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AN X-RAY STUDY OF THE CONDENSED AND SEPARATED STATES OF SCIATIC NERVE MYELIN

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SUMMARY

Low-angle X-ray diffraction patterns of peripheral nerve myelin after modification by either rehydration in various solutions or by chemical treatment have been recorded. These X-ray patterns and the previously reported modified nerve myelin patterns demonstrate that nerve myelin has at least five different states: the normal state, condensed state I and II and separated state I and II. There are two membranes per unit cell in the normal state and in states II whereas there is one membrane per unit cell in states I. Under certain conditions normal nerve can go reversibly into either of states II. With continued treatment the nerve myelin structure moves irreversibly from state II to state I and, once in state I, the nerve myelin layers cannot return to the normal state. Our results demonstrate that there is a reversible transformation between condensed state I and separated state I. Fourier profiles of nerve myelin in the normal state, condensed state I and separated state I are presented.

INTRODUCTION

The structure of sciatic nerve myelin in a direction at right angles to the surface of the myelin layers has been studied extensively by X-ray diffraction. Low-angle X-ray diffraction patterns have been recorded from live or normal nerve [1–6], nerve swollen in hypotonic solutions [6–10] and from nerve myelin modified by physical treatments such as air drying, rehydration and freezing and thawing [2, 11–13]. The structural changes which occur on swelling in hypotonic solutions are now well understood and the method of swelling has been used in the structure analysis of nerve myelin. [6, 7–10] Consequently, the phases of the first five diffraction orders of normal nerve [14] have recently been determined unambiguously and electron density profiles [14] of sciatic nerve myelin have been obtained at a resolution of 17 Å. On the other hand the X-ray diffraction patterns from modified nerve myelin have received little attention as it was not possible to interpret these patterns at the time when the pat-

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terns were first recorded [11–13]. Nevertheless, the similarity of these physical treatments to the chemical treatment of immersing the nerve specimens in a hypertonic medium has been recognized [11–13]. In this paper it will be shown that the X-ray patterns from nerve modified by physical and chemical treatments belong to a few distinct states and the relationship between these states is described.

In a recent X-ray study on rehydrated sciatic nerve the continuous Fourier transforms of the unit cell of rehydrated nerve was effectively traced [15] and the electron density profile of rehydrated nerve at a resolution of 17 Å was obtained. The Fourier profiles indicated a membrane width of about 75 Å while the adjacent rehydrated membranes were separated by a fluid layer of 20–50 Å in width.

In the present study X-ray experiments indicate that there are two different states of the rehydrated nerve. These are the separated and condensed states. The rehydrated nerve is defined to be in a separated state when adjacent membranes are separated by a fluid layer but, when the fluid layer becomes very narrow or vanishes such that the rehydrated membranes either interpenetrate at their outer surfaces or are compacted together, the membrane is defined to be in a condensed state. It would appear that only minor changes in structure occur when the membrane changes from the separated state to the condensed state or vice-versa. Moreover, these minor changes are reversible for we find that there is a reversible transformation between the separated and condensed states.

It turns out that it is necessary to further classify the two states as separated state I denoted SI and separated state II denoted SII and as condensed state I denoted CI and condensed state II denoted CII. The difference between states I and II depends on whether the unit cell contains one or two membranes. The unit cell of nerve myelin in the normal state, SII and CII contains a pair of membranes, whereas, the unit cell of nerve myelin in SI and in CI contains only one membrane. We find that our X-ray patterns of modified nerve myelin (to be described) and the various X-ray patterns of modified nerve myelin recorded by others [2, 7, 11–13, 16] all refer to nerve myelin in either the separated or condensed states. A discussion of the relationship between the separated and condensed states of nerve myelin is presented. Low resolution Fourier profiles of nerve myelin in the normal, separated and condensed states are also presented.

METHODS

X-ray diffraction

Low-angle X-ray diffraction patterns were obtained from frog and rabbit sciatic nerves after air drying and rehydration and after soaking in chemical solutions. In order to obtain rehydrated nerve specimens the freshly dissected nerves were allowed to air dry under slight tension for about 12 h. The air-dried nerves were then placed in thin-walled glass capillary tubes and allowed to rehydrate in Ringer's solution, in $CaCl_2$ solutions or in Ringer's solutions of varying concentrations for about 12 h. The chemically treated nerve specimens were obtained by soaking freshly dissected frog and rabbit sciatic nerves in $CaCl_2$ solutions (Tris buffered at pH = 7.5) or NaCl solutions (Tris buffered at pH = 7.5) and placing the specimens in thin-walled glass capillary tubes either before or after soaking.

