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PHYSICAL STUDIES OF MYELIN

II. PROTON MAGNETIC RESONANCE AND INFRARED SPECTROSCOPY

T. J. JENKINSON, V. B. KAMAT AND D. CHAPMAN

Unilever Research Laboratory Colworth/Welwyn, The Frythe, Welwyn, Herts. (Great Britain)

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SUMMARY

Myelin (ex beef brain) has been examined by wide-line and high-resolution proton magnetic resonance (PMR) and infrared spectroscopy. These techniques are used to provide information about (a) the overall organisation of the myelin membrane, and (b) specific interactions between the membrane components.

Thus,

1. The proton wide-line nuclear resonance studies of myelin in excess ^2H_2O show a spectrum very similar to that of the total lipid of myelin swollen in ^2H_2O. The lipids are known to be in a lamellar arrangement in the latter situation and hence the PMR evidence is consistent with myelin itself having a lamellar organisation. Furthermore, the infrared spectroscopic evidence shows that there is a considerable degree of planar *trans* configuration for the lipid chains within the membrane structure.

2. Sonication of myelin in ^2H_2O enables chemically shifted PMR lines to be observed with a high-resolution PMR spectrometer. By comparison of this spectrum with that of cholesterol-free myelin lipid, it can be deduced that cholesterol influences the myelin lipid chain mobility and that the cholesterol molecules themselves are prevented from having complete isotropic motion. This is in contradistinction to the situation of cholesterol when myelin is dissolved in chloroform–methanol.

INTRODUCTION

Previous studies on erythrocyte membranes have shown that proton magnetic resonance (PMR)[1] and infrared spectroscopy[2,3] can provide useful information about membrane organisation. These studies, combined with optical rotatory dispersion and circular dichroism data on erythrocyte membrane and other plasma membranes[4,5], suggest that there may be interaction between lipid hydrocarbon chains and the apolar amino acids of the membrane protein. In a previous communication (Part I) we have reported results obtained by differential thermal analysis and differential scanning calorimetric methods[6]. In the present communication we discuss PMR and infrared

Abbreviation: PMR, proton magnetic resonance.

spectroscopic results on myelin derived from beef brain and comment about the nature of lipid organisation in myelin.

MATERIALS AND METHODS

Preparation of myelin

Myelin was prepared by the method of EICHBERG *et al.*[7] with modifications described in Part I. The water-washed myelin pellets were either used immediately after preparation or stored at −15° or freeze-dried.

Lipid extraction

Samples of total lipid (cholesterol and lipid), cholesterol-free lipid (galactolipid and phospholipid), and the individual galactolipids and phospholipids were prepared as described in Part I.

Wide-line PMR spectroscopy

Wet myelin pellets were resuspended in ^2H_2O, serially dialysed against ^2H_2O and then equilibrated over saturated salt solutions in ^2H_2O. The water content of these samples was determined from their dry weights as described earlier[6]. In some instances the suspended myelin pellet, after equilibration, was sonicated under conditions previously reported[1]. Spectra were recorded under wide-line conditions on a Varian 60 MHz instrument at various temperatures.

High-resolution PMR spectroscopy

Spectra were recorded on a Perkin Elmer R-10 spectrometer (operating at 60 MHz) fitted with a variable temperature probe and a CAT (computer of average transients, Northern Scientific N.S.-544 digital memory oscilloscope). Spectra were printed out after 512 accumulations.

Spectroscopic samples were prepared as follows: (a) The wet myelin pellet was resuspended in 99.7 % ^2H_2O, equilibrated with ^2H_2O as above and sonicated. The total lipid extract, cholesterol-free lipid, phospholipids and galactolipids were also prepared as dispersions in ^2H_2O under identical conditions of sonication. (b) A solution of freeze-dried myelin was obtained in deuterated chloroform–methanol (C^2HCl_3–$C^2H_3O^2H$) (2:1, v/v). Chemical shifts are expressed in τ relative to tetramethylsilane at 10τ.

Infrared spectroscopy

Spectroscopic samples were prepared by layering (a) aqueous suspensions of myelin, (b) its total lipid extract, or (c) lipidic components on AgCl windows (1.5 cm diameter) and evaporating the water in a vacuum desiccator overnight. Some films were cast from myelin incubated in ^2H_2O at 90° for 24 h. Cells, constructed from a spacer cut from disposable AgCl and two AgCl discs, were filled with wet myelin equilibrated as above in ^2H_2O. In the latter, water was removed by vacuum evaporation until the desired water content was reached. This arrangement was clamped into a holder giving a sealed cell with path length of 0.25 mm.

