DICHROISM OF THE INFRARED SPECTRUM OF NERVE

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ABSTRACT Infrared spectra were obtained from the sciatic and optic nerves of the frog (*Rana sp.*) and the trigeminal and olfactory nerves of the garfish (*Lepisosteus osseus*). The myelinated nerves showed dichroism at several absorption peaks, particularly 1,220–1,230 cm^{-1}, but the nonmyelinated nerves showed little or no dichroism. The dichroic peaks indicate that in myelinated nerve, there is an ordered arrangement of protein and lipid molecules which was not found in nonmyelinated nerve.

INTRODUCTION

Measurement of infrared dichroism of biological materials can show the orientation of transition moments associated with the vibrations of molecular groups. This method has been used for nucleic acids (1), polypeptides and proteins (2, 3), and some carbohydrates. Infrared absorption spectra have been published for membranes (4), for nervous tissue (5), and for myelin (6), but dichroism was not found in oriented membrane films (7). Our study was undertaken because we have observed dichroic effects in the difference spectra between resting and excited nerve (MacClement and Sherebrin, in preparation).

METHODS

Most of the work was done using the sciatic nerve of the frog (*Rana sp.*) which contains both myelinated and nonmyelinated fibers. Some spectra were also obtained from frog optic nerve which is 97% unmyelinated (8) and from the trigeminal and olfactory nerves of the garfish *Lepisosteus osseus*. The garfish olfactory nerve consists of many small nonmyelinated fibers of uniform size, whereas the trigeminal nerve is mainly myelinated (9). The frog nerves were desheathed in Ringer's solution at pH 7.0, but the garfish nerves, which were dissected out as described by Easton (9), did not require desheathing. Specimens were made in the same manner as those used for measuring difference spectra (10). Live nerves were spread between AgCl or CaF_2 plates and squeezed to a thickness of about 20 μm. Nerves prepared in this way remain capable of propagating action potentials.

The spectra were recorded on one of two spectrophotometers, a Perkin-Elmer 521 and an almost identical Perkin-Elmer 621 (Perkin-Elmer Corp., Norwalk Conn.). The polarization of the transmitted light was determined by using a polarizer (Perkin-Elmer model 186–

0243, gold wire grid on AgBr) aligned parallel or perpendicular to the nerve fiber axis. The nerve fibers were oriented at 45° to the vertical to eliminate the polarization characteristics of the spectrophotometer. The slit width was governed by the slit program (1,000 × 2 μm) and scan speeds ranged from 2 to 5 cm^{-1}/s. The resolution of the spectrum was therefore about 3 cm^{-1}.

Measurements were confined to the regions 800–1,750 cm^{-1} and 2,800–3,100 cm^{-1}. Water absorption interfered with measurement below 800 cm^{-1}, above 3,100 cm^{-1}, and in the range 1,600–1,700 cm^{-1}. The region 1,750–2,800 cm^{-1} was not examined.

RESULTS AND DISCUSSION

A pair of spectra showing transmission parallel and perpendicular to the length of the frog sciatic nerve are shown in Fig. 1. Although the fibers could not be aligned perfectly, it is unlikely that the true dichroism is much greater than was found because the peak at 1,280 cm^{-1} appears only in the parallel spectrum. If the fibers were not well aligned all peaks would appear in both spectra. Virtually identical spectra were obtained from both spectrophotometers and also in an ice cooled specimen holder which kept the sample at about 10°C. Results were quite reproducible and even the slight dichroism at 1,030 cm^{-1} and 1,060 cm^{-1} was consistent. The observed dichroism cannot in general be explained by other optical effects such as scattering by the fibers. Such optical effects may give wavelength dependent differences in turbidity but are very unlikely to produce a difference at absorption peaks and almost no difference at points of maximum transmission. Spectra for all nerves were very close together at points of maximum transmission in the region 900–1,600 cm^{-1}. In myelinated nerve the points of maximum transmission did not coincide

