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Vibrational spectra and structure of myelin membranes

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Abstract. Raman and infrared spectroscopy have been simultaneously applied, for the first time, to the study of myelin membranes and their proteolipid protein (PLP) so as to obtain information on the secondary structure of proteins and the ordering of lipid chains. The vibrational spectra were recorded at physiological pH using a non-denaturing detergent (*n*-octyl-β-D-glucopyranoside) in phosphate buffer. Neither the buffer nor the detergent interfere spectroscopically with the amide bands from proteins. The spectra reveal that the predominant secondary structure in the polypeptide backbone in myelin is the helix. The proteolipid protein was found to be more disordered than the polypeptide arrangement of the myelin membrane, as deduced from the relative intensities and halfwidths of characteristic infrared amide I bands. β-form and turns are also present, the amount of these structures being higher in PLP. The study of the Raman spectra of $\nu C-C$ and $\nu C-H$ regions made it possible to obtain information on the lipid chain order.

Key words: Myelin membrane, non-denaturing detergents, lipids, proteolipid protein, Raman and infrared spectra

Introduction

Myelin is a multilamellar membrane that surrounds nerve fibres of both the central and peripheral nervous systems. A considerable amount of work has been carried out to unravel its molecular organization (Morell 1984). Evidence to date suggests that the chemical architecture of the bilayer could be similar to that of most plasma membranes. In this context, the influence of membrane proteins on the architecture and function of myelin has to be elucidated. Myelin accounts for approximately 75% of lipids and 25% of proteins, of the total dry weight of the membrane (Norton 1984). The main protein component of myelin is the so-called proteolipid protein (PLP) (Folch and Lees 1951), which makes up about 60% of the total protein fraction and is perhaps one of the models most used for the study of intrinsic proteins. It is embedded in the hydrophobic core of the lipid bilayer and is of prime importance to the study of lipid-protein and protein-protein interactions in natural and reconstituted membranes (Watts and De Pont 1985; García-Segura et al. 1986).

Pathologically, myelin is destroyed in multiple sclerosis (MS), a demyelinating disease. Studies on the physical structure of myelin suggest that MS myelin has an altered structure (Wood and Moscarello 1984). The fact that the amino acid sequence of bovine PLP is the same as in humans (Stoffel et al. 1985) stimulated our interest in the study of the conformational state of this protein and chain lipid order in normal and multiple sclerosis myelin membranes.

To our knowledge, no information is currently available on the conformational structure of the proteins in myelin itself. X-ray diffraction studies have been carried out on myelin (Kirschner et al. 1984). However, data concerning the native conformation of proteins is still unknown.

Although infrared and Raman spectroscopy provide important information on the secondary structure of proteins, these methods have been scarcely used in the study of biological membranes (Wallach and Verma 1975; Milanovich et al. 1976; Cortijo et al. 1982). Jenkinson et al. (1969) used infrared spectroscopy to study the overall organization of the membrane in myelin and found a considerable degree of planar trans configuration of lipid chains within the membrane structure. Cockle et al. (1978) prepared delipidated proteolipid protein (PLA) in

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aqueous solution and studied its conformation using circular dichroism and infrared spectroscopic techniques. They found that the percentages of helical and β-sheet structures depended on the solvent organic mixtures used in the dialysis methods. Other authors (Curatolo et al. 1978; Lavialle and Levin 1980; Verma et al. 1980) using Raman spectroscopy, determined temperature profiles that reflected lipid bilayer inter and intrachain order-disorder processes. They did not consider the conformational aspect of proteins. On the other hand, it is well known that pH significantly influences the conformation of proteins (Frushour and Koenig 1975). Most of the aforementioned studies have been carried out without considering pH conditions and using methods that modify the secondary and tertiary structure of proteins. Therefore, we have an image of the properties of denatured chains, but little information on the structures of these chains within myelin.

In recent years several detergents have proven to be useful for extracting membrane proteins without changing their structure or losing their function (Hellenius et al. 1977; Smith et al. 1984). The majority of these detergents (i.e. Triton X-100, deoxycholate, etc.) interfere spectroscopically with signals from proteins.