All infrared spectra were recorded on a Grubb Parsons GS4 double-beam grating spectrometer. A variable temperature cell (VLT Research and Industrial Instruments Co.) was used for temperature study.

RESULTS

Wide-line PMR

The wide-line PMR spectra of unsonicated myelin in excess water are shown in Figs. 1a and 1b.

Unsonicated myelin shows two wide lines of about 4–5 and 2 gauss wide (Fig. 1a). Superimposed on these lines is a narrow component, chemically shifted upfield by about 4 ppm from the central water line (Fig. 1b). The spectrum of total lipid is shown in Fig. 1c. Under identical spectrometer conditions the spectrum is similar to that of the original myelin (Figs. 1a and 1b).

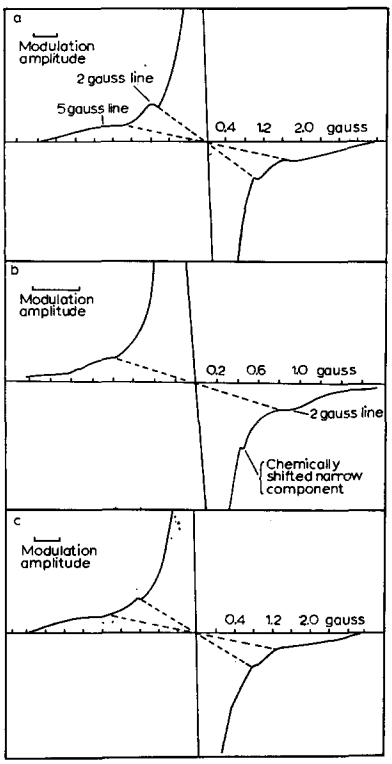


Fig. 1. (a) Wet myelin–95% ^2H_2O, 10 gauss sweep. (b) Wet myelin–95% ^2H_2O, 5 gauss sweep. (c) Total lipid extract–95% ^2H_2O, 10 gauss sweep.

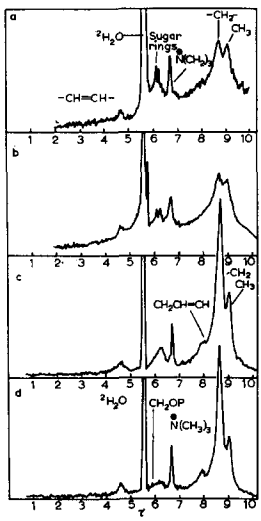


Fig. 2. (a) Sonicated myelin, 3.5% in ^2H_2O, 65°. (b) Sonicated total lipid, 2.7% in ^2H_2O, 65°. (c) Sonicated cholesterol-free lipid, 2% in ^2H_2O, 65°. (d) Sonicated phospholipid, 1.2% in ^2H_2O, 65°.

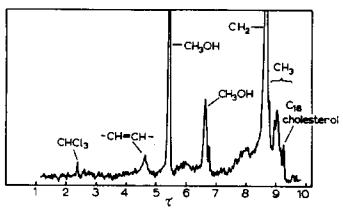
High-resolution PMR

The PMR spectra recorded at 65° of (a) myelin, (b) its total lipid extract, (c) cholesterol-free lipid (phospholipid and galactolipid), and (d) phospholipid are shown in Fig. 2.

Myelin dispersions give spectra which are consistently resolved above 65° (Fig. 2a). Samples cooled sufficiently below this temperature showed reversible loss of

resolution. Spectra recorded at higher temperatures, up to 110°, show no qualitative difference. Spectra of myelin can be observed at lower temperatures (40–55°) if the closely packed structure of myelin is modified or disrupted by freeze-drying or drying over P_2O_5.

