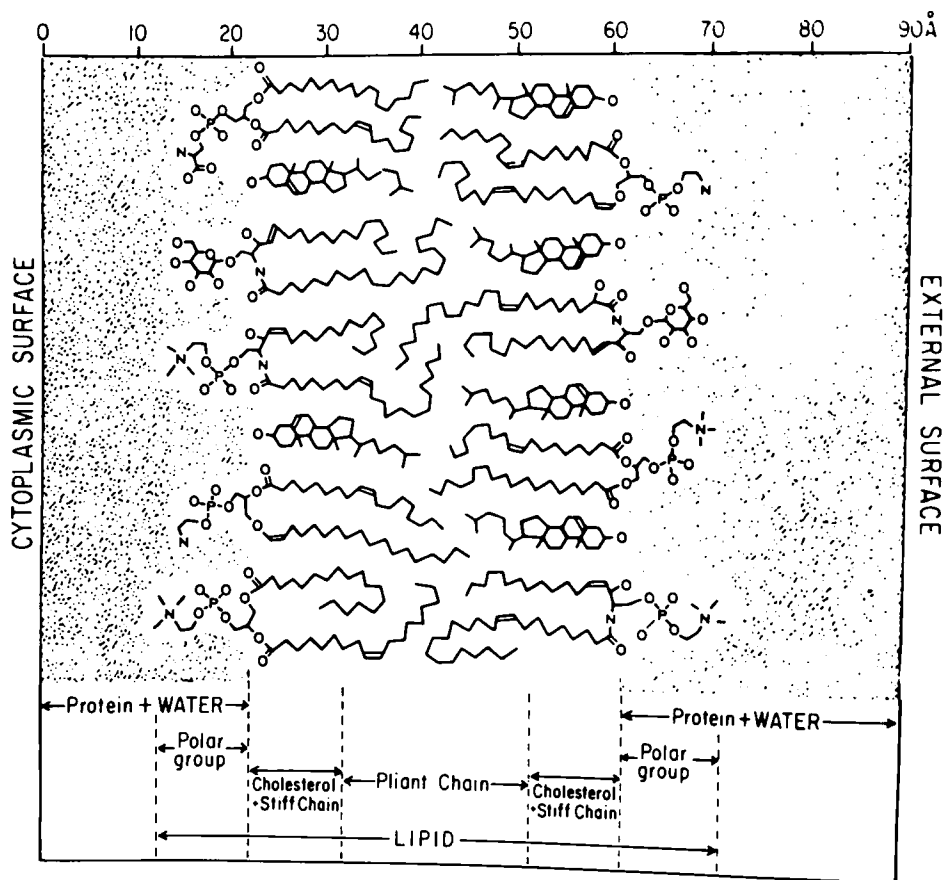


Figure 8—Organization of lipids within myelin and the dimensions of the bilayer (from Ref. 228).

a passive transfer of the PLP encephalitogenic activity. Using PLP-sensitized lymphocytes obtained from cultured lymph nodes exposed to the protein which were then reinjected into rats (*ca.* 107 cells), CNS inflammatory sites, myelin lesions and the accompanying clinical signs of demyelination were produced. In another study, Shaw *et al.* (61) have compared the sequence of PLP with the sequences of a number of viral proteins and identified several primary sequence similarities with, for example, Epstein-Barr virus, influenza A, HIV and adenovirus polypeptides. Thus, it is possible that the EAE response observed to PLP is a reflection of a potential autoimmune response similar to the relationship proposed between MBP and infecting viruses.

Myelin lipids

MOLECULAR ORGANIZATION

The myelin membrane itself is composed of a diverse collection of lipids and proteins. The lipids are arranged in a typical bilayer or bimolecular ("lamellar") form with an inner (cytoplasmic) monolayer and a corresponding outer (extracellular) monolayer (228) having a total thickness of *ca.* 40–45 Å (Fig-

ure 8). The lipid molecules are positioned such that the hydrophilic portions provide the interface between the aqueous spaces — both intra- and extracellular — and the hydrophobic core produced by the acyl chains. The bilayer dimensions remain relatively constant throughout myelin whereas the aqueous spaces differ in their degree of compaction, *i.e.*, the intracellular space is *ca.* 20–25 Å while the extracellular space is *ca.* 25–30 Å. It has been suggested that this asymmetrical packing may be a reflection of both the protein composition and different protein–lipid interactions.

The lipid composition of myelin is given in Table 2 (for reviews, see 82, 83). Cholesterol comprises the single most abundant lipid species and in normal white matter occurs in the unesterified form (83). Although there are no myelin-specific lipids *per se*, the glyco-ceramides (cerebrosides) are diagnostic of myelin in the brain such that the quantity of cerebroside is roughly proportional to the amount of myelin present (82). Another distinctive category of myelin lipids is the plasmalogens, which represent approximately 30% of the phospholipids present and occur primarily as ethanolamine phosphatides with a smaller percentage of serine and choline phosphatides (229). The plasmalogens contain fatty al-

dehydes that are linked to the glycerol backbone as alkyl ethers or α,β -alkenyl ethers and contain predominantly saturated 16:0 and 18:0 acyl chains. The nonplasmalogen phosphatidylethanolamines (PE) contain fatty acid acyl groups (18:1, 20:1, 20:4 and 22:4) which are linked via esters to the glycerol backbone. The remaining phospholipids, phosphatidylcholine (PC) and phosphatidylserine, contain ester-linked fatty acids of 16:0, 18:0, 18:1 and 18:0, 18:1, 20:4 chains, respectively (230). Cerebrosides and cerebroside sulfates occur in both the hydroxylated and nonhydroxylated forms with the major fatty acid species (aside from the ceramide portion) being 24:0 and 24:1 chains (231). Sphingomyelin fatty acids appear largely as 24:1, 18:0 and 24:0. Myelin also contains several minor lipid species (totalling < 5%) such as the gangliosides (GM₄ and GM₁) (232) and phosphatidylinositol (233).

Myelin undergoes marked developmental changes in its lipid composition as seen by the increases in cholesterol, cerebroside, phosphatidylserine, ethanolamine plasmalogens, and sphingomyelin, and a concurrent decrease in PC content with advancing age (234–236). The most rapid changes are observed in neonates (up to 6 months) followed by slower changes to a final adult or homeostatic stage that in general is not achieved in humans until 15–20 years of age. Since the myelin membrane is composed largely of lipids, these developmental changes may reflect a need to accommodate the physical alteration in the membrane organization while still maintaining the structural stability of the myelin sheath.

LIPIDS AND DEMYELINATION

Numerous investigations have stressed the involvement of lipids in demyelination both as potential autoantigens (62,237–239) and as structural variants arising from differences in the molecular species of normal vs. MS tissue (240). While there appears to be no consensus for differences, if any, of the noninvolved or grossly normal white matter between normal and MS tissue, there are some lipid abnormalities within the MS plaque and periplaque regions. The most apparent of these is a decrease in total lipid, which is consistent with the degree of myelin loss (241). The demyelinating regions also show a consistent increase in the amount of cholesterol esters (242,243), a specific decrease in PE concentration (244), and significant changes in ganglioside concentrations (particularly GM₄) (245). It remains uncertain whether these lipid variations represent a primary demyelination mechanism, or are primarily a reflection of post-breakdown events.