Wide-angle X-ray diffraction patterns were recorded using a flat plate X-ray

camera and a Philips microfocus X-ray generator. Low-angle X-ray diffraction patterns were recorded using an optically focusing X-ray camera [17] and an Elliott rotating anode microfocus X-ray generator. Most patterns were recorded using line collimation. Line collimation was obtained by using only the first mirror and a line focus on the copper anode.

The X-ray patterns were taken with nickel-filtered copper Kα radiation. The specimen-to-film distance was normally set at about 8.0 cm. Exposure times usually did not exceed 6 h. X-ray patterns were recorded on Ilford Industrial G film. The X-ray reflections obey Bragg's law: $2d \sin \theta = h\lambda$ where d is the radial repeat distance, θ is the Bragg angle, h is the diffraction order and λ is the wavelength of the X-radiation. A Joyce-Loebl microdensitometer model MKIIIC was used to obtain tracings of the patterns. The background curve was subtracted from the tracing in the usual way and the discrete intensities $I(h)$ were obtained by measuring the area under the diffraction peaks.

We use the notation that $t(x)$ is the electron density of the unit cell of width d and $T(X)$ is the Fourier transform of $t(x)$, where x, X are real and reciprocal distances. Experimentally, the integrated intensity $I(h)$ is measured, but the Fourier transform value $|T(h)|$ is needed. It is known that $|T(h)|$ for nerve myelin [18] is given by $|T(h)| = [h\, I(h)]^{\frac{1}{2}}$. Many X-ray experiments were carried out under the same conditions and a range of d values was experimentally obtained. Different sets of low-angle X-ray data recorded using the same immersion fluid were put on the same relative scale using a formula derived previously [18].

Polyacrylamide gel electrophoresis

The isolation of myelin from the frog sciatic nerve and the preparation of the myelin pellet containing the myelin proteins followed the procedures of Greenfield et al. [19]. Electrophoresis in a sodium dodecyl sulphate medium was carried out by the method of Fairbanks et al. [20]. A convenient unit (V.W.R. Scientific Company) was used and 12 disc gels were run at the same time. The gels were stained with Coomassie Blue and were densitometered in a Beckman-Gilford Instrument adapted for scanning acrylamide gels.

RESULTS

1. *Low-angle X-ray diffraction patterns*

Rehydrated nerve myelin

(*a*) *Ringer's solution.* Low-angle X-ray diffraction patterns of frog and rabbit sciatic nerve rehydrated in Ringer's solution showed the first six discrete orders of $d = 95$ Å to 125 Å. These patterns were similar to those described previously [15]. The air-dried nerve after rehydration is in SI. A reproduction of a typical diffraction pattern of rehydrated nerve (rabbit sciatic nerve) in SI is shown in Plate IA. The first three discrete orders of $d = 95$ Å are visible in the reproduction.

(*b*) *Hypotonic solutions.* Low-angle X-ray diffraction patterns of frog and rabbit sciatic nerve rehydrated in either distilled water or 0.5 times Ringer's solution showed the first three discrete orders of $d = 130$–150 Å. The nerve is in SI but with comparatively wide fluid layers. The diffraction lines ($h = 1$–3) were not as sharp as they were when rehydrated in Ringer's solution. Consequently, the higher-order diffraction ($h = 4$–6) was somewhat diffuse and the intensities were difficult to measure.

