DETECTION OF MOLECULAR MOTION IN LYOPHILIZED MYELIN BY NUCLEAR MAGNETIC RESONANCE

HAROLD LECAR, GERALD EHRENSTEIN, *and* IRVING STILLMAN

From the Laboratory of Biophysics, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT Proton resonance spectroscopy was used to determine the state of the hydrocarbon regions in lyophilized and resuspended samples of nerve myelin. Measurements of the resonance line width indicate considerable freedom of motion within the hydrocarbon moiety of the myelin samples. Sharp thermal transitions of the line width were observed, suggesting that lyophilized myelin is in a liquid crystalline state.

INTRODUCTION

Electron microscopy shows the myelin sheath of nerve to be a tightly wound spiral of Schwann cell membrane (Napolitano and Scallen, 1969; Geren, 1954). X-ray diffraction (Worthington, 1969) confirms the existence of a lamellar structure. The interior of a myelin membrane contains a 20–30 A central region of low electron density which consists mainly of the hydrocarbon chains of lipid molecules. As pointed out by Finean (1969), this narrow dimension for the lipid strata conflicts with the older picture of myelin as a paracrystalline material (Finean, 1953; van den Heuvel, 1963).

Recently, Uzman and Hedley-White (1968) have stated that the rapid turnover of various metabolites and the high degree of structural plasticity in the myelin sheath suggest that the hydrocarbon regions are fluid. Furthermore, Hubbell and McConnell (1968) have demonstrated the high mobility of small molecules in myelin using the electron spin resonance of a lipid-soluble free radical dissolved in the myelin sheath of rabbit vagus nerve.

In our experiments we determine the fluidity of the hydrocarbon chains of anhydrous myelin preparations by means of nuclear magnetic resonance (NMR) spectroscopy. For a material having a high density of protons, the main source of NMR-line broadening is the magnetic dipole-dipole interaction. The phenomenon of motional narrowing of the resonance occurs when this interaction is averaged by

increasing molecular agitation. In this way NMR has been used to study molecular motions and phase transitions in numerous hydrocarbon materials (Andrew, 1950; Lawson and Flautt, 1965).

Lyophilized myelin is a concentrated membrane preparation for which the observed proton resonance comes mainly from groups within the hydrocarbon moiety. Hence by measuring the NMR line width over a large temperature range we are able to assess the degree of fluidity of the hydrocarbon chains. The lipid moiety of biological membranes has been likened to a liquid crystal phase (Finean, 1969), and we indeed find similarities between our NMR results on lyophilized myelin and the results obtained for liquid crystals of the anhydrous fatty acid–salt soaps (Lawson and Flautt, 1965). A preliminary account of these experiments was presented at the 1967 Biophysical Society Meeting (Lecar, Ehrenstein, and Stillman, 1967).

METHODS

Myelin samples were provided by Dr. Marion Kies and Dr. John M. Davis. The lyophilized samples of bovine, rat, and guinea pig myelin were prepared by the method of Laatsch, Kies, Gordon, and Alvord (1962). One sample of human myelin prepared by the method of Autilio, Norton, and Terry (1964) was also studied. The results on all the preparations were qualitatively similar but the most detailed spectroscopic studies were performed on the bovine and guinea pig preparations. Consequently the following discussion refers specifically to those two preparations.

During the course of the preparation the chloroform-methanol extracted myelin was fractionated by ultracentrifugation from a 30% sucrose homogenate of fresh whole brain, dialyzed against distilled water (osmotic shock), rehomogenized in distilled water, and finally lyophilized to give "refined" myelin. Electron microscopy of the prelyophilized refined myelin shows mostly membranous whorls, so-called "myelin figures," and a small fraction of tiny vesicles. The myelin figures are somewhat fragmented membranous structures exhibiting the typical lamellar myelin forms. Samples of fresh, "crude" myelin showed the myelin figures less fragmented and retaining more of the axoplasm within.

The NMR line width of myelin was studied as a function of temperature. Since the temperature study covered a wide range of line widths it was necessary to use both narrow-line and wide-line NMR spectrometers. Measurements at low temperature were performed on a Varian wide-line NMR spectrometer (Varian, Palo Alto, Cal.), using a CAT (signal-averaging computer) to improve the signal-to-noise ratio. At temperatures above 35°C, we used a Varian HA-60 NMR spectrometer. Temperature uniformity throughout the sample was checked for the low temperature work by inserting a small thermocouple directly into the sample tube. In general, temperatures were measured to an accuracy of ±2°C.