Assignments of various peaks in the spectra are based on the spectra of related molecules. The spectrum of myelin at 65° has a weak absorption at 4.5–4.7τ due to –CH=CH– protons of the lipid unsaturated groups and sharper signals at 6.2 and 6.3τ due to protons in the sugar rings of the galactolipids, and at 6.7τ due to $N^+(CH_3)_3$ protons in lecithin and sphingomyelin. In addition there is a broad absorption between 8 and 9.3τ with distinct maxima at 8.7 and 9.1τ due to $-(CH_{20})-$ and $-CH_3$ protons respectively in the hydrocarbon chains. The spectrum of the total lipid extract



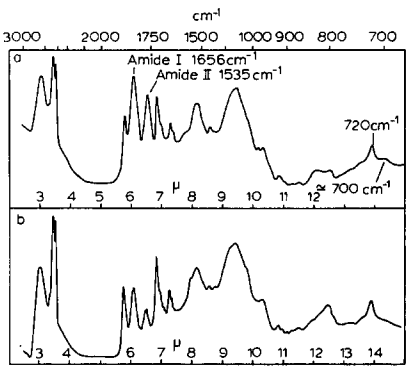


Fig. 3. Myelin in chloroform–methanol (2:1, v/v).

Fig. 4. (a) Myelin, room temperature dried AgCl film. (b) Total lipid, room temperature AgCl film.

(Fig. 2b) is very similar to that of myelin itself (Fig. 2a). The effect of cholesterol on the lipid chain signal is indicated by comparison of spectra (Figs. 2b and 2c), which show considerable narrowing in this region on removal of cholesterol.

A quantitative comparison of the spectrum of myelin with its corresponding lipid fractions shows that the area of the $N^+(CH_3)_3$ signal at 6.7τ is approximately the same. A slight broadening in the galactolipid ring proton peaks (6.2 and 6.3τ) is observed with the cholesterol-free lipids (Figs. 2b and 2c). The phospholipids (Fig. 2d) show a small absorption in this region due to the $-CH_2OP$ protons. There is also a small absorption at 7.9τ most prominent in the phospholipid spectrum primarily due to CH_2 groups α to the carbon–carbon double bonds.

The spectrum of freeze-dried myelin in C^2HCl_3–$C^2H_3O^2H$ shows sharp $(CH_2)_n$ and CH_3 resonances from the lipid chains and cholesterol. In addition there is a sharp resonance at 9.3τ which we assign specifically to C_{18} angular CH_3 groups of cholesterol (Fig. 3).

Infrared spectroscopy

The infrared spectra of dried films of myelin and its total lipid extract at room temperatures are shown in Figs. 4a and 4b. Partially hydrated spectroscopic cells containing myelin equilibrated with ^2H_2O gave spectra similar to Fig. 4a except for a slight broadening between 1660 and 1220 cm^{-1} due to H–O–H, H–O–^{2}H and ^{2}H–O–^{2}H deformations.

In general, the spectrum of myelin is similar to previously published spectra[8]. The myelin spectrum has symmetrical amide I and amide II bands from the protein at 1656 and 1535 cm^{-1}, respectively, with no distinguishable band or shoulder at 1628 cm^{-1}. There is a small absorption at 700 cm^{-1} possibly due to NH-out-of-plane deformations[9]. No further obvious peaks were detected between 700 and 400 cm^{-1}. Equilibration with ^2H_2O did not reduce the 700 cm^{-1} band although some reduction of the amide II band occurred.

No absorption at 700 cm^{-1} is observed in the spectrum of the lipid extract (Fig. 4b), although small absorptions in the amide I and II positions due to cerebrosides and sphingomyelin are present. Other features of lipidic constituents common to both spectra are the ester carbonyl C = O stretch at 1739 cm^{-1}, P = O stretch at 1226 cm^{-1}, (P)–O–C stretch at 1070 cm^{-1} (broad), P–O–(C) at 970 cm^{-1}, $\Delta 5$ sterol at 839 and 799 cm^{-1} and $(CH_2)_n$ rock at 720 cm^{-1}. Full assignments of absorptions in this region for lecithins have been given by CHAPMAN *et al.*[10].

Subjecting the films to temperatures between −150 and +150° did not alter the shape or position of the amide I band but changed the intensity of the $(CH)_2$ rocking mode at 720 cm^{-1}. Over the temperature range studied, for both myelin and the lipid extract, its intensity increased with decreasing temperature. With both myelin and the lipid extract, this band disappears on heating to about 100°.

DISCUSSION

Overall membrane organisation

Ultrastructural and other physical studies on myelin have been interpreted to support the idea that it consists of concentric layers of lamellar order organised into a tightly compact structure[11–13]. Moreover, isolated total lipid extract of myelin is shown by X-ray methods to have a lamellar arrangement (unpublished observations from this laboratory). A direct comparison of the similarity between the wide-line spectra of myelin and its total lipid extract therefore indicates that there is similar overall lipid organisation between the two systems. The occurrence of broad lines in both the spectra eliminates the possibility of a cubic phase[14]. The existence of other phases cannot be entirely ruled out, but it is extremely unlikely that the lipid organisation in the membrane should be different from that of the isolated total lipid in water since the dipole–dipole interactions in the two systems are so very similar. The existence of a hexagonal lipid phase has been reported for brain[15]- and mitochondrial lipid[16]–water systems, but only at low water content. It is not expected to be present in myelin with its full complement of water.