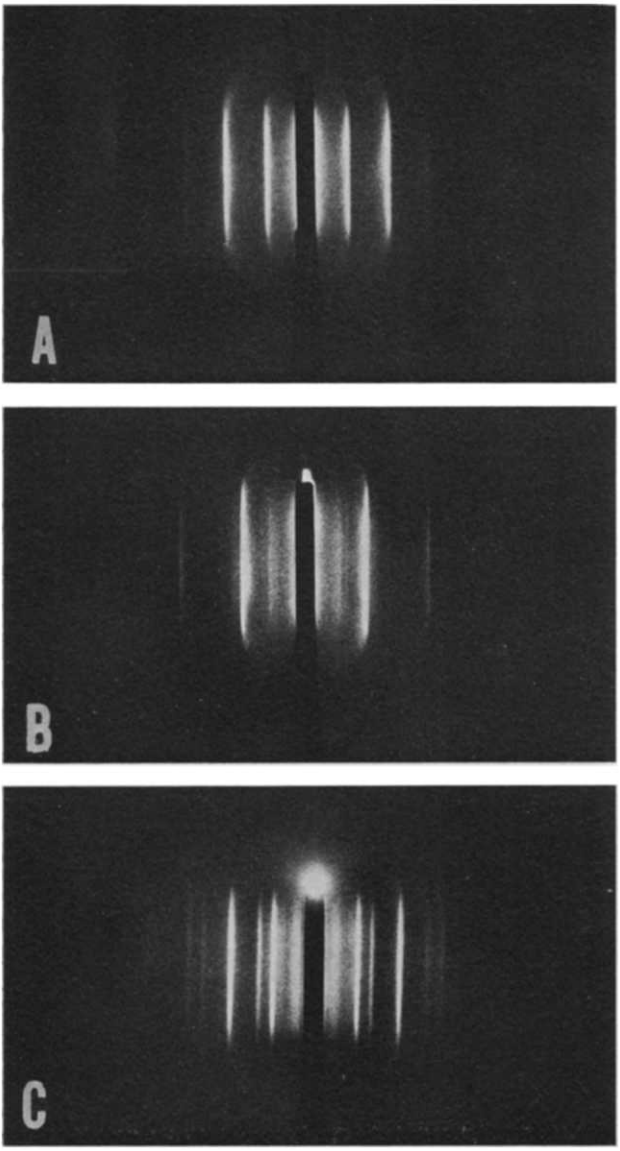

Plate I. (A) Low-angle X-ray diffraction pattern of rabbit sciatic nerve rehydrated in Ringer's solution, $d = 97$ Å. The first three orders of diffraction are visible. (B) Low-angle X-ray diffraction pattern of rabbit sciatic nerve rehydrated in 180 mM $CaCl_2$ solution, $d = 64$ Å. The first two orders of $d = 64$ Å and a diffuse reflection at $d \approx 135$ Å are visible. (C) Low-angle X-ray diffraction pattern of rabbit sciatic nerve rehydrated in 3 times Ringer's solution with $d = 95$ Å and $d = 71$ Å. The first three orders of $d = 95$ Å, the first two orders of $d = 71$ Å and a diffusion reflection at $d \approx 200$ Å are visible.

(*c*) *$CaCl_2$ solutions.* Low-angle X-ray diffraction patterns of frog and rabbit sciatic nerve rehydrated in $CaCl_2$ solutions of concentrations above 10 mM showed the first four discrete orders of $d = 64$–68 Å. There was also a diffuse reflection at $d \simeq 135$ Å. The origin of this diffuse reflection is not known. A reproduction of a typical pattern of nerve rehydrated in $CaCl_2$ solutions above 10 mM is shown in Plate 1B. The first two discrete orders of $d = 64$ Å are visible in the reproduction.

(*d*) *Hypertonic Ringer's solutions.* Low-angle X-ray diffraction patterns of frog and rabbit sciatic nerve rehydrated in hypertonic Ringer's solutions were similar to those obtained after rehydration in $CaCl_2$ solutions. The nerve after treatment with hypertonic solutions is in SI. In X-ray experiments using 3 times Ringer's solutions repeat periods of $d = 68$–71 Å were obtained.

(*e*) *Reimmersion of Ringer's-treated nerve in $CaCl_2$ solutions.* X-ray experiments showed that if the nerve previously rehydrated in Ringer's solution was soaked in $CaCl_2$ solution (concentration $\geqslant$ 10 mM) the nerve goes from SI into CI and repeat periods of $d = 64$–68 Å were recorded. Similarly, if the nerve previously rehydrated in $CaCl_2$ solution was soaked in Ringer's solution the nerve goes from CI into SI and repeat periods of $d = 95$–125 Å were recorded. It follows that there is a reversible transformation for nerve myelin between the two different states, namely SI and CI. The transformation of nerve myelin in SI to CI occurred slowly when using 3 times Ringer's solution. Rehydrated rabbit sciatic nerve in 3 times Ringer's solution after soaking for twelve hours is in SI and showed discrete orders of $d = 95$ Å. But, after soaking in 3 times Ringer's solution for another day, two distinct series of reflections were present with repeat periods of $d = 95$ Å and $d = 71$ Å. The $d = 95$ Å series derives from nerve in SI while the $d = 71$ Å series derives from nerve in CI. A reproduction of this X-ray pattern is shown in Plate IC where the first three orders of $d = 95$ Å and the first two orders of $d = 71$ Å are visible. In Plate IC the $d = 71$ Å series is just beginning to appear and on further soaking in 3 times Ringer's solution the $d = 71$ Å series of reflections will predominate.

