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Proteolipid protein: Chemistry and role in demyelinating disorders

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Our studies provide fundamental information on the structural and dynamic properties of proteolipid protein (PLP) and its role in the development, maintenance and function of CNS myelin and in the pathological processes associated with demyelinating disorders, particulary those involving autoimmune phenomena. The immunological aspects of the PLP-induced model are comparable to those of whole CNS tissue- and myelin basic protein-induced experimental allergic encephalomyelitis in other species and is similar to that of multiple sclerosis. The characteristics of the PLP-induced disease make it a useful model for understanding autoimmune processes in the nervous system and the contribution of PLP to the pathophysiology of multiple sclerosis.

Key words: proteolipid protein, myelination, demyelination, experimental allergic encephalomyelitis.

The myelin proteolipid protein (PLP) is the major protein of CNS myelin, where it is present in greater amounts than the more familiar and well-studied myelin basic protein (see [16] for review). The myelin PLP is characterized by its solubility properties [10] and is extracted from brain white matter with organic solvents, specifically 2:1 chloroform-methanol. The extract contains both protein and lipid, but it is possible to remove all of the non-covalently bound lipid. The apoprotein thus obtained retains its solubility in chloroform-methanol mixtures but can be converted to a water-soluble form under specified conditions. Thus, an important characteristic of the protein is that it shows a conformational flexibility. An additional characteristic, which will be discussed below, is that the apoprotein contains 2-4% covalently bound fatty acid [30].

The proteolipid is generally considered a structural protein and, therefore, its identification depends on its mobility on sodium dodecyl sulfate-polyacrylamide gels. However, since many proteins can migrate to the same position on a gel, for specific identification the electrophoretic separation must be combined with an immunologic probe on immunoblots. Using an immunoblot procedure, we have been able to detect as little as 250 ng PLP and thereby have confirmed that PLP is

absent from the peripheral nervous system and from non-neural tissues [21]. We have also followed the developmental appearance of PLP in the rat and found that it can first be detected in the spinal cord at 2 days and in upper brain regions at 10 days, at about the same time as myelin basic protein is detected [23].

Structural features

The primary structure of PLP was initially determined by amino acid sequencing [18] and subsequently confirmed by nucleotide sequencing [24] (Fig. 1). The protein has a molecular weight of 29,869, but its apparent molecular weight on gels is only 24-25,000. The reason for this discrepancy is that PLP has a higher zero electrophoretic mobility than the water-soluble proteins used as standards [8]. The protein contains 276 amino acids arranged in a strong domain structure of alternating hydrophobic and hydrophilic regions (Fig. 1). The protein is highly conserved during evolution with 90% or greater homology among the species thus far sequenced (bovine, human, rat and mouse).

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                                   10                                  20
Gly-Leu-Leu-Glu-Cys-Cys-Ala-Arg-Cys-Leu-Val-Gly-Ala-Pro-Phe-Ala-Ser-Leu-Val-Ala-

                                   30                                  40
Thr-Gly-Leu-Cys-Phe-Phe-Gly-Val-Ala-Leu-Phe-Cys-Gly-Cys-Gly-His-Glu-Ala-Leu-Thr-

                                   50                                  60
Gly-Thr-Glu-Lys-Leu-Ile-Glu-Thr-Tyr-Phe-Ser-Lys-Asn-Tyr-Gln-Asp-Tyr-Glu-Tyr-Leu-

                                   70                                  80
Ile-Asn-Val-Ile-His-Ala-Phe-Gln-Tyr-Val-Ile-Tyr-Gly-Thr-Ala-Ser-Phe-Phe-Phe-Leu-

                                   90                                 100
Tyr-Gly-Ala-Leu-Leu-Leu-Ala-Tyr-Gly-Phe-Tyr-Thr-Thr-Gly-Ala-Val-Arg-Gln-Ile-Phe-

                                  110                                 120
Gly-Asp-Tyr-Lys-Thr-Thr-Ile-Cys-Gly-Lys-Gly-Leu-Ser-Ala-Thr-Val-Thr-Gly-Gly-Gln-

                                  130                                 140
Lys-Gly-Arg-Gly-Ser-Arg-Gly-Gln-His-Gln-Ala-His-Ser-Leu-Glu-Arg-Val-Cys-His-Cys-