A further insight into the lipid chain organisation of myelin is obtained by infrared spectroscopy. The 720 cm^{-1} band is characteristic of four or more connected CH_2 groups in planar transconfiguration. It is present in the spectrum of hydrated myelin cells (5–50 % water) at room temperature, and in the spectra of dried films of myelin as well as its lipid extract over a wide range of temperatures. This indicates that, despite the presence of protein, there is considerable degree of order within the lipid chains of myelin. In contrast to this, with erythrocyte membranes, this band becomes distinct only at subzero temperatures, although it is distinguishable in the spectrum of erythrocyte lipids above room temperatures[3].

The amide I and amide II bands in the infrared spectrum of myelin, whether completely or partially hydrated, are typical of protein in either α-helical and/or ran-

dom coil conformation, with no detectable β-configuration[2]. The latter could, however, be induced with certain organic solvents, *e.g.* 2-chloroethanol, dimethylformamide or pyridine. Support for helicity in myelin protein is provided by the infrared and optical rotatory dispersion data published by STEIM[17].

The association between membrane components

The nature of interactions between membrane components is further revealed by the high-resolution PMR spectra of myelin and its lipidic constituents. Such chemically shifted spectra are obtained upon sonic dispersion of these materials, as shown previously in the case of erythrocyte membranes[1] and lipid–water systems[18]. Differential centrifugation of sonic dispersions of the myelin fraction shows a reduction in the fragment size. Electron microscopy of the sonicated fraction reveals profiles with a decrease in the number of stacked layers, but the same repeat of 110–130 Å is observed. This is quite different from the 50-Å repeat of the lipid extract of the material and is consistent with X-ray diffraction and electron microscopic data of FINEAN *et al.*[12], which show that repeat distances are unaltered by sonication. Although subtle effects of sonication on membrane organisation are not ruled out, it is reasonable to assume that sonication breaks the initial myelin fraction into smaller entities which retain layered structure. Such entities permit sufficiently rapid reorientation to produce narrow-line spectra[19]. This has been discussed in some detail for lecithin–water dispersions by CHAPMAN *et al.*[18].

We demonstrated earlier that cholesterol-free myelin lipid and myelin phospholipids in excess water are liquid crystalline only above 50° (ref. 6). At about 65° (Figs. 2c and 2d) the spectrum of the liquid crystalline state rather than that of the gel phase is obtained. When these spectra are compared with the spectrum of myelin total lipid (Fig. 2b) and myelin dispersions (Fig. 2a), it is clear that the presence of cholesterol has a major effect upon the lipid chain mobility. A similar effect on lipid chain mobility by cholesterol has been reported with egg-yolk lecithin[20].

It is interesting to note that neither with myelin nor with lecithin–cholesterol dispersions does cholesterol have any marked effect on the $N^+(CH_3)_3$ group associated with the signal. This suggests that the primary interaction of cholesterol is with the lipid chains. In the case of human erythrocyte membranes, an interaction between lipid chains and apolar amino acids of the membrane protein was suggested to explain the observed spectra[1]. With myelin it appears that there is no need to invoke this type of interaction.

X-ray studies on the effect of organic solvents on peripheral nerve myelin demonstrate that chloroform–methanol disrupts the lipoprotein structure[21]. This is apparently true for central myelin as the PMR spectrum of central myelin in C^2HCl_3–$C^2H_3O^2H$ (Fig. 3) shows a marked narrowing of the $(CH_2)_n$ and CH_3 signals. In addition, resonance due to the C_{18} angular CH_3 group of cholesterol at 9.3τ becomes sharp. Chemically shifted protons such as these are unresolved in the spectrum of aqueous myelin fragments. This indicates that the cholesterol is not able to move in an isotropic manner as a result of its interaction with the lipid.

Our PMR and infrared spectroscopic data on myelin and erythrocyte membrane suggests that there are differences in lipid chain organisation and lipid–protein interaction in these two membrane systems. These differences deserve careful scrutiny in future suggested models of these membranes.

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