Chemically treated nerve myelin

(*a*) *$CaCl_2$ solutions.* Low-angle X-ray diffraction patterns of frog and rabbit sciatic nerve immersed in $CaCl_2$ solutions of concentration $\geqslant$ 10 mM were of two kinds depending on the time of immersion (1 or 2 days or several (more than 2) days). When the immersion was for about 2 days (or less) discrete orders of $d = 124$–128 Å were recorded. The odd orders of diffraction had weak intensities compared to the even orders. The $h = 2$ reflection had by far the strongest intensity. The above diffraction derives from nerve myelin which is in CII. When the immersion was for several days the odd orders of $d = 124$–128 Å faded and only discrete orders of $d = 62$–64 Å remained. The $h = 1$ reflection was the most intense. The diffraction after several days immersion comes from nerve myelin which is in CI. The X-ray pattern from sciatic nerve after soaking for several days in $CaCl_2$ solutions was identical or very similar to the X-ray pattern which is shown in Plate IB. The pattern in Plate IB refers to rehydrated nerve in CI. Note that when the $CaCl_2$ solution has a concentration of less than 10 mM but somewhat greater than 1 mM the normal pattern with $d = 171$ Å for frog sciatic nerve and $d = 179$ Å for rabbit sciatic nerve was obtained.

(*b*) *Reimmersion of $CaCl_2$-treated nerve in Ringer's solution.* When the nerve previously immersed in $CaCl_2$ solution for 2 days (or less) was reimmersed in Ringer's

solution the nerve goes into the normal state and repeat periods of $d = 171$ Å for frog sciatic nerve and $d = 179$ Å for rabbit sciatic nerve were obtained. Note that the low-angle X-ray pattern [1] of peripheral nerve myelin in Ringer's solution shows the first five diffraction orders of a radial repeat distance $d = 170$–180 Å depending on the variety of nerve. When the nerve previously immersed in Ringer's solution was reimmersed in $CaCl_2$ of $\geqslant 10$ mM concentration the nerve goes into CII and repeat periods of $d = 124$–128 Å were obtained. It follows that there is a reversible transformation for nerve myelin between the normal state and CII.

(*c*) *Hypertonic NaCl solutions.* Low-angle X-ray diffraction patterns of sciatic nerve immersed in NaCl solutions of 200 mM (or less) were identical to the normal pattern. When the concentration of NaCl solution was increased to 500 mM (or higher) a low-angle X-ray diffraction pattern showing discrete orders of $d = 196$ Å was recorded. The odd orders of this pattern had weak intensities compared to the even orders. This diffraction comes from nerve myelin which is defined to be in SII. After several days of immersion the odd orders of $d = 196$ Å vanished and only discrete orders of $d = 98$ Å were recorded. This diffraction comes from nerve myelin which is defined to be in SI.

(*d*) *Hypertonic Ringer's solutions.* Low-angle X-ray diffraction patterns of frog sciatic nerve immersed in 4, 6 and 8 times Ringer's solution were recorded. The X-ray patterns were dependent on the period of immersion. A minimum time of 12 h was used to ensure reproducible patterns. Frog sciatic nerve after immersion in hypertonic Ringer's solutions for 1 or 2 days gave two series of reflections with periods of $d = 190$ Å and $d = 134$ Å. The $d = 190$ Å series comes from nerve myelin in SII while the $d = 134$ Å series comes from nerve myelin in CII. The $d = 190$ Å series showed diffraction orders $h = 1, 2, 4$ and 6 while the first four orders of $d = 134$ Å were present. Higher orders of both series were obtained using longer exposures. If the period of immersion was increased to 4 or 5 days then the odd diffraction orders of $d = 190$ Å and $d = 134$ Å disappeared. Frog sciatic nerve after immersion in hypertonic Ringer's solutions for 4–5 days gave the first three orders of $d = 95$ Å and the first two orders of $d = 67$ Å. Higher orders of both series were obtained using longer exposures. The $d = 95$ Å series comes from SI while the $d = 67$ Å series comes from CI.

(*e*) *Reimmersion of hypertonic-treated nerve in Ringer's solution.* If the frog sciatic nerve which had been immersed in hypertonic Ringer's solution for 1 or 2 days was reimmersed in Ringer's solution for 12 h or longer an X-ray pattern similar to the normal pattern of frog sciatic nerve was obtained. This normal-type pattern showed the first five orders of $d = 173$–177 Å. Our X-ray experiments with hypertonic Ringer's solution verify that nerve myelin in SII and CII moves into a normal-type state after reimmersion in Ringer's solution. The transformation is not completely reversible in that a slightly increased period was recorded.