Vibrational spectroscopic studies dealing with conformational aspects of proteins must include a comparison between infrared and Raman spectra. This comparison is the most reliable procedure to characterize the polypeptide backbone secondary structure. However, all previous works have used these techniques separately.

In this paper we have investigated for the first time the infrared and Raman spectra of natural myelin membrane and PLP in order to obtain information concerning the protein secondary structure within this biological membrane. Raman spectra in the C–C and C–H stretching regions are also recorded for myelin and its proteolipid protein. The spectra are discussed in terms of variability of protein-lipid interactions regarding the conformational state of proteins in the samples. The samples were prepared at neutral pH, using a non-denaturating detergent (*n*-octyl-β-D-glucopyranoside) (Hellenius et al. 1979; Michel 1983; Bullock and Cohen 1986) in phosphate buffer. Neither compound interfered spectroscopically with protein amide bands.

Experimental

Purification of myelin

Myelin membranes were obtained from brain white matter according to Waehneldt and Mandel (1970). All solutions used in the purification contained 1 m*M* phenylmethylsulphonylfluoride as a proteolytic inhibitor. Beef brains were obtained from recently slaughtered animals and inmediately transported to the laboratory in crushed ice. Myelin was lyophilized and stored at −30 °C.

Purification of proteolipid protein

PLP was purified from myelin, lyophilized and subsequently stored at −30 °C (Monreal 1975; Aguilar et al. 1982).

Preparation of samples for spectroscopy

Solutions of lyophilized myelin or PLP (25 mg/ml protein concentration) at pH 7.4 were prepared in 0.1 *M* phosphate buffer containing 100 m*M* *n*-octyl-β-D-glucopyranoside.

Lipid studies were carried out using lyophilized myelin or PLP suspensions in 0.1 *M* phosphate buffer, pH 7.4.

Deuterated samples were prepared with pure D_2O using the same procedures described above. Deuterations were checked by infrared spectroscopy.

Spectroscopy

Infrared spectra were recorded with a Perkin-Elmer 599 B spectrophotometer assisted by a data station (Perkin-Elmer model 3600). Myelin or PLP solutions were introduced into a wedge-shape thickness cell with silicon windows. The mean layer thickness was 8 μm. This type of wedge-shaped cell was used to avoid interference spectra caused by the high reflecting factor of silicon windows.

The buffer spectra were recorded using the same instrumental conditions as those used for the samples. The difference spectra were then obtained using the procedure described by Cortijo et al. (1982).

Raman spectra were recorded on a Jobin-Yvon Ramanor U-1000 spectrometer using the 5145 Å line of a Spectra Physics 164 argon ion laser as the excitation source. The spectral slit width and laser power were 2 cm^{-1} and 0.4 W respectively. The frequencies, calibrated with plasma lines from the laser, are reported to ± 1 cm^{-1}. Samples were transferred to capillaries with an internal diameter of 0.9–1 mm. Each Raman spectrum recorded in this work is the average of at least 5 scans.

Results and discussion

Lipids

Both C–C and C–H stretching vibration regions are very useful for determining the state of lipids since these bands reflect the random or rigid state of hydrocarbon chains.

Lipid hydrocarbon chains yield three bands in the 1,050–1,150 cm^{-1} skeletal C–C stretching mode region (Fig. 1). The sharp features at 1,065 and 1,130 cm^{-1} are assigned to all-trans chain segments and the broader third band at about 1,090 cm^{-1} is attributed to chains containing gauche configurations (Levin 1984; Levin et al. 1985). Nevertheless, it is difficult to obtain quantitative results from the chain C–C stretching mode region. The reasons for this quantitative inadequacy stem from the fact that the 1,130 cm^{-1} intensity is not linearly proportional to the number of trans bonds in a chain (Pink et al. 1980). On the other hand, this νC–C region includes a weak underlying PO_2^- symmetric stretching mode (Levin 1984). Although the intensity ratio $I_{1,130}/I_{1,090}$ is adequate for qualitative comparisons between membrane systems concerning intramolecular chain order/disorder, it is insufficient for quantitative studies of trans or gauche conformations.