In examining the potential of lipid to induce demyelination in the peripheral nervous system, Low *et al.* (246) found that interneural injections of lyso-PC (a micelle-forming derivative of PC itself), could specifically promote demyelination as detected

by severely attenuated electrical transmissions. In a related study, exogenous lipases derived either from macrophages (phagocytic cells seen at developing and expanding plaque regions) or from serum were found to be highly effective in producing demyelination (244) and were lipid-specific (*i.e.*, PE was affected to a greater extent). It is interesting and possibly significant that periplaque regions show similar decreases in PE concentrations (*vide infra*).

Apart from the sensitivity of myelin to lipid degradation products, lipid-induced demyelination may also proceed via an immune response. Carroll *et al.* (238) demonstrated that intraneural injections of cerebroside antisera produce a proportional demyelination such that low levels of serum create only slight lesions with partial remyelination, while larger doses produce extensive lesions with additional axonal degeneration. As with MS, the introduction of the anticerebroside antibodies was associated with myelin vesiculation and the presence of invading macrophages. Given the fact that cerebroside is a major component of myelin and are localized on the extracellular side of the bilayer (247), they may constitute a primary element in initiating demyelination. Support for the *in vivo* participation of anticerebroside antibodies is provided by the observation that similar antibodies have been found in the cerebro-spinal fluid (CSF) of MS patients (62). An alternate glycolipid family that may be associated with the MS immune responses is the gangliosides, which constitute the major surface lipid antigens of myelin. As with the anticerebroside, antibodies to GM₄ — a myelin specific lipid in the CNS (232) — are also found in the CSF of MS patients (62). Ganglioside characterizations in MS pathology are very limited to date, and as such comprise a potentially exciting area of future research.

Acknowledgement

This work was supported, in part, by a grant to C.M.D. from the National Multiple Sclerosis Society (USA). S.J.R. held a studentship from the Multiple Sclerosis Society of Canada. We appreciate the input and advice of Dr. Paul E. Fraser during the early stages of this work.

References

1. Blackwood W, Corsellis JAN, eds. *Greenfield's neuropathology*. London: Edward Arnold Ltd., 1976.
2. Raine, CS. The neuropathology of myelin diseases. In: Morell P., ed. *Myelin*. Pp. 259–310. New York: Plenum Press, 1984.
3. McFarlin DE, McFarland HF. Multiple sclerosis (first of two parts). *N Engl J Med* 1982; 307: 1183–251.
4. McDonald WI. Multiple sclerosis: the present position. *Acta Neurol Scand* 1983; 68: 65–76.
5. McDonald WI, Silberberg DH, eds. *Multiple sclerosis*. Boston: Butterworths, 1986.
6. Adams CWM. The onset and progression of the

- lesion in multiple sclerosis. *J Neurol Sci* 1975; **25**: 165-82.
7. Ebers GC, Vinuela FV, Feasby T, Bass B. Multifocal CT enhancement in MS. *Neurology* 1984; **34**: 341-6.
 8. Raine CS, Powers JM, Suzuki K. Acute multiple sclerosis. Conformation of "paramyxovirus-like" intranuclear inclusions. *Arch Neurol* 1974; **30**: 39-46.
 9. Adams CWM. Pathology of multiple sclerosis: progression of the lesion. *Br Med Bull* 1977; **33**: 15-20.
 10. Traugott U, Scheinberg LC, Raine CS. On the presence of Ia-positive endothelial cells and astrocytes in multiple sclerosis lesions and its relevance to antigen presentation. *J Neuroimmunol* 1985; **8**: 1-14.
 11. Hauser SL, Bhan AK, Gilles F, Kemp M, Kerr C, Heiner HL. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann Neurol* 1986; **19**: 578-87.
 12. Halonen T, Kilpelainen H, Pitkanen A, Riekkinen PJ. Lysosomal hydrolases in cerebrospinal fluid of multiple sclerosis patients. A follow-up study. *J Neurol Sci* 1987; **79**: 267-74.
 13. Bamborschke S, Heiss, W-D. Cerebrospinal fluid and peripheral blood leukocyte subsets in acute inflammation of the CNS. *J Neurol Sci* 1987; **79**: 1-12.
 14. Lumsden CE. The immunogenesis of multiple sclerosis plaque. *Brain Res* 1971; **28**: 365-90.
 15. Prineas JW, Kwon EE, Cho E-S, Scharer LR. The distribution of myelin associated glycoprotein and myelin basic protein in actively demyelinating multiple sclerosis lesions. *J Neuroimmunol* 1984; **6**: 251-64.
 16. Möller JR, Yanagisawa K, Brady RO, Tourtellotte WW, Quarles RH. Myelin-associated glycoprotein in multiple sclerosis lesions: a quantitative and qualitative analysis. *Ann Neurol* 1987; **22**: 469-74.
 17. Suzuki K, Andrews J, Waltz J, Terry R. Ultrastructural studies of multiple sclerosis. *Lab Invest* 1969; **20**: 444-54.
 18. Prineas JW, Kwon EE, Sternberber NH, Lennon VA. Remyelination in multiple sclerosis. *Ann NY Acad Sci* 1984; **436**: 11-32.
 19. Dorfman SH, Fry JM, Silberberg DH. Antiserum induced myelination inhibition *in vitro* independent of the cytolytic effects of the complement system. *Brain Res* 1979; **177**: 105-14.
 20. Silberberg DH. Pathogenesis of demyelination. In: McDonald WI, Silberberg DH, eds. *Multiple sclerosis*. Pp. 99-111. Boston: Butterworths, 1986.
 21. Gonzalez-Scarano F, Spielman RS, Nathanson N. Epidemiology. In: McDonald WI, Silberberg DH, eds. *Multiple sclerosis*. Pp. 37-55. Boston: Butterworths, 1986.
 22. Dean G, Kurtzke JF. On the risk of multiple sclerosis according to age at immigration to South Africa. *Br Med J* 1971; **3**: 725-9.
 23. Kurtzke JF, Hyllested K. Multiple sclerosis: an epidemic disease in the Faroes. *Trans Am Neurol Assoc* 1975; **100**: 213-5.
 24. Nathanson N, Miller A. Epidemiology of multiple sclerosis: critique of the evidence for a virus etiology. *Am J Epidemiol* 1978; **107**: 451-61.
 25. Kurtzke JF, Gudmundsson KR, Bergman S. Multiple sclerosis in Iceland. I. Evidence of post war epidemic. *Neurology* 1982; **32**: 143-50.
 26. Poskanzer DC, Prenney LB, Seridan JL, Kundy JY. Multiple sclerosis in the Orkney and Shetland Islands. I. Epidemiology, clinical factors, and methodology. *J Epidemiol Community Health* 1980; **34**: 229-39.
 27. Cook SD, Cromanty JI, Tapp W, Poskanzer D, Walder JD, Dowling PC. Declining incidence of multiple sclerosis in the Orkney Islands. *Neurology* 1985; **35**: 545-51.
 28. Weiner HL, Hauser SL. Neuroimmunology I: immunoregulation in neurological disease. *Ann Neurol* 1982; **11**: 437-49.
 29. Waksman BH. Immunity and the nervous system: basic tenets. *Ann Neurol* 1983; **13**: 587-91.
 30. Doniach D, Bottazzo GF, Khoury EL. Prospects in human autoimmune thyroiditis. In: Pinchera A, Doniach D, Fenzi GF, Bascheri L, eds. *Autoimmune aspects of endocrine disorders*. Pp. 25-38. London: Academic Press, 1980.
 31. Burman KD, Baker JR, Jr. Immune mechanisms in Grave's disease. *Endocr Rev* 1985; **6**: 183-201.
 32. Roitt I, Brostoff J, Male D. *Immunology*. New York: Gower Medical, 1985.
 33. Drachman DB. Myasthenia gravis. Part 1. *N Engl J Med* 1978; **298**: 136-42.
 34. Hohlfeld R, Toyka KV, Tzartos SJ, Carson W, Conti-Tronconi BM. Human T-helper lymphocytes in myasthenia gravis recognize the nicotinic receptor α subunit. *Proc Natl Acad Sci USA* 1987; **84**: 5379-83.
 35. Lampert PW. Autoimmune and virus-induced demyelinating diseases. *Am J Pathol* 1978; **91**: 175-208.
 36. Cook SD, Dowling PC. Multiple sclerosis and viruses: an overview. *Neurology* 1980; **30**: 80-91.
 37. Johnson RT, McArthur JC. Myelopathies and retroviral infections. *Ann Neurol* 1987; **21**: 113-6.
 38. Narayan O, Cork LC. Lentiviral diseases of sheep and goats: chronic pneumonia, leukoencephalomyelitis and arthritis. *Rev Infect Dis* 1985; **7**: 89-98.
 39. Stowring L, Haase AT, Petursson G, et al. Detection of visna virus antigens and RNA in glial cells in foci of demyelination. *Virology* 1985; **141**: 311-8.
 40. Gudnadottir M, Helgadottir H, Bjarnason O, Jonsdottir K. Virus isolated from the brain of a patient with multiple sclerosis. *Exp Neurol* 1964; **9**: 85-95.
 41. Burks JS, De Vald BL, Jankovsky LD, Gerdes JC. Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. *Science* 1980; **209**: 993-4.
 42. Suzumura A, Lavi E, Weiss SR, Silberberg DH. Coronavirus infection induces H-12 antigen expression on oligodendrocytes and astrocytes. *Science* 1986; **232**: 991-3.
 43. Norrby E, Link H, Olsson J-E. Measles virus antibodies in multiple sclerosis. Comparison of antibody titers in cerebrospinal fluid and serum. *Arch Neurol* 1974; **30**: 285-92.
 44. Vandvik B, Degre M. Measles virus antibodies in serum and cerebrospinal fluid in patients with multiple sclerosis and other neurological disorders, with special reference to measles antibody synthesis within the central nervous system. *J Neurol Sci* 1975; **24**: 201-19.
 45. Dal Canto MC, Lipton HL. Primary demyelination in Theiler's virus infection. An ultrastructural study. *Lab Invest* 1975; **33**: 626-37.
 46. Rodriguez M, Leibowitz JL, Lampert PW. Persistent infection of oligodendrocytes in Theiler's virus-induced encephalomyelitis. *Ann Neurol* 1983; **13**:

- 426-33.
47. Koprowski H, DeFreitas EC, Harper ME, *et al.* Multiple sclerosis and human T-cell lymphotropic retroviruses. *Nature* 1985; **318**: 154-60.
 48. Osame M, Matsumoto M, Usuku K, *et al.* Chronic progressive myelinopathy associated with elevated antibodies to human T-lymphotropic virus type I and adult T-cell leukemia-like cells. *Ann Neurol* 1987; **21**: 117-22.
 49. Vernant JC, Maurs L, Gessain A, *et al.* Endemic tropical spastic paraparesis associated with human T-lymphotropic virus type I: a clinical seroepidemiological study of 25 cases. *Ann Neurol* 1987; **21**: 123-30.
 50. Snider WD, Simpson DM, Nielsen S, Gold JWM, Metroka CE, Posner JB. Neurological complications of acquired immune deficiency syndrome analysis of 50 patients. *Ann Neurol* 1983; **14**: 403-18.
 51. Johnson RT, McArthur JC. AIDS and the brain. *Trends Neurol Sci* 1986; **9**: 91-4.
 52. Cosby SL, McQuaid S, Taylor MJ, *et al.* Examination of eight cases of multiple sclerosis and 56 neurological and non-neurological controls for genomic sequences of measles virus, canine distemper virus, simian virus 5 and rubella virus. *J Gen Virol* 1989; **70**: 2027-36.
 53. Oldstone MBA. Molecular mimicry and autoimmune disease. *Cell* 1987; **50**: 819-20.
 54. Jahnke U, Fischer EH, Alvord EC, Jr. Sequence homology between certain viral proteins and proteins related to encephalomyelitis and neuritis. *Science* 1985; **229**: 282-4.
 55. Fujinami RS, Oldstone MBA. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* 1985; **230**: 1043-5.
 56. Spitler LE, von Muller CM, Fudenberg HH, Eylar EH. Experimental allergic encephalitis. Dissociation of cellular immunity to brain protein and disease production. *J Exp Med* 1972; **136**: 156-74.
 57. Maugh TH. The EAE model: a tentative connection to multiple sclerosis. *Science* 1977; **195**: 969-71.
 58. Sires LR, Hruby S, Alvord EC, Jr., *et al.* Species restrictions of a monoclonal antibody reacting with residues 130 to 137 in encephalitogenic myelin basic protein. *Science* 1981; **214**: 87-9.
 59. Lazarus KJ, Hashim GA, Varitek VA, Jr., Paterson PY, Day ED. A rabbit B cell determinant for a conserved portion of myelin basic protein, rabbit encephalitogenic sequence 65-74. *J Immunol* 1983; **131**: 275-81.
 60. Fritz RB, Chou C-HJ. Epitopes of peptide 43-99 of guinea pig myelin basic protein: localization with monoclonal antibodies. *J Immunol* 1983; **130**: 2180-2.
 61. Shaw S-Y, Laursen RA, Lees MB. Analogous amino acid sequences in myelin proteolipid and viral proteins. *FEBS Lett* 1986; **207**: 266-70.
 62. Kasai N, Pachner AR, Yu RK. Anti-glycolipid antibodies and their immune complexes in multiple sclerosis. *J Neurol Sci* 1986; **75**: 33-42.
 63. Waksman BH. Mechanisms in multiple sclerosis. *Nature* 1985; **318**: 104-5.
 64. Morell P, ed. *Myelin*. New York: Plenum Press, 1977.
 65. Morell P, ed. *Myelin*. New York: Plenum Press, 1984.
 66. Quinn PJ, Gounaris K, Sen A, Williams WP. In: Wintermans JFGM, Kuiper PJC, eds. *Biochemistry and metabolism of lipids*. Amsterdam: Elsevier Biomedical, 1982.
 67. Gounaris K, Brain APR, Quinn PJ, Williams WP. Structural and functional changes associated with heat-induced phase-separations of non-bilayer lipids in chloroplast thylakoid membranes. *FEBS Lett* 1983; **153**: 47-52.
 68. Wrigglesworth JM, Packer L, Branton D. Organization of mitochondrial structure as revealed by freeze-etching. *Biochim Biophys Acta* 1970; **205**: 125-35.
 69. Palmer JM, Hall DO. The mitochondrial membrane system. *Prog Biophys Biophys Chem* 1972; **24**: 125-76.
 70. Raker E. Membranes of mitochondria and chloroplasts. *American Chemical Society Monograph 165*, New York: Van Nostrand Reinhold, 1970.
 71. Wallace BA. Comparison of bacteriorhodopsin and rhodopsin molecular structure. *Methods Enzymol* 1982; **88**: 447-62.
 72. Blackwood W. Normal structure and general pathology of the nerve cell and neuroglia. In: Blackwood W, Corsellis JAN, eds. *Greenfield's neuropathology*. Pp. 1-42. London: Edward Arnold Ltd., 1976.
 73. Davison AW, Peters A. Biochemistry of the myelin sheath. In: Davison AW, Peters A, eds. *Myelination*. Pp. 80-161. Springfield, IL: Thomas, 1970.
 74. Raine CS. Morphological aspects of myelin and myelination. In: Morell P, ed. *Myelin*. Pp. 1-41. New York: Plenum Press, 1977.
 75. Caley DW, Butler AB. Formation of central and peripheral myelin sheaths in the rat: an electron microscopic study. *Am J Anat* 1974; **140**: 339-46.
 76. Peters A. Observations on the connexions between myelin sheaths and glial cells in the optic nerve of young rats. *J Anat* 1964; **98**: 125-36.
 77. Bunge RP, Glass P. Some observations on myelin-glial relationships and on the etiology of the cerebrospinal fluid exchange lesion. *Ann NY Acad Sci* 1965; **122**: 15-22.
 78. Blakemore WF, Crang AJ, Evans RJ. The effect of chemical injury on oligodendrocytes. In: Mims CA, Cuzner ML, Kelly RE, eds. *Viruses and demyelinating diseases*. Pp. 167-90. London: Academic Press, 1983.
 79. Sato S, Fujita N, Kurihara T, Kuwano R, Sakimura K, Takahashi Y, Miyatake T. cDNA cloning and amino acid sequence for human myelin-associated glycoprotein. *Biochem Biophys Res Commun* 1989; **163**: 1473-80.
 80. Yoshida K, Kohsaka S, Nii S, *et al.* Subcultured astrocytes suppress the proliferation of neuroblasts *in vitro*. *Neurosci Lett* 1986; **70**: 34-9.
 81. Liuzzi FJ, Lasek RJ. Astrocytes block axonal regeneration in mammals by activating the physiological stop pathway. *Science* 1987; **237**: 642-5.
 82. Norton WT. Isolation and characterization of myelin. In: Morell P, ed. *Myelin*. Pp. 161-90. New York: Plenum Press, 1977.
 83. Rumsby MG. Organization and structure in central-nerve myelin. *Biochem Soc Trans* 1978; **6**: 448-62.
 84. Boggs JM, Moscarello MA, Papahadjopoulos D. Structural organization of myelin - role of lipid-protein interactions determined in model systems. In: Jost P, Griffith OH, eds. *Lipid-protein interactions*, Vol.

