Neurochemical Research, Vol. 19, No. 8, 1994, pp. 959–966

Plasmolipin: The Other Myelin Proteolipid. A Review of Studies on its Structure, Expression, and Function*

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(Accepted March 16, 1994)

KEY WORDS: Plasmolipin; myelin; proteolipids.

Proteolipids were discovered by Marjorie B. Lees and Jordi Folch-Pi in 1951 (1) when they isolated the myelin proteolipid protein (PLP). PLP is a major constituent of CNS myelin where it represents at least 40% of the total myelin protein. Since then, proteolipids have been associated with a variety of other membrane domains and organelles (2-4). However with few exceptions (5) all proteolipids described thus far show little homology with each other. In this review we describe the properties of a proteolipid, plasmolipin, which is restricted to specific plasma membranes of kidney and brain (6,7). Plasmolipin is particularly relevant to a Neurochemical Research issue dedicated to Marjorie Lees, since the studies on this protein were inspired by her work on PLP and, like PLP, it is a major myelin protein (8). This review covers our knowledge to date of plasmolipin and describes its structure, localization, development, distribution within the myelin complex, phylogenic profile and putative function.

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* Special issue dedicated to Dr. Marjorie B. Lees.

Abbreviations: CNPase, cyclic nucleotide phosphohydrolase; MAG, myelin associated glycoprotein; MAP-1B, microtubule associated protein 1B; MBP, myelin basic protein; hsp-70, the 70 kD heat shock protein; Na^+, K^+ ATPase, sodium and potassium-stimulated adenosinetriphosphatase; PLP, proteolipid protein.

1) Properties of Plasmolipin in Lipid Bilayers

Plasmolipin was first described in the early 1980s and was originally isolated from canine and bovine kidney plasma membrane (9,10). Originally this protein was referred to as PMPLP, plasma membrane proteolipid protein. Initial purification from canine kidney indicated that this protein exists as two low molecular weight polypeptides now determined to have molecular masses of 17-18 kD. Addition of plasmolipin to lipid bilayers showed that this protein was capable of forming cation-specific ion channels (9). Further analysis indicated that K^+ was needed to be present for channel formation. However, once the channel has formed in KCl it allows the movement of Na^+ as well as K^+ but at approximately 1/3 the rate of K^+. These data distinguish plasmolipin from PLP in that the latter is not cation-selective and channel formation does not require K but requires Na instead (11). These differences indicate that neither protein is forming a channel merely based on their amphipathic characteristics but on a basis specific to the structure of each protein.

The incremental addition of plasmolipin to lipid bilayers indicated two important characteristics (9). First, at low concentrations of the protein the channels were small, showing single channel conductances of about 10pS with no detectable voltage dependency. In con-

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trast, when plasmolipin levels bathing the bilayer exceeded 1 μg/ml, the channels were much larger, 100pS, and exhibited voltage dependency, closing at potentials greater than 30mV. Second, comparison of conductance changes with the molar concentration of plasmolipin added to the bilayer, indicated that these channels were formed by a plasmolipin oligomer comprised of either three or six monomers. Additional studies with purified subunits indicated that both subunits needed to be present for channel formation. Thus, a trimer made of each plasmolipin subunit was a possible channel structure. This ambiguity has in part been answered by structural studies described below.

2) Structural Studies on Plasmolipin

Plasmolipin is typical of proteolipids in that it can be extracted from membranes by neutral chloroform methanol (CM). Once delipidated, i.e., the apoprotein with noncovalently bound lipids are removed, the protein can be converted to a water soluble form by solvent exchange under a stream of N_2. The early structural studies on PLP (12,13) indicated that this conversion led to a marked change in secondary and tertiary structure.

a. Fluorescence Emission. Plasmolipin also exhibits the property of structural flexibility (10). This was initially studied using the intrinsic tryptophan fluorescence which has an emission spectrum between 330-360nm, with the maximum dependent on the polarity of the tryptophan environment. The lower the wavelength (λ) of the emission maximum the more hydrophobic the environment. For plasmolipin, the tryptophan fluorescent emission spectrum in water was a symmetrical peak at 335 nm (Figure 1) indicating that, in water, all the tryptophan residues are buried in an intense hydrophobic field. In CM the protein appears to open up resulting in higher λ emission maxima at 345 and 355nm indicating that tryptophan residues have shifted into more polar environments. Addition of the protein to liposomes gave an emission spectrum similar to that seen in CM indicating that the tryptophan residues of plasmolipin exist both within the bilayer and close to the bilayer surface, exposed to an aqueous (polar) environment. Recent molecular cloning of plasmolipin and deduction of the amino acid sequence predict a distribution of tryptophan residues in the bilayer similar to that inferred from these fluorescent experiments (14).

b. Circular Dichroism. Structural flexibility was also observed in circular dichroism (CD) studies (10). In water the spectrum was indicative of a large hydrophobic core excluding the aqueous solvent and giving a profile dominated by α helix with some β structure and random coil.