The Raman spectra of myelin and its proteolipid (MPLP) in the 1,050–1,150 cm^{-1} region exhibit similar general features (Fig. 1). However, the relative intensity $I_{1,130}/I_{1,090}$ for MPLP is significantly lower than the myelin ratio, indicating that lipid hydrocarbon chains in MPLP contain more gauche configurations than myelin membranes.

The 2,800–3,000 cm^{-1} region exhibits a number of bands that correspond to C–H stretching vibrations and their interactions with H–C–H deformation overtones of lipid hydrocarbon chains. The intense bands at 2,845–2,850 cm^{-1} and 2,880–2,890 cm^{-1} have been assigned to methylene symmetric and asymmetric C–H stretching respectively (Larsson and Rand 1973; Verma and Wallach 1977; Snyder et al. 1980; MacPhail et al. 1984). These bands are sensitive not only to chain configuration, but also to the intermolecular order. The intensity of the $\nu_{as}CH_2$ Raman band is markedly enhanced by Fermi resonance interactions between symmetric C–H stretching fundamentals and H–C–H overtones (Verma and Wallach 1977; Levin 1984; MacPhail et al. 1984). The lipid hydrocarbon chain disorder causes the asymmetric CH_2 stretching mode to be out of resonance, resulting in a reduction of the intensity (Verma et al. 1980). In contrast, only slight changes of the intensity of the ν_sCH_2 band caused by structural changes of the lipid hydrocarbon chains were observed. Myelin (Fig. 1) shows prominent sharp bands at 2,848, 2,884 and 2,930 cm^{-1}. The first two bands represent symmetric and antisymmetric CH_2 stretching respectively (Table 1). Since the proteolipid apoprotein only shows a weak

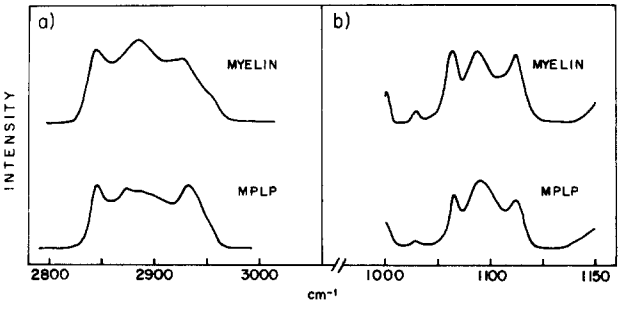

Fig. 1. Raman spectra of myelin and proteolipid protein from myelin membrane (MPLP) in phosphate buffer, pH 7.4. **a** C–H and **b** C–C stretching regions

Table 1. Raman frequencies of myelin membranes

	Tentative assignments	
cm^{-1}	Protein	Lipid
3,292 w	Amide A	
2,985 sh	$\nu_a(CH_3)$	$\nu_a(CH_3)$
2,930 vs	$\nu_s(CH_3)$ and $\nu_a(CH_2)$	$\nu_s(CH_3)$
2,884 vs	$\nu_s(CH_2)$	$\nu_a(CH_2)$
2,848 vs		$\nu_s(CH_2)$
1,749 w		ν(C=O) esters
1,670 sh	Amide I (β)	ν(C=C) (trans)
1,658 s	Amide I (α)	ν(C=C) (cis)
1,453 sh 1,441 vs	$\delta_s(CH_2)$ and $\delta_s(CH_3)$	$\delta_s(CH_2)$ and $\delta_s(CH_3)$
1,343 m	δ(C–H), Phe	
1,303 m	CH_2 twist	CH_2 twist
1,274 m		δ(=C–H)
1,265 sh	Amide III (α)	
1,237 vw	Amide III (β)	
1,157 vw	CH_3 rock	
1,131 m		ν(C–C) (trans)
1,110 w		
1,087 m		ν(C–C) (gauche) and $\nu_s(PO_2^-)$
1,065 m		ν(C–C) (trans)
1,033 vw	Phe	
1,005 m	Phe, Trp	
950 w	ν(C–C_α) (α)	
878 vw	Trp	
852 vw	Tyr	
828 vw	Tyr	$\nu_a(PO_2)$ diester
808 vw		
763 w	Trp	$\nu_s(PO_2)$ diester
730 vw	Amide V	
703 w	ν(C–S) (Met)	
609 vw	Trp	
551 w	ν(S–S) (TGT)	