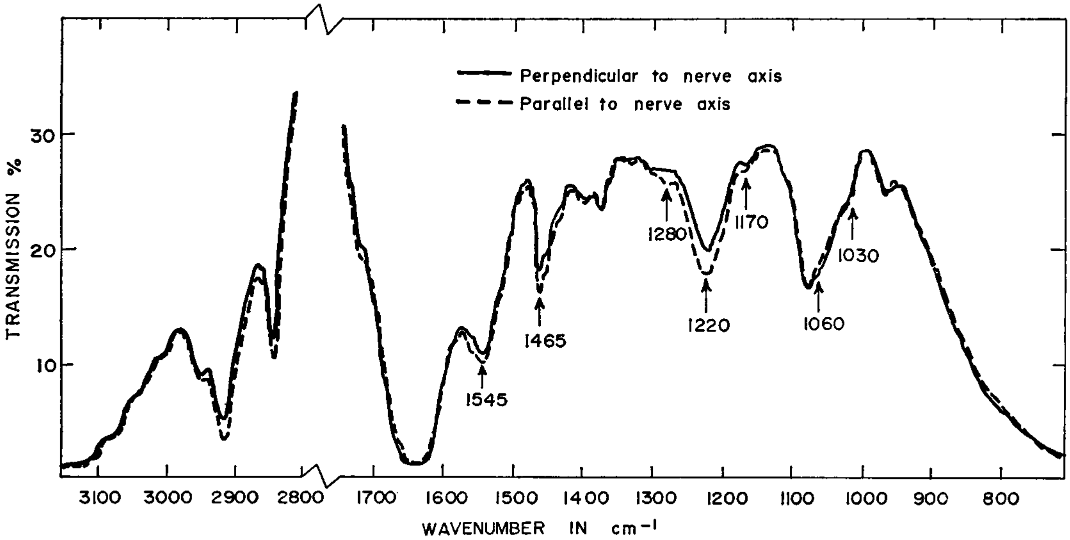

FIGURE 1 The infrared spectrum of frog sciatic nerve. The solid line shows transmission with the electric vector perpendicular to the length of the nerve fibers and the dotted line shows transmission parallel to the length of the nerve fibers.

between 2,800 and 3,000 cm^{-1} and dichroism could be questioned. Dichroism of shoulders on some strongly dichroic bands was also questionable.

Both the myelinated nerves had a strongly dichroic peak at about 1,220–1,230 cm^{-1} and specimens of the garfish trigeminal nerve which had been left in the specimen holder for a few hours developed parallel dichrosim at 1,455 and 1,465 cm^{-1}. In contrast, the garfish olfactory nerve, although it was the easiest nerve to make into good specimens with apparently parallel fibers, showed only slight dichroism in two bands and leaving the specimen in the holder produced no increase in dichroism. It appears that dichroic peaks may be associated with myelin. No dichroism was found in the frog optic nerve, probably because it was very difficult to desheath this nerve and arrange the fibers in the specimen holder. The spectrum is included because the nerve contains relatively little myelin, and comparison with the nonmyelinated garfish olfactory nerve shows some differences between these two nerves and the corresponding myelinated nerves. The myelinated nerves show stronger absorption at 1,720, 1,435, 1,375, and 970 cm^{-1}, as well as greater dichroism at 1,220–1,230 cm^{-1}. Band assignments were made on the assumption that the nerve consists mainly of lipid and protein. The C—H stretching modes between 2,800 and 3,000 cm^{-1} and the C—H bending modes between 1,450 and 1,465 cm^{-1} are characteristic of straight chain hydrocarbons (11) and would be expected in phospholipids. Phospholipid bands associated with the phosphate group occur at 1,230, 1,170, 1,090, 1,060, and 1,030 cm^{-1} (12). The characteristic absorption bands of protein are associated with the polypeptide chain and occur at 1,660–1,640 cm^{-1} (Amide I), 1,570–1,530 cm^{-1} (Amide II), and 1,300–1,240 cm^{-1} (Amide III) (13). The absorption bands of all four nerves are summarised in Table I together with observed dichroism and probable assignments.

The dichroism of the myelinated nerves indicates an ordered molecular arrangement which is not found in nonmyelinated nerves. The dichroism of the peak at 1,220–1,230 cm^{-1} gives some information about the orientation of the phosphate groups in phospholipid molecules. This line has been assigned to either a P=O stretch or an antisymmetric PO_2^- stretch. The angle between the average transition moment and the membrane surface may be calculated. The membrane can be regarded as a long cylinder so that the average transition moment has three components: p, perpendicular to the membrane; q, parallel to the cylinder axis; and r, tangential to the membrane surface and perpendicular to q and p. The magnitude of the average transition moment m is given by

$$m^2 = p^2 + q^2 + r^2. \qquad (1)$$

The dichroic ratio R is defined as the ratio of the optical density measured with the electric vector parallel to the nerve to that measured with the electric vector perpendicular to the nerve. The optical density is proportional to the square of the transition moment. The average transition moment parallel to the nerve is q but