                                  150                                 160
Leu-Gly-Lys-Trp-Leu-Gly-His-Pro-Asp-Lys-Phe-Val-Gly-Ile-Thr-Tyr-Ala-Leu-Thr-Val-

                                  170                                 180
Val-Trp-Leu-Leu-Val-Phe-Ala-Cys-Ser-Ala-Val-Pro-Val-Tyr-Ile-Tyr-Phe-Asn-Thr-Trp-

                                  190                                 200
Thr-Thr-Cys-Gln-Ser-Ile-Ala-Ala-Pro-Ser-Lys-Thr-Ser-Ala-Ser-Ile-Gly-Thr-Leu-Cys-

                                  210                                 220
Ala-Asp-Ala-Arg-Met-Tyr-Gly-Val-Leu-Pro-Trp-Asn-Ala-Phe-Pro-Gly-Lys-Val-Cys-Gly-

                                  230                                 240
Ser-Asn-Leu-Leu-Ser-Ile-Cys-Lys-Thr-Ala-Glu-Phe-Gln-Met-Thr-Phe-His-Leu-Phe-Ile-

                                  250                                 260
Ala-Ala-Phe-Val-Gly-Ala-Ala-Ala-Thr-Leu-Val-Ser-Leu-Val-Thr-Phe-Met-Ile-Ala-Ala-

                                  270
Thr-Tyr-Asn-Phe-Ala-Val-Leu-Lys-Leu-Met-Gly-Arg-Gly-Thr-Lys-Phe
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Fig. 1. Amino acid sequence of bovine white matter proteolipid protein. Hydrophobic regions are underlined

As a consequence of our knowledge of the amino acid sequence, it has been possible to develop a model of the orientation of PLP in the myelin membrane (Fig. 2) [15]. The model contains 3 helical trans-membrane segments which pass through the bilayer and two cis-membrane segments which enter and leave on the same side of the membrane. An alternate model has been proposed by Stoffel and collaborators [29]. The two models are similar in the orientation of the carboxyl terminal part of the molecule, residues 190 to 276. They are also similar in the localization of the amino terminus on the extracellular face and in the location of free sulfhydryl groups in hydrophilic loops. The major differences derive from our placement of a cis segment at the amino terminus, whereas Stoffel places it close to the middle of the molecule. In our model a β turn at proline residue 14 leads to the alignment of cysteine residues opposite one another to form 3 pairs of disulfide linkages. Since most of the cys residues of the protein are in the disulfide rather than the free thiol form, the proposed structure appears realistic. As a consequence, in our model the highly charged basic region (residues 90-151) is cytoplasmic, whereas it is extracellularly localized by Stoffel et al. At this point, both models remain hypothetical. Nevertheless, they are important since they bring to the fore several new features of the molecule: a) PLP shows internal homology, e. g. the transmembrane segments show a repeating structure [14]; b) a portion of the molecule shows structural similarity to myelin basic protein [19]; and c) the covalently bound fatty acid (see below) is located in a hydrophilic region. The fatty acid would increase the hydrophobicity of this region and could act to hold together the multilamellar myelin structure. Both models place this region at the extracellular face of the membrane, and our experimental evidence confirms this location [20].