Abbreviations: s, m, w: strong, medium and weak intensity; v: very; sh: shoulder; Phe, Trp, Tyr, Met: phenylalanine, tryptophan, tyrosine and methionine

band at 2,892 cm^{-1} and lipid CH_2 groups are predominant in the samples (Monreal et al., unpublished results), we conclude that the 2,848 and 2,884 cm^{-1} Raman bands arise mainly from lipid components. We have qualitatively compared the Raman spectra of the intermolecular order of lipid chains (Fig. 1). The spectrum of MPLP shows a higher $I_{2,848}/I_{2,884}$ ratio, suggesting that the intermolecular order of lipid hydrocarbon chains of MPLP is smaller than in myelin. A possible explanation could be that during PLP purification some essential lipids (acidic phospholipids, etc.) are removed. This loss may be the cause of the relative hydrocarbon lipid chain disorder in MPLP. Precise causes are currently under investigation.

We also note that lipid chains in MPLP become more disordered at pH's below neutrality. Our preliminary spectroscopic results show an increase of the proton random coil structure. This increase probably induces the lipid chain disorder that results from denaturation at low pH values.

Finally, our Raman spectra of myelin in the $\nu C-C$ and $\nu C-H$ regions show that hydrocarbon chains are more ordered than erythrocyte membranes (Wallach and Verma 1975). This can be expected because of the high content of cholesterol and long chain fatty acids and lower amounts of polyunsaturated fatty acids (O'Brien and Sampson 1965).

Proteins

The amide bands of the vibrational spectra of proteins reflect more clearly the secondary structure of these compounds, particularly when comparing infrared and Raman amide I bands that appear in the 1,700–1,600 cm^{-1} spectral range. These bands are primarily associated with the stretching vibrations of peptide carbonyl groups (de Lozé et al. 1978; Tu 1982). When ordered structures are present, a splitting occurs between the intense amide I bands in both infrared and Raman spectra. This splitting is relatively small for α-helices and big for β-structures. On the other hand, there should be no splitting between these bands for random coil structures. The only possible difficulty may arise from the overlapping of the amide I bands with $\nu C=C$ vibrations. The use of other amide bands may allow one to detect α, β and random coil structures (Parker 1971; Tu 1982).

The most prominent amide I bands in the infrared spectra appear at different frequencies from those of Raman amide I bands. Thus, there seems to be some splitting between the infrared and Raman amide I modes, which may suggest the existence of ordered secondary structures in the samples. Table 2 lists the frequencies in the 1,700–1,600 cm^{-1} region of myelin and MPLP. In addition to the infrared bands corresponding to the α-helix of undeuterated samples near 1,653 cm^{-1} (Figs. 2 and 3), both samples show bands around 1,638–1,635 cm^{-1}. Our decision to assign these infrared bands to alpha and beta structures respectively is based on the spectra of proteins with very high α- and β-contents (Byler and Susi 1986).

A difficulty in the assignment of the infrared amide I bands is the existence of $\nu C=C$ vibrations from lipids. However, the average intensity of these $\nu C=C$ modes is relatively weak and only visible near 1,625 cm^{-1} when the myelin lipid extract contains less than 5 wt% of proteins (Monreal et al., unpublished results). The infrared bands near 1,635 cm^{-1} can be assigned to β-structures. Raman shoulders situated near 1,670 cm^{-1}, indicative of β-sheet, cannot be unambiguously assigned to specific protein secondary structure due to the contribution of lipid C=C groups. Nevertheless, typical infrared bands near 1,635 and 1,692 cm^{-1} (amide I) may be caused by a β-structure. The fact that the α-helix is the predominant protein conformation in myelin is deduced not only from the amide I bands of the undeuterated sample (Table 2), but also from the amide III Raman band at 1,265 cm^{-1} (Fig. 4 and Table 1). This band shifts to lower frequencies upon