TABLE I
PROBABLE ASSIGNMENTS OF INFRARED ABSORPTION BANDS

Myelinated		Nonmyelinated		Vibration mode
Frog sciatic	Garfish trigeminal	Frog optic	Garfish olfactory	
2,950		2,955	2,950	As. CH_3 str. (11)
2,920‖	2,920	2,925	2,920	As. CH_2 str. (11)
2,860 wb			2,866 wb⊥	Sym. CH_3 str. (11)
2,845 ‖*	2,850 ‖*	2,850	2,850	Sym. CH_2 str. (11)
1,720 wb	1,720			$C{=}O$ str.
1,555 wb‖				Amide II (13)
1,545‖	1,545	1,550	1,545	
1,465‖	1,465	1,465	1,465	$-CH_2$ -sc. (11)
1,455 sh‖.	1,455 sh	1,455	1,450	$-CH_2$ -bend
1,435 sh‖*	1,438			
1,400	1,400 wb	1,395	1,397	
1,375	1,375	1,375 wb		
1,280‖	1,250 sh‖*	1,300		Amide III (13)
1,235‖	1,220‖	1,230	1,230 wd‖	As. PO_2^- str. (1) or $P{=}O$ str. (12)
	1,190 sh‖*			
1,170 sh⊥*	1,170 wb	1,170	1,160 sh	$C{-}O{-}C$ str. (12)
1,080	1,080	1,080	1,080	Sym. PO_2^- str. (1) or $P{-}O^-$ (bend?) (12)
1,060 wb⊥				$P{-}O{-}C$ str. (12)
1,030 wb‖	1,020 wd‖		1,040 wb	$P{-}OH$ (bend?) (12)
965	970	970 sh	970 sh	CH out of plane bend in $-CH{=}CH^-$ (14)

Sym., symmetric; as., antisymmetric; str., stretch; sc., scissors; sh, shoulder; wb, very weak band; wd, weak dichroism; ⊥, perpendicular dichroism; ‖, parallel dichroism; *, possible dichroism.

the perpendicular component is more complex. At the edge of the cylinder the component *r* is parallel to the direction of propagation of infrared radiation and causes no absorption, similarly at the center *p* is parallel to the direction of propagation. The average transition moment responsible for absorption in this direction is given by the square root of $(p^2 + r^2)/2$. The dichroic ratio is therefore

$$R = q^2/[\tfrac{1}{2}(p^2 + r^2)]. \tag{2}$$

It is very unlikely that the phosphate groups are aligned parallel to any specific direction in the plane of the membrane surface, particularly as current opinions state that the membrane has semifluid properties (15). One can therefore assume that the two components *q* and *r* which lie in the plane of the membrane are equal.

$$q = r. \tag{3}$$

The cosine of the angle θ between the average transition moment m and the membrane normal is p/m, and using equations 1, 2, and 3 one can eliminate p, q, and r and obtain the expression $\cos\theta = [(2 - R)/(2 + R)]^{1/2}$. If the line shows parallel dichroism, R is greater than one and the angle θ is greater than 55°. The angle between the average transition moment and the membrane surface is $90° - \theta$ so parallel dichroism shows that the angle between the average transition moment and the membrane surface is less than 35°. The value of the dichroic ratio for the frog sciatic nerve was found from the spectrum in Fig. 1 to be 1.30 so that the angle between the average transition moment and the membrane surface is 27°. The slight perpendicular dichroism at 1,060 cm^{-1} indicates that the average transition moment of the P—O—C groups in phospholipids is at an angle of more than 35° to the membrane surface. Similarly the transition moment of the P—OH bending mode (1,030 cm^{-1}) is at an angle of less than 35° to the membrane surface.

These features of the dichroic spectra of nerve are qualitatively consistent with the accepted model of the lipid bilayer. It was also noted that the CH_2 out-of-plane bending modes at 1,465 and 1,455 cm^{-1} in the frog sciatic nerve show parallel dichroism indicating that the CH bonds lie roughly parallel to the membrane surface. The bands at 2,845 and 2,920 cm^{-1} are due to CH_2 stretching modes and should show parallel dichroism, but examination of the parts of maximum transmission shows that although the dichroism at 2,920 cm^{-1} is probably real, the dichroism at 2,845 cm^{-1} may be artifact. In the garfish trigeminal nerve dichroism at 2,850 cm^{-1} is also questionable. The 2,920 cm^{-1} band did not show any dichroism in the garfish trigeminal nerve unless it had been left in the specimen holder for several hours, which is consistent with the appearance of parallel dichroism at 1,455–1,465 cm^{-1} in old specimens of this nerve.

In the frog sciatic nerve there is a highly dichroic band at 1,280 cm^{-1} which has been assigned to the Amide III band of protein. The dichroic ratio of this band cannot be estimated accurately but appears to be greater than two. According to the previous analysis a dichroic ratio of two shows that the transition moment lies parallel to the surface of the membrane, but for a dichroic ratio greater than two the model for an isotropic membrane fails, and the transition moment must be oriented nearly parallel to the length of the nerve. Since the transition moment of the Amide III band lies parallel to the length of the extended polypeptide chain, the protein responsible for this band probably lies in straight chains whose axis is parallel to the length of the nerve (3).

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