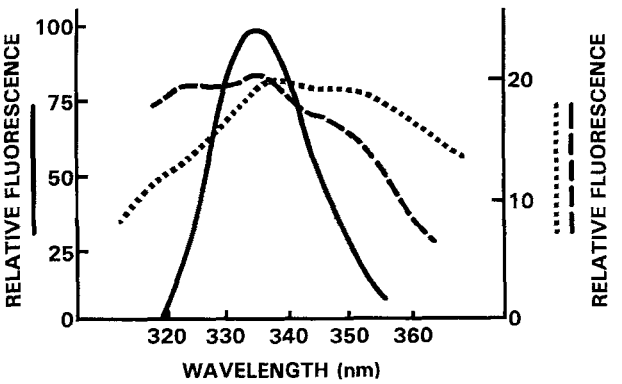

Fig. 1. Fluorescent spectra of plasmolipin in water, Chloroform-Methanol and Liposomes. Excitation in water (—) was at 280nm, with a protein concentration of 0.05 mg/mL. Excitation in CM (---) and in liposomes (■■■) was at 295nm, with a protein concentration of 0.12mg/ml. The excitation λ does not affect the emission spectra, but was used in CM and liposomes to avoid interference of solvent and lipid. The peak fluorescence in water was arbitrarily taken as 100%. The data in this figure are taken from reference (10) and used with permission of the author.

Table I. Effect of Solvents on Secondary Structure of Plasmolipin

Solvent	% α helix	% β structure	% random coil
H_2O	73	14	12
Phosphatidyl choline/ cholesterol liposome	62	2	35

Insertion of plasmolipin into liposomes gave a significant structural change showing a small decrease in α-helix, a loss of β structure and an increase in random coil (Table I). This is consistent with a structure comprised mostly of transmembrane hydrophobic segments with short extramembranous random coil segments. Based upon its amino acid sequence, the hydropathy plot of plasmolipin shows a similar pattern comprised of four hydrophobic transmembrane segments interspersed with short extramembraneous domains (14) and predicts a general membrane topology as illustrated in Figure 2.

c. Oligomeric Structure. The oligomeric structure of proteins can be inferred by the stoichiometry of binding of specific fluorescent probes such as 8-anilino-1-naphthalenesulfanate (ANS) which has been useful in the study of the quaternary structure of plasmolipin. ANS binds to proteins with 1:1 stoichiometry. Based upon the ratio of [ANS]/[plasmolipin] binding in H_2O it was calculated that plasmolipin exists as an hexamer in this solvent. In CM, it was calculated to be a dimer (10).

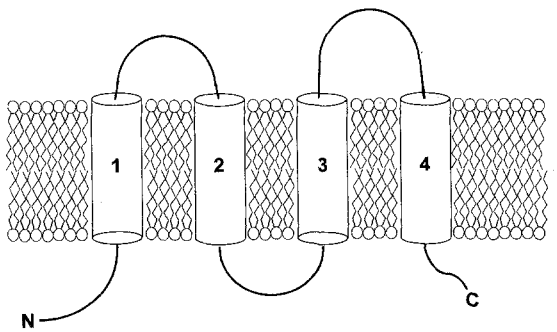

Fig. 2. Membrane topology of plasmolipin. The topological arrangement of plasmolipin within a membrane is derived from the amino acid sequence and hydropathy plot and is depicted as a series of transmembrane segments (hollow tubes) and short extramembranous segments (solid black lines).