Table 2. Raman and infrared frequencies in the 1,700–1,600 cm^{-1} region

H_2O solvent		D_2O solvent		Tentative assignment
IR	Raman	IR	Raman	
		Myelin		
1,692 sh 1,680 sh		1,692 vw		Amide I (β)
	1,670 sh	1,670 sh	1,669 sh	$\nu C=C$, Amide I (β)
1,665 sh				Amide I (turns and random)
1,652 s	1,658 s	1,642 s	1,652 s	Amide I (α)
1,635 s		1,630 s		Amide I (β)
		MPLP		
1,692 sh 1,680 sh				Amide I (β)
	1,670 sh	1,670 sh	1,670 sh	$\nu C=C$, Amide I (β)
1,665 s				Amide I (turns and random)
1,653 s	1,657 s	1,640 s	1,649 s	Amide I (α)
1,635 s		1,635 s		Amide I (β)
1,612 sh		1,612 sh		Amide I (β)

Abbreviations: s (strong), sh (shoulder), vw (very weak), MPLP (proteolipid protein from myelin membrane)

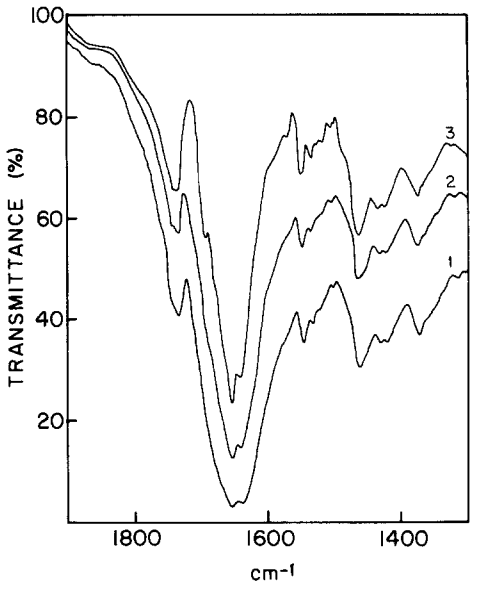

Fig. 2. Difference infrared spectra for aqueous solution (pH 7.4) of myelin membrane. The spectrum of buffer was multiplied by the following factors before the subtraction: spectrum 1, 0.81; 2, 0.93; 3, 1.0

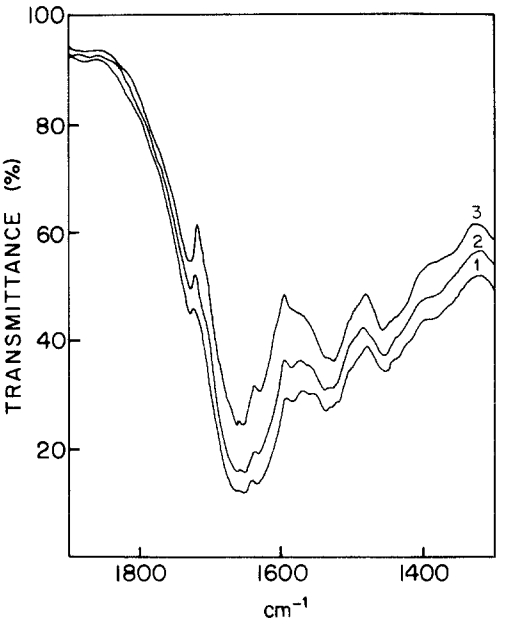

Fig. 3. Difference infrared spectra for aqueous solution (pH 7.4) of proteolipid protein from myelin membrane. The spectrum of buffer was multiplied by the following factors before the subtraction: spectrum 1, 0.81; 2, 0.93; 3, 1.0

deuteration of the sample. No model protein of α-helical configuration has exhibited a Raman amide III mode below 1,250 cm^{-1}. Hence, the amide III region of these Raman spectra is consistent with amide I bands suggesting the presence of considerable α-helix. The Raman spectra also show characteristic α-helical C−C stretching vibrations near 950 cm^{-1} (Table 1).

Correlations between the location of the S−S stretching frequency and the CS−SC dihedral angle in the protein C−S−S−C moiety have been described (Van Wart et al. 1973). The spectra of a number of model compounds indicate that the gauche-gauche-gauche configuration appears at approximately 510 cm^{-1}, trans-gauche-gauche at 525 cm^{-1}, and trans-gauche-trans at 540 cm^{-1}. The spectrum in Fig. 4 contains a band at 551 cm^{-1}, which can be tentatively assigned to the C−S−S−C moiety. The relative strength of this band indicates that disulfide bonds of membrane proteins are predominantly trans-gauche-trans.