Since it was known that insufficient equilibration time was an important source of error in NMR studies of liquid crystals (Lawson and Flautt, 1965), all temperatures were maintained for at least 2 hr to provide enough time for the sample to reach thermal equilibrium. During the equilibration time, the line width was monitored until it remained constant. For the low temperature experiments, the samples were held in liquid nitrogen overnight.

One source of uncertainty in a heterogeneous material, such as a membrane, is the possibility that a significant part of the signal does not come from the hydrocarbon moiety. From the measured ratios of protein to cholesterol to phospholipid (O'Brien, 1965) we have esti-

mated that about 90% of the membrane protons are in the hydrocarbon moiety. However, we must consider the possibility that if the NMR signal from the hydrocarbon protons were greatly broadened, the net signal could come primarily from protein protons. If this were the case, then complete melting of the sample should increase the integrated intensity of the resonance signal by a factor of 10 or more. Melting the sample increased the signal by about a factor of 2, indicating that at least 80% of the signal in the state of intermediate line width comes from the hydrocarbon moiety.

It was necessary to study lyophilized myelin rather than native myelin because we could not obtain samples sufficiently concentrated to have detectable resonances without lyophilization. To obtain a qualitative idea of the effects of lyophilization and hydration, we also studied the spectrum of myelin resuspended in D_2O.

RESULTS

The proton resonances of the lyophilized myelin samples were observed over a temperature range −196 to +160°C. Typical spectra obtained for bovine myelin are shown in Fig. 1. In Fig. 1 *a* we see a low temperature resonance, taken at −196°C. Fig. 1 *b* shows a typical room temperature resonance. These two figures show the first derivative of the absorption line. The line width is taken as the separation between positive and negative peaks of the derivative signal shape. Figs. 1 *c* and *d* show the narrower resonance lines recorded at higher temperatures. The narrow lines themselves, rather than their derivatives, are displayed in the latter two figures.

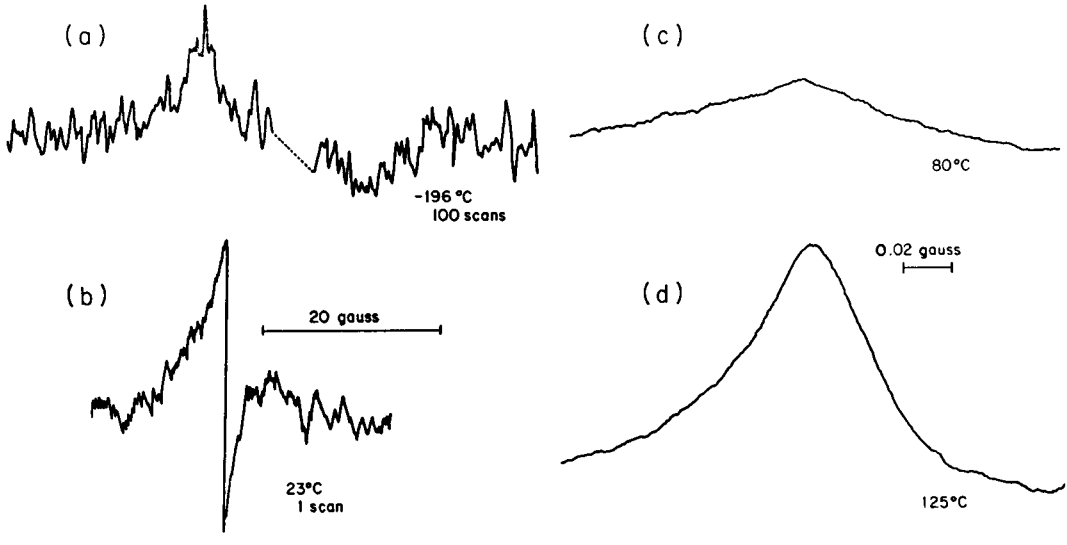

FIGURE 1 Typical proton resonance spectra of lyophilized bovine myelin. Figs. 1 *a* and *b* are derivative spectra using a wide-line NMR spectrometer. The dotted portion of Fig. 1 *a* was retouched and showed a proton line caused by water condensation on the outside of the sample tube. Figs. 1 *c* and *d* are high resolution spectra at higher temperatures taken on a conventional high resolution spectrometer.