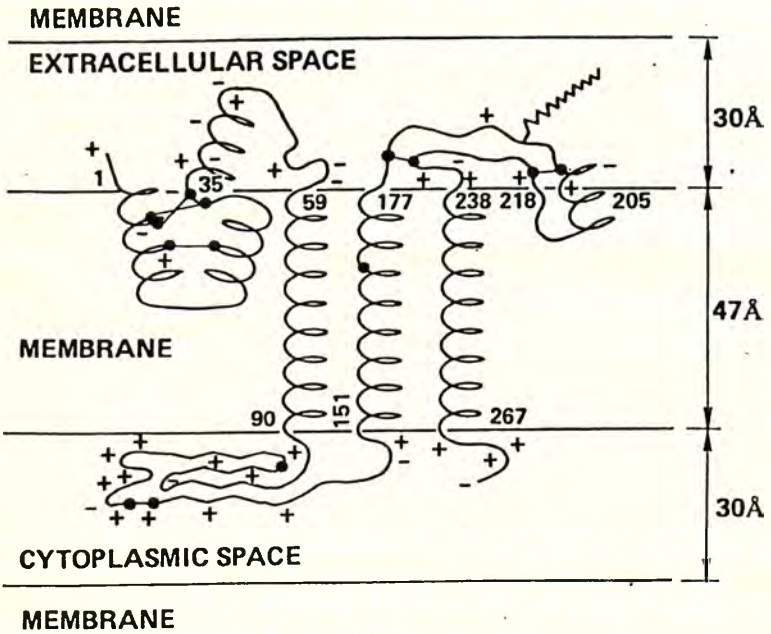

Fig. 2. Proposed orientation of the proteolipid in the myelin membrane, modified from Laursen et al. [15]. Numbers refer to residue numbers in the linear sequence of the protein

Acylation

PLP contains approximately 2 moles of covalently bound fatty acids per mole of protein, and these occur at specific sites in the protein. Thus far, only serine or threonine residue 198 has been identified as an acylated site. The fatty acids can be cleaved by hydroxylamine and are, therefore, bound in an O-ester linkage. The predominant fatty acid is palmitic (55%), with lesser amounts of oleic (26%) and stearic (19%) [30].

Acylation is the only known post-translational modification of PLP. Since acylation continues after protein synthesis is inhibited by cycloheximide, and since transport from the Golgi to the myelin membrane is not blocked with monensin or colchicine [6, 31], it appears that acylation takes place after synthesis, transport and incorporation of the protein into the myelin membrane. With Dr. Oscar Bizzozero, we have been studying the mechanism of the acylation reaction. First, we demonstrated that activated fatty acid is the immediate donor and that acylation occurs within myelin [1]. The in vitro acylation showed all the properties of an enzymatic reaction including Michaelis-Menten kinetics, fatty acid specificity and site specificity [3, 4]. Our more recent studies have shown that under suitable conditions, isolated, deacylated PLP can be acylated by palmitoyl-CoA with no requirement for the addition of any subcellular fraction or other source of enzyme [5]. Thus, fatty acid addition to PLP appears to be an autoacylation process, i.e., the protein acts as both the substrate and the acylating enzyme; a separate enzyme is not required. The mechanism by which the autoacylation occurs is not as yet known, but sulfhydryl groups and the conformational flexibility of the protein may be involved.

The covalently bound fatty acids may be of importance in certain demyelinating diseases. Adrenoleukodystrophy is an X-linked progressive neurological disorder characterized by demyelination, inflammation and an accumulation of very long chain fatty acid esters (C 24-30). PLP from adrenoleukodystrophy autopsy material shows a 2- to 3-fold increase in saturated C25 to C27 fatty acids and more than an 8-fold increase in C28 to C30 fatty acids, at the expense of oleic acid [2]. These fatty acid abnormalities do not represent the primary defect, but they may explain part of the pathophysiology of adrenoleukodystrophy. A study of this disease may well lead to important clues concerning the normal function of the covalently bound fatty acid. It is also of interest that among all of the genetic disorders of myelin adrenoleukodystrophy is the only one in which there is an inflammatory response. Thus, an understanding of this desease may be relevant to multiple sclerosis.

Role of PLP in experimental allergic encephalomyelitis (EAE)

It has long been recognized that myelin basic protein does not account for all the effects of whole tissue in the induction of EAE and that other components are involved. As the most abundant CNS myelin protein, PLP can be expected to play a role in autoimmune responses in the nervous system. Such speculation is reinforced by the fact that we have demonstrated that regions of the PLP molecule are on the extracellular face of the myelin membrane where they could be exposed to either normal or invading components of the immune system. A role for PLP in EAE was first suggested in the 1950s (see [12] for reviews of early studies), but at that time the presence of contaminating myelin basic protein could not be convincingly ruled out. However, modern methodological tools have

now demonstrated unequivocally that PLP preparations are free of myelin basic protein [17] and that PLP *per se* has an encephalitogenic effect [7, 26, 33, 36, 37, 38].