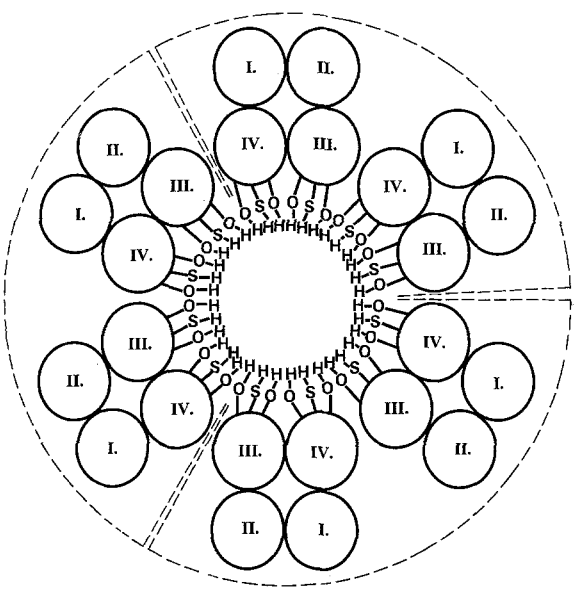

Fig. 3. Proposed quaternary structure of plasmolipin. A quaternary structure of plasmolipin is proposed which consists of three sets of plasmolipin units defined by dashed lines. The plasmolipin unit consists of 2 monomers each containing four transmembrane segments I-IV. The model given is as viewed from the extracellular face. The SH's represent the location of cysteine residues.

These data indicate that two oligomeric states can exist, an hexamer and dimer. The trimeric structure inferred from the in vitro electrophysiological studies, described above, may be an hexamer comprised of 3 sets of dimers. Based on the four transmembrane segments predicted by the plasmolipin sequence we propose a model (Figure 3), that incorporates the concept of a trimer, each made of two plasmolipin monomers, resulting in a protein complex with 24 transmembrane segments, a common motif in K^+ channels (15).

3) Localization and Developmental Expression of Plasmolipin

Plasmolipin is found in brain (16), peripheral nerve (17) and kidney (6), i.e., tissues with a high demand for ion homeostasis to maintain functional capacity. In the kidney, it is restricted to the apical surface of the tubular cells and is concentrated in the proximal tubules (Figure 4A). It differs from Na^+, K^+ ATPase which is present on the basolateral surface (Figure 4B). The levels in kidney increase dramatically during the first four weeks postnatally.

Staining of mixed primary glial cultures (18) with

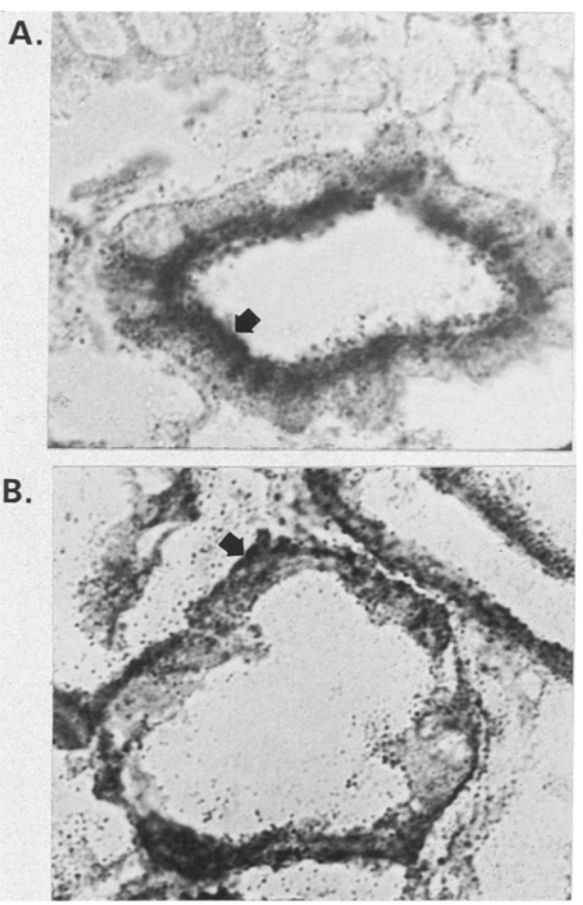

Fig. 4. Immunocytochemical staining of plasmolipin and Na^+, K^+ ATPase in rat kidney. Immunocytochemical staining of plasmolipin (A) and Na^+, K^+ ATPase (B) was carried out as previously described (6). The antibodies to Na^+, K^+ ATPase were used at a 1:500 dilution, and were a generous gift of Dr. George Siegal, Loyola University.