Definite spectral differences between myelin and MPLP can be observed in Figs. 2 and 3. These refer to conformational states of the proteins in the samples. MPLP originates a relative increase of the infrared 1,665 cm^{-1} band which is probably due to turns and random coils (Byler and Susi 1986). All these differences concerning the conformational state of the proteins may be explained as follows. In MPLP the lipid content is about 40%, which may not be sufficient to prevent protein aggregation. These aggrega-

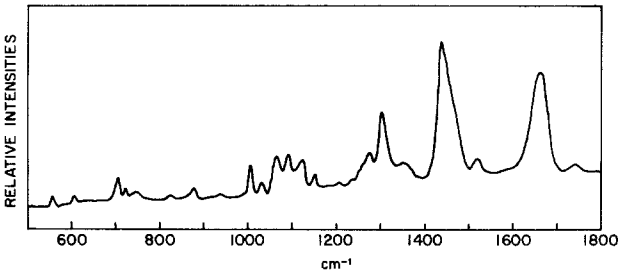

Fig. 4. Raman spectrum of myelin membrane in phosphate buffer (pH 7.4)

tion processes involve the formation of both intermolecular bonds and β-sheet polypeptide chains, as well as partial helix to coil transition (Frushour and Koenig 1975). When aggregated, the all-trans hydrocarbon conformations of the "boundary" lipids are not retained by the irregular shape of the protein. Lipid chains then adopt a greater intra- and intermolecular disorder as shown in Fig. 1. Aggregation can explain why the lipid and protein conformational states in MPLP are more disordered than in myelin.

In the infrared spectra of deuterated samples (Figs. 5 and 6) we also noted a relative increase of the β-sheet 1,635 cm^{-1} band in comparison with the spectra of non-deuterated samples. This was also observed in previous works using heavy water (Wallach and Verma 1975). As occurs in the non-deuterated samples, the halfwidth of the amide I infrared bands in myelin is smaller than those of

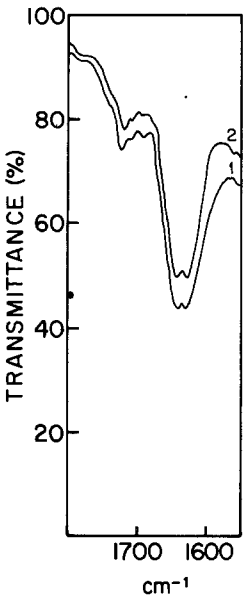

Fig. 5. Difference infrared spectra for aqueous solution (pD 7.4) of myelin membrane. The spectrum of buffer was multiplied by the following factors before the subtraction: spectrum 1, 0.93; 2, 1.0

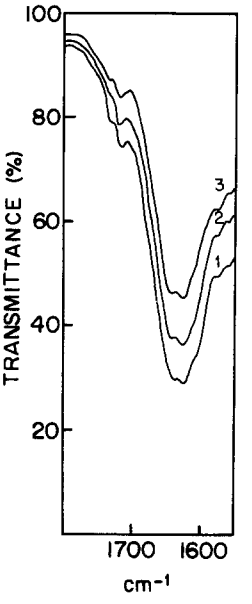

Fig. 6. Difference infrared spectra for aqueous solution (pD 7.4) of proteolipid protein from myelin membrane. The spectrum of buffer was multiplied by the following factors before the subtraction: spectrum 1, 0.81; 2, 0.93; 3, 1.0

membrane, indicating that the protein conformational structures are more ordered in the membrane.

In summary, we have studied for the first time the secondary structure of proteins in myelin using experimental conditions (non-denaturing detergent and physiological pH) that do not alter the native conformations of the proteins. Our vibrational spectra reveal that the average polypeptide arrangement in myelin is mainly helical. The same results were obtained using solid samples (Ramos et al. 1986). This conformation was also found in erythrocyte ghosts (Wallach and Verma 1975). Lippert et al. (1975) estimated an α-helix content of 40–45% with little β-conformation. Milanovich et al. (1976) working with membranes from Dutch belt rabbit erythrocytes, estimated considerable α-helix content based on amide I and III intensities. These authors point out a slight shoulder at 1,645 cm^{-1} suggesting the presence of β-structures. Membrane proteins seem to have high α-helical content when interacting with the hydrocarbon region of the bilayer (Boggs and Moscarello 1978), since this would permit shielding of polar groups including the peptide bond.