A normal-type pattern was not obtained from nerve previously soaked in hypertonic Ringer's solution for 4 to 5 days after reimmersion in Ringer's solution. The nerve after 4 or 5 days in hypertonic Ringer's solution is in SI and CI. After reimmersion in Ringer's solution the nerve moves solely into SI and only the $d = 95$ Å series of reflection remained.

2. *Wide-angle X-ray diffraction pattern*

Wide-angle X-ray diffraction patterns of rehydrated sciatic nerve specimens

were very similar to the well-known wide-angle X-ray pattern of normal nerve in Ringer's solution. This X-ray pattern of normal nerve [1, 2, 11] shows a somewhat diffuse 4.7 Å reflection which is arced on the meridian. The sciatic nerves, whether in the normal state, separated state I and II and condensed state I and II all showed a 4.7 Å reflection which had the same appearance in each case. Thus, it can be concluded that the arrangement and alignment of hydrocarbon chains in the myelin layers are very similar for the five different states of nerve myelin.

3. Polyacrylamide gel patterns

Gels were run using frog sciatic nerves as the source of myelin proteins. The gel patterns of normal nerve showed four main bands after staining with Coomassie Blue. The appearance of these bands for frog sciatic nerve was similar to those obtained for other sciatic nerves [19]. The four bands are designed P_0, X, P_1 and P_2 where P_0 is the major protein band of high molecular weight, X is of moderate molecular weight and P_1 and P_2 are the lower molecular weight basic protein fractions. Our gel patterns of air-dried nerves, rehydrated nerves, frozen below -20 °C and thawed nerves all showed the same four bands of normal nerve. Moreover, the densitometer traces of these gels which were obtained from the modified nerves were very similar to the traces from normal nerve.

The gel electrophoresis densitometer trace give the molecular weight distribution of myelin proteins in the myelin pellet. That is, the peak position of the band gives the molecular weight while the area under the band gives the percentage occupancy. The observation that the traces of normal and modified nerves were very similar indicates that the physical treatments of air-drying and rehydration and of freezing and thawing did not change the molecular weight distribution of the myelin proteins.

Note that the gels measure the overall molecular weight distribution of proteins in the myelin pellet. The gels are therefore not sensitive to any rearrangement of proteins either in the myelin sheath or in the myelin pellet. On the other hand, the gels are sensitive to a loss of protein components which may occur during the physical treatments. Any protein component which might be released from the myelin sheath will become separated from the myelin pellet during the isolation procedures because of its higher density. Our results, therefore, indicate that there is no loss of protein components from the myelin sheath during the physical treatments of air-drying and rehydration and of freezing and thawing.

CORRELATION WITH PREVIOUS WORK

Five different states of nerve myelin have been distinguished as a result of our present X-ray experiments. These are the normal state, separated state I and II and condensed state I and II. Two other states of nerve myelin namely, the swollen state [7–10] and the subnormal state, [10] have been previously described. Previous X-ray patterns of modified nerve myelin [2, 7, 11–13, 16] are identified as arising from nerve myelin in either the separated or the condensed state. A schematic diagram indicating the relationship between the five states of nerve myelin is shown in Fig. 1.

The low-angle X-ray patterns of sciatic nerves obtained after immersion in hypertonic solutions [7, 10, 13], after freezing below -20 °C and thawing [13], and after rehydration [2, 11, 12, 15] refer to nerve myelin in either SI or SII. Our X-ray

experiments indicate that nerve myelin after rehydration goes straight into SI whereas nerve myelin treated with hypertonic solutions first goes into SII but, after further soaking, goes into SI.

The low-angle X-ray patterns of nerves after freezing [13] below −20 °C and after immersion of normal nerves in dimethylsulfoxide in Ringer's solution [16] refer to nerve myelin in the condensed state. The frozen nerve patterns [13] indicate that nerve myelin goes straight into CI. The dimethylsulfoxide treated nerve patterns [16] and our patterns of nerve in $CaCl_2$ solutions indicate that nerve myelin first goes into CII but, after further soaking, goes into CI.