affinity-purified antibodies demonstrated the presence of plasmolipin galactosyl cerebroside (GC) positive cells but not in GFAP positive cells. Plasmolipin staining was first observed at about 8 days in vitro (DIV) as sparse granules of fluorescence in only some of the GC-positive cells. By 14 DIV, plasmolipin staining was intense and evenly distributed throughout the cell body and processes. In mature glial cultures, plasmolipin remained mostly in the cell body and processes, but was not prominent throughout the MBP-positive membrane sheaths (Figure 5). Immunoblot analysis of homogenates from primary glial cultures showed that plasmolipin levels gradually increased between one and four weeks in culture. Similar increases in plasmolipin were found in cultures enriched in oligodendroglia, although the relative levels were higher in the enriched cultures (18). These observations indicate that oligodendroglial cells express plasmolipin during differentiation in culture and predicts its presence in myelin.

Plasmolipin is highly enriched in isolated myelin with quantitation indicating it represents 3-5% of myelin protein in the CNS (7,10). The presence of plasmolipin in myelin has been demonstrated in several ways (8). Immunoblot analysis of different brain regions revealed that plasmolipin levels were higher in regions rich in white matter. Plasmolipin levels in myelin were progressively enriched during five cycles of myelin purification, similar to the enrichment of PLP and MBP. When myelin or brain white matter was extracted with chloroform-methanol the extracts contained, in addition to PLP, a significant amount of plasmolipin. Comparison of CNS and PNS myelin (Figure 6) indicate that plasmolipin is similarly enriched in the PNS (17). Immunocytochemical staining of brain sections showed a restriction of the protein to myelinated tracts with an absence of staining of neuronal cell bodies in the granular and purkinje cell layers (Figure 7). Staining was also absent from the molecular layer indicating that non-myelinated axons do not contain this protein.

Developmental studies in rat brain (6) are also consistent with plasmolipin as a myelin protein with plasmolipin levels increasing more than 7-fold between the first and fourth post-natal week. The biosynthesis of plasmolipin was studied during this period of rapid ac-

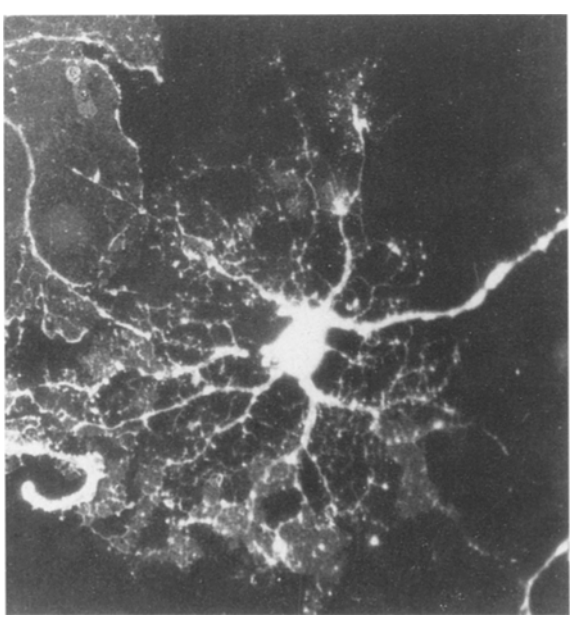

Fig. 5. Immunofluorescent staining of plasmolipin in mouse oligodendrocyte cultures. Enriched oligodendrocyte cultures were prepared and stained by immunofluorescence for plasmolipin as described previously (18) and photographed at 500X.