For the last several years we have been studying the effects of immunization of rabbits with proteolipid apoprotein, along with complete Freund's adjuvant [7, 36]. Symptoms appeared one to six months after immunization and were characterized first by hind limb weakness and ataxia and then by flaccid paralysis, progressing to spastic paralysis and incontinence. In most animals the disease followed a chronic progressive course, but a relapsing course was observed in several rabbits. More recently, the disease has developed consistently 3 to 8 weeks after immunization. When the rabbits first showed clinical symptoms, a positive delayed type hypersensitivity was observed with the induration and erythema greatest in the sickest animals [34]. Histologically, the disease was characterized by meningitis with mononuclear lymphocytes accompanied by demyelination and reactive gliosis. The infiltrates were perivascular, and quantitation showed that sicker animals had a greater amount of infiltration. Immunocytochemical studies using antibodies against T cells and Ia showed both perivascular and diffuse infiltrates [28]. Our more recent studies have shown similar responses to PLP in mice [33]. S/JL mice show an acute form of the disease, whereas other strains show a more chronic form. Other investigators have also shown similar results in mice, Lewis rats and Hartley guinea pigs [37, 38]. Our studies with mice indicated that: a) the susceptibility of various strains of mice to PLP-induced EAE is not controlled solely by immune response genes; b) the strain susceptibility to PLP differs from that of myelin basic protein; and c) the varied clinical, histologic and genetic responses involved in PLP-induced EAE in mice may be comparable to the varied expression of multiple sclerosis in humans (T u o h y, unpublished).

Two characteristics of PLP may be relevant to its possible contribution to the complex series of events leading to multiple sclerosis. Since PLP is a transmembrane protein, exposed regions occur on both the cytoplasmic and the extracellular faces of the myelin membrane. The extracellular regions in particular provide potential for the involvement of mechanisms different from those found for myelin basic protein which is localized exclusively to the cytoplasmic face. Furthermore, we have found that the sequences of the exposed regions have extensive analogies with many viral sequences, even more than does myelin basic protein [27]. Thus, PLP may share similar epitopes with many different viruses. For example, an adenovirus protein contains a sequence identical to 7 of the first 9 amino acids at the amino terminus of PLP and 11 of the first 19 amino acids:

PLP (1-20) GLLECCARCLVGAPFASLV
Adenovirus (561-579) GLLECHCRCNLCTPHRSLV.

The amino terminus and the region consisting of residues 135-153 show similarities to a particularly large number of viruses including Epstein-Barr virus and rodent polyoma viruses. Other regions show similarities to human adult T cell leukemia viruses, influenza virus and even the AIDS virus, although the latter is not as striking as some of the other similarities. These findings suggest that "molecular mimicry" may be occurring and that immunologic cross-reactivities between virus-induced antibodies or T cells and analogous epitopes in myelin proteolipid could be involved in the pathophysiology of multiple sclerosis of post-infections demyelinating syndromes.

Very little information is available on PLP in multuple sclerosis. Immunoreactive PLP activity has been demonstrated in CSF of patients with neurologic diseases [32], but since myelin fragments have been identified in spinal fluid sediments of multiple sclerosis patients, this is perhaps not surprising.

Johnson et al. [11] found that peripheral blood lymphocytes from four of 19 multuple sclerosis patients showed a proliferative response to PLP but only after removal of suppressor cells. This suggests that at least in some patients the balance between suppressor and stimulator cells may be important in the appearance of a cell-mediated response to PLP.

Molecular biological studies

Molecular biological approaches to the study of PLP have recently progressed rapidly and provide new insights not only into PLP synthesis, but also into myelination in general. The PLP gene has been localized to the X-chromosome in the human and the mouse [35]. The gene consists of seven exons encompassing 17 kilobases of DNA [9]. In addition to conservation of the coding region, the regulatory regions also appear to be highly conserved [22]. It has now been shown that the primary abnormality in one of the mouse dysmyelination mutants, namely the jimpy mouse, involves PLP [25]. The size of the PLP mRNA is shorter in the jimpy than in the wild type mouse, and this results in a deletion of amino acids 207-231 and the production of a highly abnormal protein as a result of a frame-shift. The importance of PLP to the regulation of myelin formation is indicated by the low levels of myelin basic protein mRNA and the essentially complete lack of myelin in the jimpy mice.

Pelizaeus-Merzbacher disease, a severe X-linked dysmyelinating disease, is the only human disorder in which proteolipid protein is implicated as a primary defect. Low levels of other myelin proteins can be detected, but PLP appears to be completely absent [13]. The nature of the gene defect remains to be elucidated.

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