The corresponding width should be measured between the inflection points of the absorption curve. Since these points are difficult to locate, we measured the width at half-maximum and multiplied by the correction factor 1.2, valid for gaussian line shapes.

Fig. 2 shows the resonance line width for bovine myelin as a function of temperature. At low temperatures, the line width is approximately 20 gauss, which is typical for a rigid hydrocarbon material. As temperature is increased, there are two sharp transitions. The first transition occurs in the physiological range, between 25 and 40°C. In this transition the line width decreases by more than two orders of magnitude. At higher temperatures, the line width remains constant at 0.08 gauss over a temperature range of about 100°C. This broad temperature region of constant line width intermediate between that of a rigid solid and a mobile liquid indicates the existence of a mesomorphic state. Above ~140°C there is a visible melting of the sample to a brownish liquid bead. During this transition the line width decreases further by a factor of 1.9.

Fig. 3 shows the high temperature region of the line width vs. temperature curve for a sample of lyophilized guinea pig myelin. The mesomorphic state line width and the melting point are approximately the same as for the bovine sample.

Although we did not make a detailed study of the effects of hydration, we did find that hydration of the myelin affected the state of motion of the hydrocarbon chains. In particular, the line width for guinea pig myelin resuspended in D_2O was found to be 0.02–0.03 gauss in the temperature range from 35 to 90°C. As shown

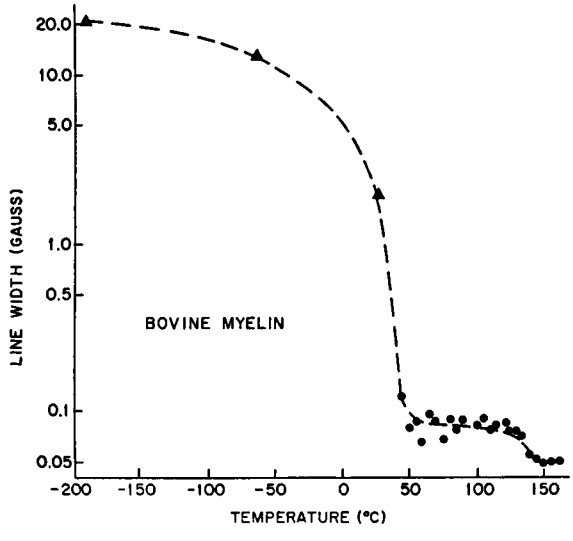

FIGURE 2 Peak-to-peak line width plotted as a function of temperature for lyophilized bovine myelin. Triangles taken with wide-line spectrometer, circles with high resolution instrument. The major line narrowing occurs between 23 and 40°C. A second line width transition coincides with the melting of the sample at 140°C.

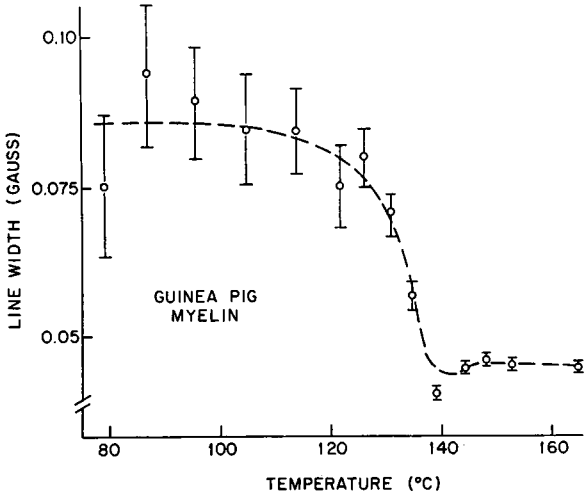

FIGURE 3 High temperature region of line width vs. temperature plot for lyophilized guinea pig myelin. The transition shown in this figure is the melting transition. The transition to the state of intermediate line width is not shown, but occurred at a temperature below 40°C.

in Fig. 3, lyophilized guinea pig myelin in this temperature range has a line width of about 0.08 gauss.

DISCUSSION

The proton resonance line widths observed for lyophilized myelin show that the hydrocarbon chains have considerable freedom of motion at temperatures within the physiological range. Generally, smectic liquid crystals become more fluid when hydrated (Gray, 1962). Chapman (1968) shows that this is also true for phospholipids. Thus, it seems probable that hydrated myelin has relatively fluid hydrocarbon regions at even lower temperatures than the anhydrous preparation. Indeed our experiments with myelin resuspended in D_2O show the hydrocarbon chain regions to be essentially liquid at physiological temperatures.