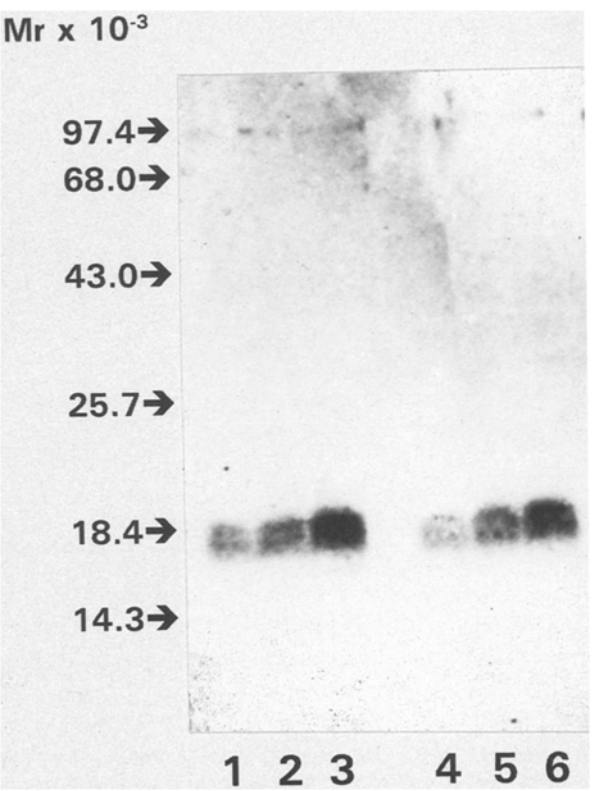

Fig. 6. The level of plasmolipin in purified rat myelin isolated from CNS and PNS. Myelin was isolated and 1, 2 and 5μg protein from CNS (forebrain), lanes 1, 2 and 3, respectively; and PNS (sciatic nerve), lanes 4, 5 and 6 respectively, were analyzed by Western blot. Electrophoresis was carried out on 14% polyacrylamide gels. Under these conditions one should note the separation of plasmolipin subunits. The figure is taken from reference (17) and used with permission of the author.

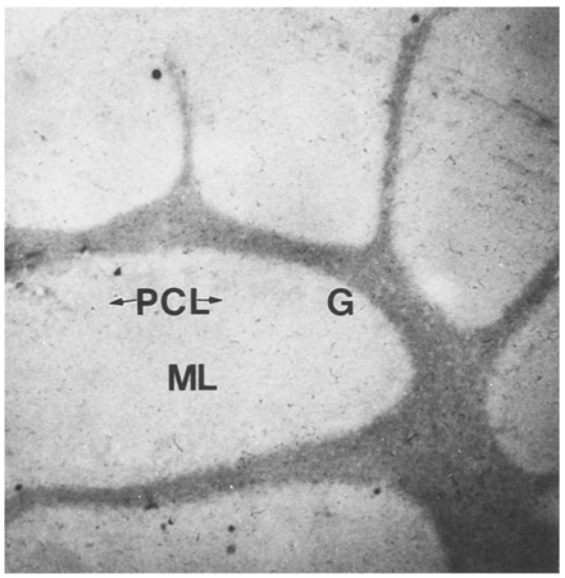

Fig. 7. Immunocytochemical staining of rat cerebellum. The cerebellum was rapidly removed trimmed and frozen in Tissue-Tek OCT media chilled in isopentane and dry ice. The tissue sections (8μ) were placed on gelatin coated slides air dried and stored at −70C. The sections were treated with cold acetone (−20 C) prior to immunocytochemical staining. Endogenous peroxidase activity was eliminated with H_2O_2 and the sections reacted with anti-plasmolipin. The immune complex was visualized with peroxidase-anti-peroxidase as previously described (6). ML is molecular layer; G is the granule cell layer; PCL is the Purkinje cell layer; WM is white matter. Sections were photographed at 100 X.

cumulation. In vitro translation was carried out with free and bound polysomes isolated at postnatal day 16 and plasmolipin purified from the translation mixture by immunoprecipitation. The results demonstrate that plasmolipin is synthesized on membrane-bound polysomes and is inserted into membrane by a co-translational event without evidence of proteolytic processing, i.e. removal of a signal sequence (16).

Subcellular fractionation studies reveal sites of plasmolipin other than compact myelin (7,19,20). Separation of membranes from myelinated nerve tract into fractions highly enriched in compact myelin, periaxolemmal-myelin and axolemma show that plasmolipin is equally distributed among these fractions (7). These data are striking since in the same study proteins such as PLP and MBP were restricted to compact myelin, while MAG and CNPase were highly enriched only in periaxolemmal-myelin. Moveover, axon-specific fodrin, MAP-1B and Na-Channel were all enriched in the axolemmal fraction. These data indicate that plasmolipin exhibits a unique distribution within myelin (compact myelin and periaxolemmal-myelin) and that it appears to become incorporated into the axolemma.