2. Pp. 1-51. New York: J. Wiley and Sons, 1982.
85. Boggs JM, Moscarello MA. Structural organization of the human myelin membrane. *Biochim Biophys Acta* 1978; **515**: 1-21.
 86. Carnegie PR, Moore WJ. Myelin basic protein. In: Bradshaw RA, Schneider DM, eds. *Proteins of the nervous system*. Pp. 119-43. New York: Raven Press, 1980.
 87. Harris R, Findlay JBC. Investigation of the organization of the major proteins in bovine myelin membranes. Use of chemical probes and bifunctional crosslinking reagents. *Biochim Biophys Acta* 1983; **732**: 75-82.
 88. Eylar RH, Brostoff S, Hashim G, Caccam J, Burnett P. Basic A1 protein of the myelin membrane. *J Biol Chem* 1971; **246**: 5770-84.
 89. Carnegie PR. Amino acid sequence of the encephalitogenic protein of human myelin. *Biochem J* 1971; **123**: 57-67.
 90. Martenson RE, Deibler GE, Kies MW. Comparison of amino-acid sequences of hypothalamic peptide, brain-specific histone and myelin basic protein. *Nature* 1971; **234**: 87-9.
 91. Martenson RE. Prediction of the secondary structure of myelin basic protein. *J Neurochem* 1981; **36**: 1543-60.
 92. Small DH, Carnegie PR. *In vivo* methylation of an arginine in chicken myelin basic protein. *J Neurochem* 1981; **38**: 184-90.
 93. Carnegie PR, Kemp BE, Dunkley PR, Murray AW. Phosphorylation of myelin basic protein by an adenosine 3':5'-cyclic monophosphate-dependent protein kinase. *Biochem J* 1973; **135**: 569-72.
 94. Miyamoto E, Kakiuchi S. *In vitro* and *in vivo* phosphorylation of myelin basic protein by exogenous and endogenous adenosine 3':5'-monophosphate-dependent protein kinase in brain. *J Biol Chem* 1974; **249**: 2769-77.
 95. Steck AJ, Appel SH. Phosphorylation of myelin basic protein. *J Biol Chem* 1974; **249**: 5416-20.
 96. Turner RS, Chou C-HJ, Kibler RF, Kuo JF. Basic protein in brain myelin is phosphorylated by endogenous phospholipid-sensitive Ca²⁺-dependent protein kinase. *J Neurochem* 1982; **39**: 1397-404.
 97. Kishimoto A, Nishiyama K, Nakanishi H, et al. Studies on the phosphorylation of myelin basic protein by protein kinase C and adenosine 3':5'-monophosphate-dependent protein kinase. *J Biol Chem* 1985; **260**: 12492-9.
 98. Ulmer JB, Braun PE. Chloroform markedly stimulates the phosphorylation of myelin basic proteins. *Biochem Biophys Res Commun* 1987; **146**: 1084-8.
 99. Chan K-FJ, Moscarello MA, Stoner GL, Webster HdeF. A novel fragmentation of human myelin basic protein: identification of phosphorylated domains. *Biochem Biophys Res Commun* 1987; **144**: 1287-95.
 100. Hagopian A, Westall FC, Whitehead JS, Eylar EH. Glycosylation of the A1 protein from myelin by a polypeptide N-acetylgalactosaminyltransferase. Identification of the receptor sequence. *J Biol Chem* 1971; **246**: 2519-23.
 101. Cruz TF, Moscarello MA. Identification of the major sites of enzymic glycosylation of myelin basic protein. *Biochim Biophys Acta* 1983; **760**: 403-10.
 102. Brostoff S, Eylar EH. Localization of methylated arginine in the A1 protein from myelin. *Proc Natl Acad Sci USA* 1971; **68**: 765-9.
 103. Chou FC-H, Chou C-HJ, Shapira R, Kibler RF. Basis of microheterogeneity of myelin basic protein. *J Biol Chem* 1976; **251**: 2671-9.
 104. Martenson RE, Law JM, Deibler GE. Identification of multiple *in vivo* phosphorylation sites in rabbit myelin basic protein. *J Biol Chem* 1983; **254**: 930-7.
 105. Deber CM, Cheifetz S, Moscarello MA. Microheterogeneity of bovine myelin basic protein studied by nuclear magnetic resonance spectroscopy. *Biopolymers* 1983; **22**: 377-80.
 106. Smyth DS, Utsumi S. Structure at the hinge region in rabbit immunoglobulin-G. *Nature* 1967; **216**: 332-5.
 107. Caamano CA, Zand R. Homologous sequences in cholera toxin A and B subunits to peptide domains in myelin basic protein. *FEBS Lett* 1989; **252**: 88-90.
 108. Martenson RE, Deibler GE, Kies MW. Microheterogeneity of guinea pig myelin basic protein. *J Biol Chem* 1969; **244**: 4261-3.
 109. Deibler GE, Martenson RE, Kramer AJ, Kies MW. The contribution of phosphorylation and loss of COOH-terminal arginine to the microheterogeneity of myelin basic protein. *J Biol Chem* 1975; **250**: 7931-8.
 110. Wood DD, Moscarello MA. The isolation, characterization, and lipid-aggregating properties of a citrulline containing myelin basic protein. *J Biol Chem* 1989; **264**: 5121-7.
 111. Martenson RE, Kramer AJ, Deibler GE. Microheterogeneity and phosphate content of myelin basic protein from 'freeze-blown' guinea-pig brains. *J Neurochem* 1976; **27**: 1529-31.
 112. Wu N-C, Ahmad J. Calcium- and cyclic AMP-regulated protein kinases of bovine central nervous system myelin. *Biochem J* 1984; **218**: 923-32.
 113. Endo T, Hidaka H. Ca²⁺-Calmodulin dependent phosphorylation of myelin isolated from rabbit brain. *Biochem Biophys Res Commun* 1980; **97**: 553-8.
 114. Chan K-FJ. Ganglioside-modulated protein phosphorylation. Partial purification and characterization of a ganglioside-inhibited protein kinase in brain. *J Biol Chem* 1988; **263**: 568-74.
 115. Yang S-D, Liu J-S, Fong Y-L, Yu J-S. Endogenous brain protein phosphatases in the brain myelin. *J Neurochem* 1987; **48**: 160-6.
 116. Vandenheede JR, Van Lint J, Vanden Abeele C, Merlevede W. Interaction of myelin basic protein with the different components of the ATP, Mg-dependent protein phosphatase system. *FEBS Lett* 1987; **211**: 190-4.
 117. Ulmer JB, Braun PE. *In vivo* phosphorylation of myelin basic proteins in developing mouse brain: evidence that phosphorylation is an early event in myelin formation. *Dev Neurosci* 1984; **6**: 345-55.
 118. Ulmer JB, Braun PE. *In vivo* phosphorylation of myelin basic proteins: age related differences in ³²P incorporation. *Dev Biol* 1986; **117**: 493-501.
 119. Schulz P, Cruz TF, Moscarello MA. Endogenous phosphorylation of basic protein of varying degrees of compaction. *Biochemistry* 1988; **27**: 7793-9.
 120. Vartanian T, Szuchet S, Dawson G, Campagnoni AT. Oligodendrocyte adhesion activates protein kinase C-mediated phosphorylation of myelin basic protein. *Science* 1986; **234**: 1395-8.
 121. Su HD, Kemp BE, Turner RS, Kuo JF. Synthetic myelin basic protein peptide analogs are specific inhibitors of phospholipid/calcium-dependent protein