The higher content of random coil, β-turns and β-structure in MPLP suggest the existence of some protein-protein interactions producing some disorder of the polypeptide arrangement. This disorder is probably due to the fact that the low lipid content does not prevent the protein molecules from interacting with each other. These interactions result in a disordering of the hydrocarbon lipid chains in MPLP as described above. On the other hand, our results indicate that β-structure and random coil are also present in both types of samples, but their spectra do not allow a quantitative estimate of the secondary structure due to the contribution of lipid C=C vibrations. We are presently studying membrane systems using difference spectroscopy.

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References

Aguilar JS, Cózar M, Criado M, Monreal J (1982) Method for lyophilizing brain proteolipid preparations that increases subsequent solubilization by detergents. J Neurochem 39: 1733–1736

Boggs JM, Moscarello MA (1978) Effect of basic protein from human central nervous system myelin on lipid bilayer structure. J Membr Biol 39:75–96

Bullock JO, Cohen FS (1986) Octyl-glucoside promotes incorporation of channels into neutral planar phospholipid bilayers. Studies with colicin Ia. Biochim Biophys Acta 856: 101–108

Byler DM, Susi H (1986) Examination of the secondary structure of proteins by deconvolved FTIR spectra. Biopolymers 25:469–487

Cockle SA, Epand RM, Boggs JM, Moscarello MA (1978) Circular dichroism studies on lipid-protein complexes of a hydrophobic myelin protein. Biochemistry 17:624–629

Cortijo M, Alonso A, Gómez-Fernández JC, Chapman D (1982) Intrinsic protein-lipid interactions. Infrared spectroscopic studies of gramicidin A, bacteriorhodopsin and Ca^{2+}-ATPase in biomembranes and reconstituted systems. J Mol Biol 157:597–618

Curatolo W, Verma SP, Sakura JD, Small DM, Shipley GG, Wallach DFH (1978) Structural effects of myelin proteolipid apoprotein on phospholipids: a Raman spectroscopic study. Biochemistry 17:1802–1807

De Lozé C, Baron MH, Fillaux F (1978) Interactions of the CONH group in solution. Interpretation of the infrared and Raman spectra in relationship to secondary structures of peptides and proteins. J Chem Phys 75:631–649

Folch-Pi J, Lees M (1951) Proteolipids, a new type of tissue lipoproteins. Their isolation from brain. J Biol Chem 191: 807–817

Frushour BG, Koenig JL (1975) Raman spectroscopy of proteins. In: Clark, RJH, Hester RE (eds) Advances in infrared and Raman spectroscopy. John Wiley and Sons, New York, pp 35–97

García-Segura LM, de Cózar M, Moreno MC, Monreal J (1986) Freeze-fracture characterization of proteolipid protein and basic protein of central nervous system myelin incorporated to liposomes. Brain Res 380: 261–266

Hellenius A, Fries E, Kartenbeck (1977) Reconstitution of Semliki fores virus membrane. J Cell Biol 75: 866–880

Hellenius A, McCaslin DR, Fries E, Tanford CH (1979) Properties of detergents. In: Fleischer S, Packer L (eds) Methods in enzymology, vol 56. Academic Press, New York, pp 734–749

Jenkinson TJ, Kamat VB, Chapman D (1969) Physical studies of myelin II. Proton magnetic resonance and infrared spectroscopy. Biochim Biophys Acta 183: 427–433

Kirschner D, Ganser AL, Caspar DLD (1984) Diffraction studies of molecular organization and membrane interactions in myelin. In: Morell P (ed) Myelin. Plenum Press, New York, pp 51–95

Larsson K, Rand RP (1973) Detection of changes in the environment of hydrocarbon chains by Raman spectroscopy and its application to lipid-protein systems. Biochim Biophys Acta 326: 245–255