There is a reversible transformation between nerve myelin in the normal state and nerve myelin in CII. This transformation was first obtained with nerve in dimethylsulfoxide in Ringer's solution [16] and our X-ray experiments with nerve in $CaCl_2$ solutions confirm that there is a reversible transformation between the normal state and CII

It has been reported [7, 13] that there is a reversible transformation between nerve myelin in the normal state and nerve myelin in hypertonic solutions provided that the ionic strength had been maintained at a relatively low value. Our X-ray experiments with frog sciatic nerves in hypertonic Ringer's solutions confirm that there is a reversible transformation provided that nerve myelin is in SII and not SI. Note that the nerve myelin moves from SII to SI if immersed in hypertonic Ringer's solutions for more than 2 days The transformation from SII to normal is not perfect in that a period of $d = 173$–176 Å is regained instead of the normal period of $d = 171$ Å. The intensity variation of the regained normal pattern is, however, very similar to the intensity pattern of normal nerve.

The X-ray observation that there is a reversible transformation between nerve myelin in SI and CI provides the link between these two states. Thus, normal nerve moves into either SII or CII depending on treatment. With continued treatment the nerve myelin structure moves irreversibly into state I from state II. There is, however, a reversible transformation between SI and CI.

Note that the schematic diagram in Fig. 1 is a simplified presentation, for after certain treatments nerve myelin can often go partly into the separated state while the remainder goes into the condensed state. This is evident from our X-ray experi-

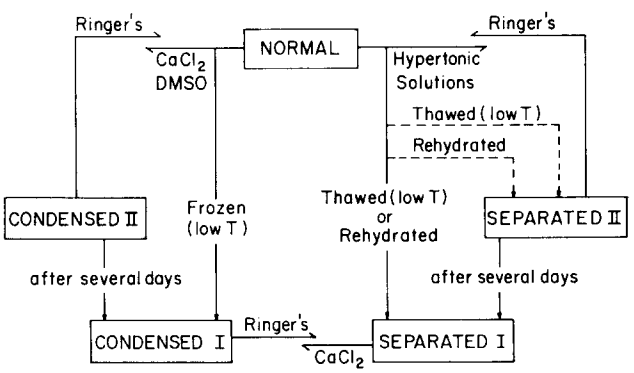

Fig. 1. Schematic diagram showing the relationships between the normal, separated and condensed states of nerve myelin.

ments on rehydrated nerve in hypertonic solutions (Plate IC) and on nerves immersed in hypertonic Ringer's solutions. Previous X-ray patterns [10] of nerve in hypertonic solutions and our present experiments distinguish between the two states. In the earlier work on modified nerve [2, 7, 11–13] the existence of nerve myelin in the condensed state was not fully recognized and the origin of the $d = 62$–68 Å reflection was in doubt. It was tentatively thought that this reflection was due to a lipid-phase separation [2] which occurred during the hypertonic-type treatments. It is now evident that this reflection arises from an assembly of myelin layers which have been condensed together.

FOURIER PROFILES

The Fourier series representation for $t(x)$, the electron density of the unit cell, is given by

$$(2/d)\sum_{1}^{h}\{\pm\}\,|T(h)|\cos 2\pi hx/d$$

where $\{\pm\}$ refers to the phase of the Fourier transform value $|T(h)|$ and where it is assumed that the unit cell contains a center of symmetry. The experimental data points $|T(h)|$ for nerve rehydrated in Ringer's solution and for nerve rehydrated in $CaCl_2$ solutions are plotted in Fig. 2, values of X in the range of $0 < X < 0.032$ Å^{-1} are shown. The data points all appear to lie on the same Fourier transform curve. The experimental data points for nerve rehydrated in Ringer's solution have been previously described [15] and the phase of this region was shown to be minus. Thus the phases for the first two reflections of $d = 62$–64 Å are the same and are also minus.

The Fourier profile of rabbit sciatic nerve in Ringer's solution was computed using $(--+-+-)$ phases [14] for the first five orders of $d = 179$ Å and is shown in Fig. 3A. The resolution of the Fourier synthesis in Fig. 3A is 18 Å. In Fig. 3A the membrane is slightly asymmetric about its center and it has a slightly higher density on the cytoplasmic side as noted previously [14]. The Fourier profile of rabbit sciatic

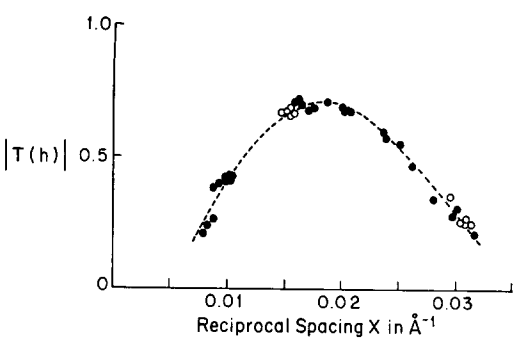

Fig. 2. Low-angle x-ray diffraction data from rehydrated nerve myelin in Ringer's solution and in $CaCl_2$ solutions. The Fourier transform values $|T(h)|$ for nerve rehydrated in Ringer's solution are represented by small black dots (●) whereas the corresponding values for nerve rehydrated in $CaCl_2$ solutions are represented by open circles (○). The data points are plotted as a function of reciprocal space distance X and extend out to $X \approx 0.032$ Å^{-1}. The continuous transform (dotted line) has been drawn in by eye.