- kinase (protein kinase C). *Biochem Biophys Res Commun* 1986; **134**: 78–84.
122. Deshmukh DS, Kuizon S, Brockerhoff H. Mutual stimulation by phosphatidylinositol-4-phosphate and myelin basic protein of their phosphorylation by the kinases solubilized from rat brain myelin. *Life Sci* 1984; **34**: 259–64.
 123. Chandekar LP, Paik WK, Kim S. Studies on myelin basic protein methylation during mouse brain development. *Biochem J* 1986; **240**: 471–9.
 124. Jacobson W, Grandy G, Sidman RL. Experimental subacute combined degeneration of the cord in mice. *J Pathol* 1973; **109**: pxiii–pxiv (abstr.).
 125. Young PR, Vacante DA, Waickus CM. Mechanism of the interaction between myelin basic protein and the myelin membrane: the role of arginine methylation. *Biochem Biophys Res Commun* 1987; **145**: 1112–8.
 126. Young JD, Tsuchiya D, Sandlin DE, Holroyde MJ. Enzymic O-glycosylation of synthetic peptides from sequences in basic myelin protein. *Biochemistry* 1979; **18**: 4444–8.
 127. Mononen I, Karjalainen E. Structural comparison of protein sequences around potential N-glycosylation sites. *Biochim Biophys Acta* 1984; **788**: 364–7.
 128. Fraser PE, Deber CM. Structure and function of the proline-rich region of myelin basic protein. *Biochemistry* 1985; **24**: 4593–8.
 129. Persaud R, Boggs JM, Wood DD, Moscarello MA. Interaction of glycosylated human myelin basic protein with lipid bilayers. *Biochemistry* 1989; **28**: 4209–16.
 130. De Ferra F, Engh H, Hudson L, et al. Alternative splicing accounts for the four forms of myelin basic protein. *Cell* 1985; **43**: 721–7.
 131. Takahashi N, Roach A, Teplow DB, Prusiner SB, Hood L. Cloning and characterization of the myelin basic protein gene from mouse: one gene can encode both the 14 Kd and 18.5 Kd MBPs by the alternate use of exons. *Cell* 1985; **42**: 139–48.
 132. Kamholz J, de Ferra F, Puckett C, Lazzarini R. Identification of three forms of human myelin basic protein by cDNA cloning. *Proc Natl Acad Sci USA* 1986; **83**: 4962–6.
 133. Miskimins R, Ebato H, Seyfried TN, Yu RK. Molecular basis for heterosis for myelin basic protein content in mice. *Proc Natl Acad Sci USA* 1986; **83**: 1532–5.
 134. Newman S, Kitamura K, Campagnoni AT. Identification of a cDNA coding for a fifth form of myelin basic protein in mouse. *Proc Natl Acad Sci USA* 1987; **84**: 886–90.
 135. Chapman BE, Moore WJ. Conformation of myelin basic protein in aqueous solution from nuclear magnetic resonance spectroscopy. *Biochem Biophys Res Commun* 1976; **73**: 758–65.
 136. Littlemore LAT. N.M.R. studies on myelin basic protein. II ^1H N.M.R. studies of the protein and constituent peptides in aqueous solutions. *Aust J Chem* 1978; **31**: 2387–98.
 137. Littlemore LAT, Ledeen R. N.M.R. studies of myelin basic protein. III. Interaction of the protein with lipid micelles by ^1H and ^{31}P N.M.R. *Aust J Chem* 1979; **32**: 2631–6.
 138. Gow A, Smith R. The thermodynamically stable state of myelin basic protein in aqueous solution is a flexible coil. *Biochem J* 1989; **257**: 535–40.
 139. Whitaker JN, Chou C-HJ, Chou FC-H, Kibler RF. Molecular internalization of A region of myelin basic protein. *J Exp Med* 1977; **146**: 317–31.
 140. Jones AJS, Epand RM. Effect of microheterogeneity on the structure and function of the myelin basic protein. *Biochim Biophys Acta* 1980; **625**: 165–78.
 141. Randall CS, Zand R. Spectroscopic assessment of secondary and tertiary structure in myelin basic protein. *Biochemistry* 1985; **24**: 1998–2004.
 142. Randall CS, Zand R. Microcalorimetric studies of the heats of solution of bovine myelin basic protein. *Biochim Biophys Acta* 1985; **831**: 242–8.
 143. Anthony JS, Moscarello MA. A conformation change induced in the basic encephalitogen by lipids. *Biochim Biophys Acta* 1971; **243**: 429–33.
 144. Liebes LF, Zand R, Phillips WD. Solution behaviour. Circular dichroism and 220 MHz PMR studies of the bovine myelin basic protein. *Biochim Biophys Acta* 1975; **405**: 27–39.
 145. Keniry MA, Smith R. Circular dichroic analysis of the secondary structure of myelin basic protein and derived peptides bound to detergents and to lipid vesicles. *Biochim Biophys Acta* 1979; **578**: 381–91.
 146. Keniry MA, Smith R. Dependence on lipid structure of the coil-to-helix transition of bovine myelin basic protein. *Biochim Biophys Acta* 1981; **668**: 107–18.
 147. Eylar EH, Thompson M. Allergic encephalomyelitis: the physicochemical properties of the basic protein encephalitogen from bovine spinal cord. *Arch Biochem Biophys* 1969; **129**: 469–79.
 148. Chao IP, Einstein ER. Physical properties of bovine encephalitogenic protein: molecular weight and conformation. *J Neurochem* 1970; **17**: 1121–32.
 149. Stoner GL. Predicted folding of β -structure in myelin basic protein. *J Neurochem* 1984; **43**: 443–7.
 150. Surewicz WK, Moscarello MA, Mantsch HH. Fourier transform infrared spectroscopic investigation of the interaction between myelin basic protein and dimyristoylphosphatidylglycerol bilayers. *Biochemistry* 1987; **26**: 3881–6.
 151. Deber CM, Moscarello MA, Wood DD. Conformational studies on ^{13}C -enriched human and bovine myelin basic protein, in solution and incorporated into liposomes. *Biochemistry* 1978; **17**: 898–903.
 152. Fraser PE, Rand RP, Deber CM. Bilayer-stabilizing properties of myelin basic protein in dioleoylphosphatidylethanolamine systems. *Biochim Biophys Acta* 1989; **983**: 23–9.
 153. Rand RP, Fuller N, Lis LJ. Myelin swelling and measurement of forces between myelin membranes. *Nature* 1979; **279**: 258–60.
 154. Kirschner DA, Ganser AL. Compact myelin exists in the absence of basic protein in the shiverer mutant mouse. *Nature* 1980; **283**: 207–10.
 155. Readhead C, Popko B, Takahashi N, et al. Expression of a myelin basic protein gene in transgenic shiverer mice: correction of the dysmyelinating phenotype. *Cell* 1987; **48**: 703–12.
 156. Dupouey P, Jacque C, Bourre JM, Cesselin F, Privat A, Baumann N. Immunohistochemical studies of myelin basic protein in shiverer mouse devoid of major dense line of myelin. *Neurosci Lett* 1979; **12**: 113–8.
 157. Sternberger NH, Itoyama Y, Kies MW, Webster HdeF. Myelin basic protein demonstrated immunocytochemically in oligodendroglia prior to myelin sheath formation. *Proc Natl Acad Sci USA* 1978; **75**:

- 2521-4.
158. Imamoto K, LeBlond CP. Radioautographic investigation of gliogenesis in the corpus callosum of young rats. *J Comp Neurol* 1978; **180**: 139-64.
 159. Zeller NK, Behar TN, Dubois-Dalcq ME, Lazzarini RA. The timely expression of myelin basic protein gene in cultured rat brain oligodendrocytes is independent of continuous neuronal influences. *J Neurosci* 1985; **5**: 2955-62.
 160. Kristensson K, Holmes KV, Duchala CS, Zeller NK, Lazzarini RA, Dubois-Dalcq M. Increased levels of myelin basic protein transcripts gene in virus-induced demyelination. *Nature* 1986; **322**: 544-7.
 161. Lampe PD, Nelsestuen GL. Myelin basic protein-enhanced fusion of membranes. *Biochim Biophys Acta* 1982; **693**: 320-5.
 162. Boggs JM, Moscarello MA, Papahadjopoulos D. Phase separation of acidic and neutral phospholipids induced by human myelin basic protein. *Biochemistry* 1977; **16**: 5420-6.
 163. Gould RM, London Y. Specific interaction of central nervous system myelin basic protein with lipids. Effects of basic protein in glucose leakage from liposomes. *Biochim Biophys Acta* 1972; **290**: 200-18.
 164. Stollery JG, Boggs JM, Moscarello MA, Deber CM. Direct observation by carbon-13 nuclear magnetic resonance of membrane-bound human myelin basic protein. *Biochemistry* 1980; **19**: 2391-6.
 165. Hughes DW, Stollery JG, Moscarello MA, Deber CM. Binding of myelin basic protein to phospholipid micelles. *J Biol Chem* 1982; **257**: 4698-700.
 166. Smith R. ¹H-nuclear magnetic resonance study of the association of the basic protein of central nervous system myelin with lysophosphatidylcholine. *Biophys Chem* 1982; **16**: 347-54.
 167. Smith R, Cornell BA, Keniry MA, Separovic F. ³¹P Nuclear magnetic resonance studies of the association of basic proteins with multilayers of diacyl phosphatidylserine. *Biochim Biophys Acta* 1983; **732**: 492-8.
 168. Boggs JM, Stollery JG, Moscarello MA. Effect of lipid environment on the motion of a spin-label covalently bound to myelin basic protein. *Biochemistry* 1980; **19**: 1226-33.
 169. Boggs JM, Wood DD, Moscarello MA. Hydrophobic and electrostatic interactions of myelin basic protein with lipid. Participation of N-terminal and C-terminal portions. *Biochemistry* 1981; **20**: 1065-73.
 170. Boggs JM, Stamp D, Moscarello MA. Interaction of myelin basic protein with dipalmitoylphosphatidylglycerol: dependence on the lipid phase and investigation of a metastable state. *Biochemistry* 1981; **20**: 6066-72.
 171. Vadas EB, Melancon P, Braun PE, Galley WC. Phosphorescence studies of the interaction of myelin basic protein with phosphatidylserine vesicles. *Biochemistry* 1981; **20**: 3110-6.
 172. Jones AJS, Rumsby MG. The intrinsic fluorescence characteristics of the myelin basic protein. *J Neurochem* 1975; **25**: 565-72.
 173. MacNaughtan W, Snook KA, Caspi E, Franks NP. An X-ray diffraction analysis of oriented lipid multilayers containing basic proteins. *Biochim Biophys Acta* 1985; **818**: 132-48.
 174. Deber CM, Hughes DW, Fraser PE, Pawagi AB, Moscarello MA. Binding of normal and multiple sclerosis-derived myelin basic protein to phospholipid vesicles: effects of membrane head group and bilayer regions. *Arch Biochem Biophys* 1986; **245**: 455-63.
 175. Golds EE, Braun PE. Protein association and basic protein conformation in the myelin membrane. *J Biol Chem* 1978; **253**: 8162-70.
 176. Golds EE, Braun PE. Crosslinking studies on the conformation and dimerization of myelin basic protein in solution. *J Biol Chem* 1978; **253**: 8171-7.
 177. Smith R. Self-association of myelin basic protein: enhancement by detergents and lipids. *Biochemistry* 1982; **21**: 2697-701.
 178. Moskaitis JE, Shriver LC, Campagnoni AT. The association of myelin basic protein with itself and other proteins. *Neurochem Res* 1987; **12**: 409-17.
 179. Smith R. Noncovalent cross-linking of lipid bilayers by myelin basic protein - a possible role in myelin formation. *Biochim Biophys Acta* 1977; **470**: 170-84.
 180. Mendz GL, Moore WJ, Brown LR, Martenson RE. Interaction of myelin basic protein with micelles of dodecylphosphocholine. *Biochemistry* 1984; **23**: 6041-6.
 181. Mendz GL, Moore WJ, Kaplin IJ, et al. Characterization of dodecylphosphocholine/myelin basic protein complexes. *Biochemistry* 1988; **27**: 379-86.
 182. Brady GW, Fein DB, Wood DD, Moscarello MA. The role of charge microheterogeneity of human myelin basic protein in the formation of phosphatidylglycerol multilayers. *Biochem Biophys Res Commun* 1985; **126**: 1161-5.
 183. Moscarello MA, Brady GW, Fein DB, Wood DD, Cruz TF. The role of charge microheterogeneity of basic protein in the formation and maintenance of the multilayered structure of myelin: a possible role in multiple sclerosis. *J Neurosci Res* 1986; **15**: 87-99.
 184. Cheifetz S, Moscarello MA. Effect of bovine basic protein charge microheterogeneity on protein-induced aggregation of unilamellar vesicles containing a mixture of acidic and neutral phospholipids. *Biochemistry* 1985; **24**: 1909-14.
 185. Cheifetz S, Boggs JM, Moscarello MA. Increase in vesicle permeability mediated by myelin basic protein: effect of phosphorylation of basic protein. *Biochemistry* 1985; **24**: 5170-5.
 186. Pettinelli CB, McFarlin DE. Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after *in vitro* activation of lymph node cells by myelin basic protein: requirement for Lyt 1+2- T lymphocytes. *J Immunol* 1981; **127**: 1420-3.
 187. Swanborg R. Autoimmune effector cells. V. A monoclonal antibody specific for rat helper T lymphocytes inhibits adoptive transfer of autoimmune encephalomyelitis. *J Immunol* 1983; **130**: 1503-5.
 188. Ellerman KE, Powers JM, Brostoff SW. A suppressor T-lymphocyte cell line for autoimmune encephalomyelitis. *Nature* 1988; **331**: 265-7.
 189. Zamvil S, Nelson P, Trotter J, et al. T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. *Nature* 1985; **317**: 355-8.
 190. Price WS, Mendz GL, Martenson E. Conformation of a heptadecapeptide comprising the segment encephalitogenic in rhesus monkey. *Biochemistry* 1988; **27**: 8990-9.
 191. Moscarello MA. Chemical and physical properties of