The accumulation of plasmolipin in axolemma may reflect the transfer of this protein from periaxolemmal sites. If plasmolipin were to be transferred to the axon from periaxolemmal-myelin this would not be unique. A mechanism involving vesicle mediated transport has already been postulated for inducible HSP-70 in squid (21) and data suggest a similar mechanism in mammals (22). Similarly, the localization of transferrin to oligodendrocytes in many areas of the CNS (23) suggests that the iron required by the neuron may eneter the neuronal compartment from the oligodendroglial/myelin complex. A vesicular uptake mechanism into the axon has been observed by several investigators who noted endocytotic profiles which enclose periaxolemmal-myelin membrane along with the axolemma (24,25). Purification of clathrin coated vesicles from white matter reveal that plasmolipin is the most abundant intrinsic membrane protein within the vesicle (19). The only other protein present in these vesicles which is unambiguously derived from myelin is the paranodal marker CNPase (19,20). These vesicles are heterogenous with respect to the plasma membrane from which they are derived and probably include oligodendroglial plasma membrane sites as well as periaxolemmal myelin, but electron microscopic evidence indicates the latter is an important source. The enrichment of the axonal cytoskeletal protein MAP-1B (26) on these vesicles indicate further that sites along the axons represent a likely site for the formation of these vesicles. We propose that plasmolipin enters the axon by an endocytotic mechanism and that it becomes inserted in the axolemma when the sorting of the vesicles leads to exocytosis and fusion of the vesicle membrane with the axolemma.

3) Phylogenic Profile

Phylogenic analysis of plasmolipin expression underscores the unique nature of this protein. Using two different polyclonal antibodies plasmolipin was found to be restricted to the mammalian nervous system (17). Western blots of purified myelin protein (Figure 8) showed that mammals but not birds, reptiles, amphibian or fish express an immune reactive plasmolipin. While such analysis has its limitations, i.e., the protein could be present but the dominant epitopes be sufficiently altered so as to preclude reactivity, it should be noted that polyclonal antibodies to the other major myelin proteins show a clear lineage in the CNS going back to amphibians (27,28). If a progenitor to plasmolipin or an homologous protein exists in these lower species, it must

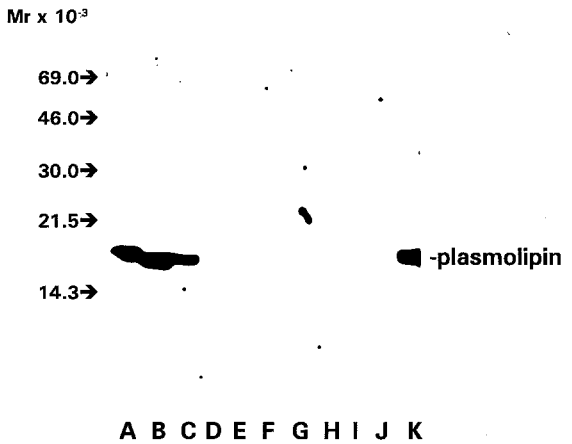

Fig. 8. Phylogenic analysis of plasmolipin in vertebrate myelin. Plasmolipin was analyzed by Western blot. Human, lane A; bovine, lane B; rat, lane C; chicken, lane D; lizard, lane E; turtle, lane F; frog, lane G; goldfish, lane H; shark, lane I; skate, lane J; human PNS; lane K. All lanes contained 10 μg of myelin protein, except the first three lanes that contained 5 μg of rat myelin. Electrophoresis was carried out with 13% polyacrylamide gels. This figure is taken from reference (17) and used with permission of the author.