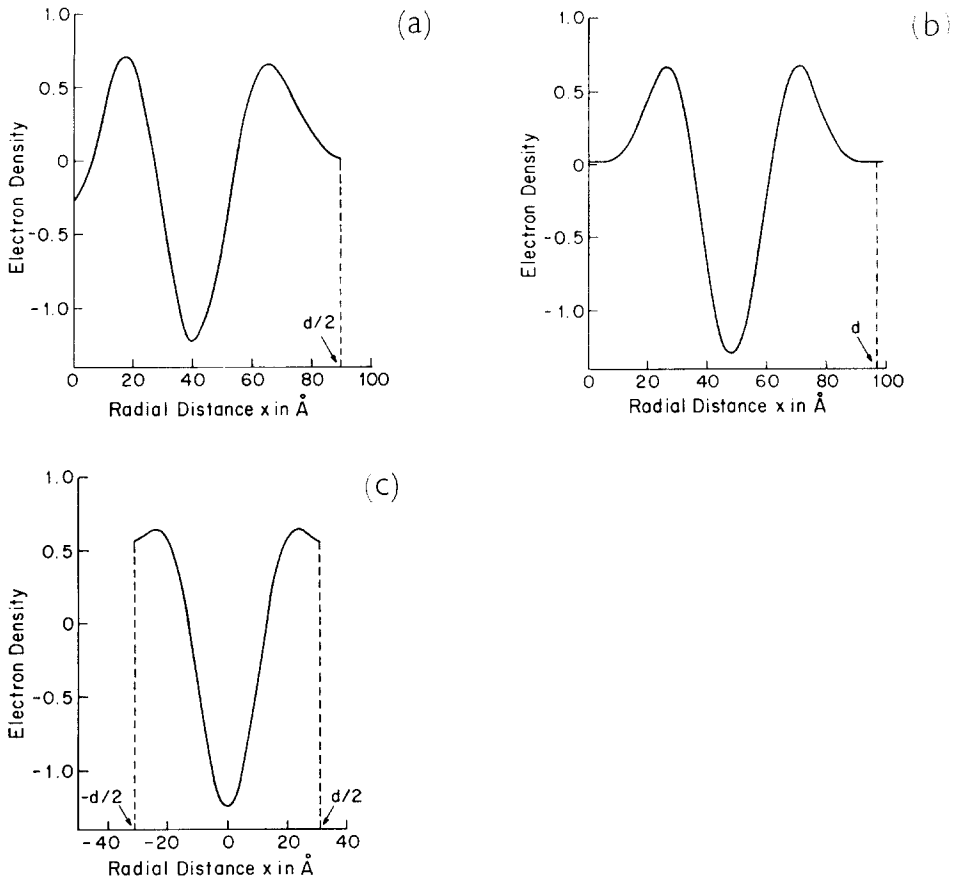

Fig. 3 (A) Fourier series representation for rabbit sciatic nerve in Ringer's solution computed using the first five orders of $d = 179$ Å and using the phase choice $(-, +, +, -, -)$. The unit cell contains two membranes but only half of the unit cell is shown. The origin of the unit cell ($x = 0$) is at the center of the cytoplasmic fluid layer and $x = d/2$ defines the center of the extracellular fluid layer. (B) Fourier series representation for rabbit sciatic nerve rehydrated in Ringer's solution computed using the first three orders of $d = 97$ Å and using negative phases. The unit cell contains one membrane which is symmetric about its center. The membrane is centrally placed within the unit cell. The origin of the unit cell ($x = 0$) is at the center of the fluid layer and $x = d$ is at the center of the adjacent fluid layer. (C) Fourier series representation for rabbit sciatic nerve rehydrated in $CaCl_2$ solution computed using the first two orders of $d = 64$ Å and using negative phases. The unit cell contains one membrane which is symmetric about its center. The origin of the unit cell ($x = 0$) is placed at the center of the hydrocarbon chain region and $x = \pm d/2$ defines the center of the narrow fluid layers between adjacent membranes.