- myelin proteins. In: Bronner F, Kleinzeller A, eds. *Current topics in membranes and transport*, Vol. 8. Pp. 1-28. New York: Academic Press, 1976.
192. Folch J, Lees M. Proteolipids, a new type of tissue lipoproteins. *J Biol Chem* 1951; 191: 807-17.
 193. Lees MB, Chao BH, Lin L-FH, Samiullah M, Laursen RA. Amino acid sequence of bovine white matter proteolipid. *Arch Biochem Biophys* 1983; 226: 643-56.
 194. Jolles J, Nussbaum J-L, Jolles P. Enzymic and chemical fragmentation of the apoprotein of the major rat brain myelin proteolipid. Sequence data. *Biochim Biophys Acta* 1983; 742: 33-8.
 195. Milner RJ, Lai C, Nave K-A, Lenoir D, Ogata J, Sutcliffe JG. Nucleotide sequences of two mRNAs for rat brain myelin proteolipid protein. *Cell* 1985; 42: 931-9.
 196. Willard HF, Riordan JR. Assignment of the gene for myelin proteolipid protein to the X chromosome: implications for X-linked myelin disorders. *Science* 1985; 230: 940-2.
 197. Naismith AL, Hoffman-Chudzik E, Tsui L-C, Riordan JR. Study of the expression of myelin proteolipid protein (lipophilin) using a cloned complementary DNA. *Nucl Acids Res* 1985; 13: 7413-25.
 198. Yamamura T, Namikawa T, Endoh M, Kunishita T, Tabira T. Passive transfer of experimental allergic encephalomyelitis induced by proteolipid apoprotein. *J Neurol Sci* 1986; 76: 269-75.
 199. Trotter JL, Clark HB, Collins KG, Wegeschiede CL, Scarpellini JD. Myelin proteolipid protein induces demyelinating disease in mice. *J Neurol Sci* 1987; 79: 173-88.
 200. Laursen RA, Samiullah M, Lees MB. The structure of bovine brain myelin proteolipid and its organization in myelin. *Proc Natl Acad Sci USA* 1984; 81: 2912-6.
 201. Stoffel W, Hillen H, Giersiefen H. Structure and molecular arrangement of proteolipid protein of central nervous system myelin. *Proc Natl Acad Sci USA* 1984; 81: 5012-6.
 202. Edwards AM, Ross NW, Ulmer JB, Braun PE. Interaction of myelin basic protein and proteolipid protein. *J Neurol Res* 1989; 22: 97-102.
 203. Simons R, Alon N, Riordan JR. Human myelin DM-20 proteolipid protein deletion defined by cDNA sequence. *Biochem Biophys Res Commun* 1987; 146: 666-71.
 204. Weise MJ. Hydrophobic regions in myelin proteins characterized through analysis of "hydrophobic" profiles. *J Neurochem* 1985; 44: 163-70.
 205. Stoffel W, Subkowski T, Jander S. Topology of proteolipid protein in the myelin membrane of central nervous system. *Hoppe Seyler's Z. Physiol Chem* 1989; 370: 165-76.
 206. Kahan I, Moscarello MA. Identification of membrane-embedded domains of lipophilin from human myelin. *Biochemistry* 1985; 24: 538-44.
 207. Kyte J, Doolittle RF. A simple method for displaying the hydrophobic character of a protein. *J Mol Biol* 1982; 157: 105-32.
 208. Schulz GE, Schirmer RH. *Principles of protein structure*. Pp. 66-106. Heidelberg: Springer-Verlag, 1979.
 209. Surewicz WK, Moscarello MA, Mantsch HH. Secondary structure of the hydrophobic myelin protein in a lipid environment as determined by Fourier-transform infrared spectrometry. *J Biol Chem* 1987; 262: 8598-402.
 210. Cockle SA, Epand RM, Stollery JG, Moscarello MA. Nature of cysteinyl residues in lipophilin from human myelin. *J Biol Chem* 1980; 255: 9182-8.
 211. Papahadjopoulos D, Vail WJ, Moscarello MA. Interaction of a purified hydrophobic protein from myelin with phospholipid membranes: studies on ultrastructure, phase transitions and permeability. *J Membr Biol* 1975; 22: 143-64.
 212. Boggs JM, Vail WJ, Moscarello MA. Preparation and properties of vesicles of a purified hydrophobic myelin protein and phospholipid: a spin label study. *Biochim Biophys Acta* 1976; 448: 517-30.
 213. Meier P, Sachse J-H, Brophy PJ, Marsh D, Kothe G. Integral membrane proteins significantly decrease the molecular motion in lipid bilayers: a deuterium NMR relaxation study of membranes containing myelin proteolipid apoprotein. *Proc Natl Acad Sci USA* 1987; 84: 3704-8.
 214. Braun PE, Radin NS. Interaction of lipids with a membrane structural protein from myelin. *Biochemistry* 1969; 8: 4310-8.
 215. Stoffyn P, Folch-Pi J. On the type of linkage binding fatty acids present in brain white matter proteolipid apoprotein. *Biochem Biophys Res Commun* 1971; 44: 157-61.
 216. Townsend LE, Agrawal D, Benjamins JA, Agrawal HC. *In vitro* acylation of rat brain myelin proteolipid protein. *J Biol Chem* 1982; 257: 9745-50.
 217. Yoshimura T, Agrawal D, Agrawal HC. Cell-free acylation of rat brain myelin proteolipid protein and DM-20. *Biochem J* 1987; 246: 611-7.
 218. Stoffel W, Hillen H, Schroeder W, Deutzmann R. The primary structure of bovine brain myelin lipophilin (proteolipid apoprotein). *Hoppe Seyler's Z. Physiol Chem* 1983; 364: 1455-66.
 219. Bizzozero OA, McGarry JG, Lees MB. Autoacylation of myelin proteolipid protein with acyl coenzyme A. *J Biol Chem* 1987; 262: 13550-7.
 220. O'Brien PJ, St Jules RS, Reedy TS, Bazan NG, Katz M. Acylation of disc membrane rhodopsin may be nonenzymatic. *J Biol Chem* 1987; 262: 5210-5.
 221. Diehl H-J, Schaich M, Budzinski R-M, Stoffel W. Individual exons encode the integral membrane domains of human myelin proteolipid protein. *Proc Natl Acad Sci USA* 1986; 83: 9807-11.
 222. Nave KA, Lai C, Bloom FE, Milner RJ. Jimpy mutant mouse: a 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in RNA splicing. *Proc Natl Acad Sci USA* 1986; 83: 9264-8.
 223. Hudson LD, Berndt JA, Puckett C, Kozak CA, Lazarini RA. Aberrant splicing of proteolipid protein mRNA in the dysmyelinating jimpy mutant mouse. *Proc Natl Acad Sci USA* 1987; 84: 1454-8.
 224. Duncan ID, Hammang JP, Trapp BD. Abnormal compact myelin in the myelin-deficient rat: absence of proteolipid protein correlates with a defect in the intraperiod line. *Proc Natl Acad Sci USA* 1987; 84: 6287-91.
 225. Trotter JL, Clark HB. Contamination of proteolipid protein with basic protein. *Ann Neurol* 1984; 16: 513-4.
 226. Hashim GA, Wood DD, Moscarello MA. Myelin-lipophilin induced experimental allergic encephalo-