Lavialle F, Levin IW (1980) Raman spectroscopic study of the interactions of dimyristoyl- and 1-palmitoyl-2-oleoyl-phosphatidylcholine liposomes with myelin proteolipid apoprotein. Biochemistry 19: 6044–6050

Levin IW (1984) Vibrational spectroscopy of membrane assemblies. In: Clark RJH, Hester RE (eds) Advances in infrared and Raman spectroscopy. John Wiley and Sons, New York, pp 1–48

Levin IW, Keihn E, Harris WC (1985) A Raman spectroscopic study on the effect of cholesterol on lipid packing in diether phosphatidylcholine bilayer dispersions. Biochim Biophys Acta 820: 40–47

Lippert JL, Gorczyca LE, Meiklejohn G (1975) A laser Raman spectroscopic investigation of phospholipid and protein configurations in hemoglobin-free erythrocyte ghosts. Biochim Biophys Acta 382: 51–57

MacPhail RA, Strauss HL, Snyder RG, Elliger CA (1984) C–H stretching modes and the structure of n-alkyl chains. 2. Long, all-trans chains. J Phys Chem 88: 334–341

Michel H (1983) Crystallization of membrane proteins. Trends Biochem Sci 8: 56–59

Milanovich FP, Shore B, Harney RC (1976) Raman spectroscopic analysis of Dutch Belt rabbit erythrocyte ghosts. Chem Phys Lipids 17: 79–84

Monreal J (1975) Chromatographic fraction of brain white matter proteolipid. J Neurochem 25: 539–541

Morell P (1984) Myelin. Plenum Press, New York

Norton WT (1984) Oligodendroglia. Advances in neurochemistry, vol 5. Plenum Press, New York, pp 47–75

O'Brien J, Sampson EL (1965) Lipid composition of the normal human brain: gray matter, white matter and myelin. J Lipid Res 6: 537–544

Parker FS (1971) Applications of infrared spectroscopy in biochemistry, biology and medicine. Hilger, London

Pink DA, Green TJ, Chapman D (1980) Raman scattering in bilayer of saturated phosphatidylcholines. Experiment and theory. Biochemistry 19: 349–356

Ramos JM, Carmona P, Monreal J, Chapman D (1986) Raman and infrared spectroscopic study of myelin membranes. J Mol Struct 143: 461–464

Smith R, Cook J, Dickens PA (1984) Structure of the proteolipid protein extracted from bovine central nervous system myelin with non-denaturing detergents. J Neurochem 42: 306–313

Snyder RG, Scherer JR, Gaber BP (1980) Effects of chain packing and chain mobility on the Raman spectra of biomembranes. Biochim Biophys Acta 601: 47–53

Stoffel W, Giersiefen H, Hillen H, Schroeder W, Tunggal B (1985) Amino acid sequence of human and bovine brain myelin proteolipid protein (lipophilin) is completely conserved. Biol Chem Hoppe-Seyler 366: 627–635

Tu A (1982) Raman spectroscopy in biology. John Wiley and Sons, New York

Van Wart HE, Lewis A, Scheraga HA, Saeva FD (1973) Disulfide bond dihedral angles from Raman spectroscopy. Proc Natl Acad Sci USA 70: 2619–2623

Verma SP, Wallach DFH (1977) Raman spectra of some saturated, unsaturated and deuterated C_{18} fatty acids in the HCH-deformation and CH-stretching regions. Biochim Biophys Acta 486: 217–227

Verma SP, Wallach DFH, Sakura JD (1980) Raman analysis of the thermotropic behavior of lecithin-fatty acid systems and of their interaction with proteolipid apoprotein. Biochemistry 19: 574–579

Waehneldt, TV, Mandel P (1970) Proteins of rat brain myelin. Extraction with sodium dodecylsulphate and electrophoresis on analytical and preparative scale. FEBS Lett 9: 209–212

Wallach DFH, Verma SP (1975) Raman and resonance Raman scattering by erythrocyte ghosts. Biochim Biophys Acta 382: 542–551

Watts A, De Pont JHM (1985) Progress in protein-lipid interactions, vol 1. Elsevier, Amsterdam, pp 1–56

Wood DD, Moscarello MA (1984) Is the myelin membrane abnormal in multiple sclerosis? J Membr Biol 79: 195–201