The most striking features of the line width as a function of temperature are the relatively sharp transitions seen in Figs. 2 and 3. These transitions are similar to the ones observed for the alkali salts of long-chain fatty acids (Lawson and Flautt, 1965). The line width transitions in the fatty-acid–salts reflect the mesomorphic phase transitions during which the lamellar structure is preserved despite an increase in molecular motion within the lamellae.

We may make a further comparison between the mesomorphic state of myelin and the smectic liquid-crystal state of the anhydrous soaps. Both have NMR line widths of the order of 10^{-2} gauss, indicating similar correlation times for reorientation of the protons. However, the myelin mesomorphic phases exist at lower tem-

peratures than the corresponding soap phases so that the barrier to the reorientational motion is lower in myelin.

Thermally induced changes in nerve myelin structure have been observed by X-ray diffraction (Elkes and Finean, 1952). These transitions occur in the same temperature range as those we observed in lyophilized myelin. It is of interest to inquire whether the X-ray changes were caused by protein denaturation or structural changes in the lipid moiety. Our results do not permit us to comment about protein modifications, but they do suggest that the X-ray intensity changes could be explained by lipid phase transitions.

In contrast to our results on lyophilized myelin, the recent NMR studies of erythrocyte ghosts by Chapman, Kamat, de Gier, and Penkett (1968) showed a broad resonance line for intact membranes. From this the authors inferred a rigid membrane structure. We do not know whether these differences indicate intrinsic structural differences between the two membranes, (which certainly have different phospholipid composition—van Deenan, 1965; O'Brien, 1965) or whether they merely reflect differences in the preparations.

We would like to thank Dr. Marion Kies and Dr. John M. Davis of NIMH for their assistance in providing the myelin samples.
We would also like to thank Dr. E. T. Becker of NIAMD and Dr. L. Bennet of the National Bureau of Standards for the use of their NMR spectrometers.
We are particularly indebted to Mr. R. Bradley for his generous assistance.

Received for publication 13 March 1970 and in revised form 28 September 1970.

REFERENCES

ANDREW, E. R. 1950. *J. Chem. Phys.* **18**:607.
AUTILIO, L. A., W. T. NORTON, and R. D. TERRY. 1964. *J. Neurochem.* **11**:17.
CHAPMAN, D. 1968. *In* Membrane Models and the Formation of Biological Membranes. L. Bolis and B. A. Phethica, editors. North Holland Publishing Company, Amsterdam.
CHAPMAN, D., V. B. KAMAT, J. DE GIER, and S. A. PENKETT. 1968. *J. Mol. Biol.* **31**:101.
DEENEN, L. L. M. VAN. 1965. *Prog. Chem. Fats Other Lipids.* **8** (Pt. 1).
ELKES, J., and J. B. FINEAN. 1952. *Exp. Cell Res.* **4**:69.
FINEAN, J. B. 1953. *Experientia.* **9**:17.
FINEAN, J. B. 1969. *Quart. Rev. Biophys.* **2**:1.
GEREN, B. B. 1954. *Exp. Cell Res.* **7**:558.
GRAY, G. W. 1962. Molecular Structure and Properties of Liquid Crystals. Academic Press, Inc., New York.
HEUVEL, F. A. VAN DEN. 1963. *J. Amer. Oil Chem. Soc.* **40**:455.
HUBBELL, W. L., and H. M. MCCONNELL. 1968. *Proc. Nat. Acad. Sci. U.S.A.* **61**:12.
LAATSCH, R. H., M. W. KIES, S. GORDON, and E. C. ALVORD. 1962. *J. Exp. Med.* **115**:777.
LAWSON, K. D., and T. J. FLAUTT. 1965. *J. Phys. Chem.* **69**:4256.
LECAR, H., G. EHRENSTEIN, and I. STILLMAN. 1967. *Biophys. Soc. Annu. Meet. Abstr.* **7**:A65.
NAPOLITANO, L. M., and T. J. SCALLEN. 1969. *Anat. Rec.* **163**:1.
O'BRIEN, J. S. 1965. *Science (Washington).* **147**:1099.
UZMAN, B. G., and E. T. HEDLEY-WHITE. 1968. *J. Gen. Physiol.* **51**(5, Pt. 2):8s.
WORTHINGTON, C. R. 1969. *Proc. Nat. Acad. Sci. U.S.A.* **63**:604.