nerve rehydrated in Ringer's solution was computed using minus phases for the first three orders of $d = 97$ Å and is shown in Figure 3B. The resolution of the Fourier synthesis in Fig. 3B is 16 Å. The membrane is symmetric about its center. The rehydrated membrane (in Ringer's) is about 75 Å wide and therefore it has the same width as the normal nerve membrane. The Fourier profile of rabbit sciatic nerve rehydrated in $CaCl_2$ solutions was computed using minus phases for the first two orders of $d = 64$ Å and is shown in Fig. 3C. The resolution of the Fourier synthesis in Figure 3C is

16 Å. In Figure 3C the membrane is symmetric about its center. The rehydrated membrane ($CaCl_2$ solutions) is narrower than the 75 Å width of normal and rehydrated (Ringer's) nerve membrane by about 11 Å. Comparison of the Fourier profiles in Fig. 3A, B and C shows that the central low density regions are identical or very similar. Thus, when nerve myelin goes into CI the membrane becomes narrower but with little or no change in the central lipid hydrocarbon region. The outer layers of the nerve myelin membrane in the condensed state appear to have an increased electron density suggesting that the outer layers are compacted together.

Fourier profiles of nerve myelin in either SII or CII have not been obtained because the phases of the odd-order reflections are not known for certain. The odd-order reflections have very weak intensities so that the Fourier profiles will resemble those in Figures 3B and C. The weak odd-orders of diffraction imply that either the individual membranes are slightly asymmetric about their centers or that the extracellular or cytoplasmic fluid layers have slightly different widths. There will also be an ambiguity in identifying the cytoplasmic and extracellular fluid layers.

DISCUSSION

The present X-ray experiments together with the previous X-ray patterns of modified nerve specimens [2, 7, 11–13, 16] demonstrate that nerve myelin has at least five different states. Normal nerve can reversibly go into either condensed state II or separated state II. On continued treatment the nerve goes irreversibly from state II to state I. Although this step from II to I is irreversible, our results indicate that the myelin layers undergo only small or minor changes in structure.

After nerve myelin moves from state II to state I, the unit cell contains a single membrane which is symmetric about its center. It is not known exactly how the normal asymmetric nerve membrane can become symmetric. The case of rehydrated nerve in Ringer's solution is considered. It would seem that changes in the protein arrangement rather than the lipid arrangement are responsible. The Fourier profiles and the wide-angle X-ray diffraction patterns do not detect any change in the lipid hydrocarbon distribution in the central low-density parts of the normal and rehydrated nerve membrane. But, from the Fourier profiles, some change in the electron density of the outer layers of the rehydrated membrane takes place. In an earlier discussion [15] of the structural changes leading to a symmetric rehydrated membrane, the possibility of removal of some of the protein from the membrane during rehydration was mentioned. This possibility now seems remote for the gel patterns show no apparent loss in any one of the four main protein components. Moreover, the reversible transformation between SI and CI would seem unlikely if part of the protein fraction was removed when nerve moves from state II to state I. The remaining possibility that there is some protein rearrangement when the normal asymmetric membrane becomes symmetric now seems the more likely explanation.

The present X-ray experiments verify that the myelin layers can exist in a condensed state and it has been deduced that only minor structural changes occur when the normal membrane is compacted together. The condensed membrane (state I) is narrower than the normal membrane by about 7–11 Å. The Fourier profiles and the wide-angle X-ray diffraction patterns do not detect any change in the lipid hydrocarbon distribution in the central parts of the condensed membrane when compared to

the normal membrane. The gel patterns show no apparent loss in any one of the four main protein components. Presumably some protein rearrangement occurs when the condensed membrane becomes symmetric in state I. The structural changes which occur when nerve moves from CI to SI, or vice versa, are reversible. These changes refer to the narrowing of the fluid channel between adjacent membranes and to a possible closer packing of molecular components in the outer layers of the nerve myelin membrane.

Whether nerve myelin is in the normal, separated or condensed state depends primarily on the width of the fluid layers between membranes. The differences in the widths of fluid layers also account for why nerve myelin can exist in either a swollen state or a subnormal state [10]. The key factor determining the physical state of the myelin layers (whether normal, separated, condensed, swollen or subnormal) no doubt depends on the surface charge of the outer layers of the nerve membrane and the ionic composition of the fluid layers as noted previously [10].

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