- myelitis. *Neurochem Res* 1980; **5**: 1137-45.
227. Cambi F, Lees MB, Williams RM, Macklin WB. Chronic experimental allergic encephalomyelitis produced by bovine proteolipid apoprotein: immunological studies in rabbits. *Ann Neurol* 1983; **13**: 303-8.
 228. Kirschner DA, Caspar DLD. Diffraction studies of molecular organization in myelin. In: Morell P, ed. *Myelin*. Pp. 51-89. New York: Plenum Press, 1977.
 229. Horrocks LA. The alk-1-enyl group content of mammalian myelin phosphoglycerides by quantitative two-dimensional thin layer chromatography. *J Lipid Res* 1968; **9**: 469-74.
 230. Sun GY, Samorajski T. Age differences in the acyl group composition of phosphoglycerides in myelin isolated from the brain of the rhesus monkey. *Biochim Biophys Acta* 1973; **316**: 19-24.
 231. Curatolo W. Thermal behavior of fractionated and unfractionated bovine brain cerebroside. *Biochemistry* 1982; **21**: 1761-4.
 232. Ledeen RW, Cochran FB, Yu RK, Samuels FG, Haley JE. Gangliosides of the CNS myelin membrane. *Adv Exp Med Biol* 1980; **125**: 167-76.
 233. Hauser G, Eichberg J. The subcellular distribution of polyphosphoinositides in myelinated and unmyelinated rat brain. *Biochim Biophys Acta* 1973; **326**: 210-7.
 234. Svennerholm L, Vanier MT, Jungbjer B. Changes in fatty acid composition of human brain myelin lipids during maturation. *J Neurochem* 1978; **30**: 1383-90.
 235. Svennerholm L, Vanier MT. Lipid and fatty acid composition of human cerebral myelin during development. *Adv Exp Biol Med* 1979; **100**: 27-41.
 236. Boggs JM, Rangaraj G. Changes in the composition of two molecular species of ethanolamine plasmalogen in normal human myelin during development. *Biochim Biophys Acta* 1984; **793**: 313-6.
 237. Hosein ZZ, Gilbert JJ, Strejan GH. The role of myelin lipids in experimental allergic encephalomyelitis. In: Alvord EC, Jr., ed. *Experimental allergic encephalomyelitis: a useful model for multiple sclerosis*. Pp. 49-54. New York: Alan R. Liss, Inc., 1984.
 238. Carroll WM, Jennings AR, Mastaglia FL. Experimental demyelinating optic neuropathy induced by intraneural injection of galactocerebroside antiserum. *J Neurol Sci* 1984; **65**: 125-35.
 239. Ariga T, Kohriyama T, Freddo L, et al. Characterization of sulfated glucuronic acid containing glycolipids reacting with IgM M-proteins in patients with neuropathy. *J Biol Chem* 1987; **262**: 848-53.
 240. Wood DD, Moscarello MA. Is the myelin membrane abnormal in multiple sclerosis? *J Membrane Biol* 1984; **79**: 195-201.
 241. Davison AW, Wajda M. Cerebral lipids in multiple sclerosis. *J Neurochem* 1962; **9**: 427-32.
 242. Wender M, Filipek-Wender H, Stanislawski J. Cholesteryl esters of the brain in demyelinating diseases. *Clin Chim Acta* 1974; **54**: 269-75.
 243. Shah SN, Johnson RC. Activity levels of cholesterol ester metabolizing enzymes in brain in multiple sclerosis: correlation with cholesterol ester concentrations. *Exp Neurol* 1980; **68**: 601-4.
 244. Trotter J, Smith ME. Macrophage-mediated demyelination: the role of phospholipases and antibody. In: Alvord EC, Jr., ed. *Experimental allergic encephalomyelitis: a useful model for multiple sclerosis*. Pp. 55-60. New York: Alan R. Liss, Inc., 1984.
 245. Yu RK, Ledeen RW, Eng LF. Ganglioside abnormalities in multiple sclerosis. *J Neurochem* 1974; **23**: 169-74.
 246. Low PA, Schmelzer JD, Yao JK, Dyck PJ, Parthasarathy S, Baumann WJ. Structural specificity in demyelination induced by lysophospholipids. *Biochim Biophys Acta* 1983; **754**: 298-304.
 247. Weiner HL, Hauser SL. Neuroimmunology II: antigenic specificity of the nervous system. *Ann Neurol* 1982; **12**: 499-509.
 248. Turner RS, Raynor RL, Mazzei GJ, Kuo JF. Developmental studies of phospholipid-sensitive Ca^{2+} -dependent protein kinase and its substrates of phosphoprotein phosphatases in rat brain. *Proc Natl Acad Sci USA* 1984; **81**: 3143-7.