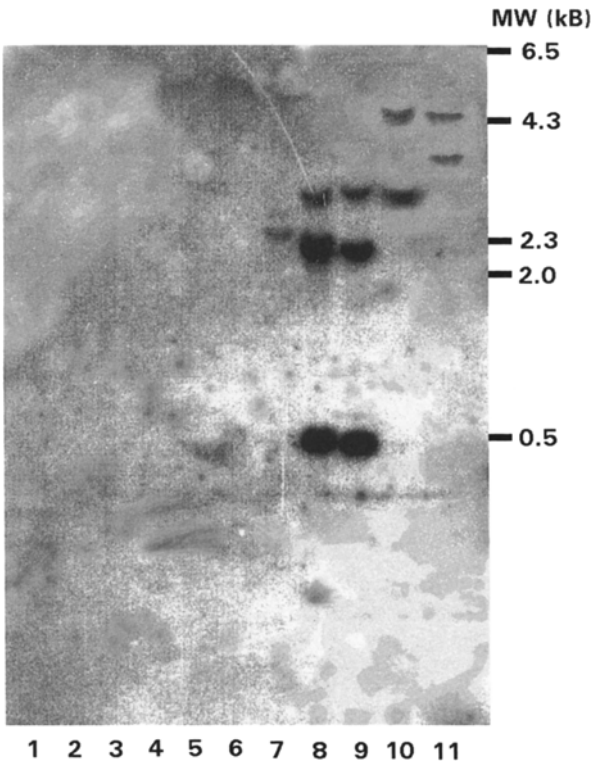

Fig. 9. Southern blot analysis of DNA from various vertebrate species. DNA from different species was digested with Hind III, separated on 1% agarose gels, transferred to nylon membranes and hybridized with a ^{32}P-labeled 1.2kb cDNA probe containing the full coding region of plasmolipin. Lanes 1-6 are DNA from fruitfly, fish, frog, two species of snake and chicken, respectively. Lanes 7-11 are DNA from cow, mouse, rat, chimpanzee and human, respectively.

have been significantly altered so as to abolish antibody reactivity.

The molecular cloning of plasmolipin allowed the phylogenic analysis to be extended to the DNA level. Southern blot analysis with DNA was prepared from mammalian and non-mammalian species and probed at high stringency with a plasmolipin cDNA that included the full coding region. The results showed no detectable homology with non-mammalian species (Figure 9). We conclude that plasmolipin represents a genetic element which is relatively new and not highly conserved. Consistent with this view is the variability in the reactivity of the rat cDNA probe with non-rodent mammalian species reacting strongly with DNA fragments from rats and mice, but weakly and in a different pattern with bovine and primate DNA.

The expression of plasmolipin does not appear to reflect the evolution of the brain per se since an identical phylogenic pattern is observed in the peripheral nervous system (17). Plasmolipin was undetectable in the PNS of species other than mammals where, as illustrated in Figure 7, it is present in levels comparable to that in the CNS suggesting further that its function is unique to mammals.

4) Function

Although we do not know the exact biological function of plasmolipin, its capacity to form voltage dependent K^+ channels suggests that this may be the basis of its in vivo activity. The localization of plasmolipin to renal epithelia and oligodendroglia and its absence from excitable cells such as neurons and skeletal muscle, suggest plasmolipin does not subserve fast K^+ channel activity involved in the control of excitability or neurotransmission. Plasmolipin may therefore belong to a family of slow K^+ channels usually associated with fluid volume and/or intracellular pH regulation (29-31). With the segregation of carbonic anhydrase to myelin, and away from the axon, acidification must be considered as an ongoing process in the myelin complex. The extent of the acidification and its relationship to fluid volume regulation probably depends on a variety of physiological and potentially pathophysiological events. The expression of ion channel proteins which facilitate fluid movement would be important for the maintenance of myelin integrity. In this context, the evolution of plasmolipin and its expression in mammalian myelin may be

of great value. The importance of fluid regulation in the oligodendroglial/myelin complex is underscored by the pivotal role vacuolization plays in most myelinolytic conditions and diseases.

An insight into the control of plasmolipin function in mammalian myelin may come from new molecular evolution data (see Campagnoni et al, this Issue). Dr. Campagnoni data show that the sequence of PLP in the Zebra Finch, differs from the mammalian PLP in 29 amino acid residues. What is striking is that many of these sites reside in the exon where mutations are most deleterious (32). The fact that Zebra Finch have both normal levels of myelin and PLP suggests that PLP mutations in mammals may be deleterious not due to altered PLP structure or function per se but because of other factors which are unique to mammalian myelin. The restriction of plasmolipin to mammalian myelin allows one to speculate that these two proteolipids, PLP and plasmolipin, may functionally interact and that as a result PLP affects plasmolipin function in compact myelin.

Future study on plasmolipin function and the basis for its regulation will clarify our knowledge of this protein and its role in myelin dynamics. If as we postulate, its function is related to fluid volume regulation the study of plasmolipin will be an important focus for understanding the control of myelin integrity.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Marjorie B. Lees for her warmth and support that she has extended to us through the years. The authors also wish to thank their colleagues who have contributed greatly to the studies cited in this review. In alphabetical order: M. Beard, S. Blau, O. Bizzozero, E. Cochary, C.J. Flynn, J. Konola, C.E. Nolan, G. Romano-Clark and T. Shea. This work was supported in part by grants from NSF, IBN 9296101, The Milton Fund (Harvard Medical School), NIH, NS 24894 and the New York State Office of Mental Health.

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