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DISORDER IS CHARACTERISTIC OF NERVE MYELIN *

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Summary

An X-ray diffraction pattern from the myelin in frog sciatic nerve has been obtained using the intense synchrotron radiation from the storage ring, SPEAR. Data with good statistical accuracy are obtained in a few minutes by using a scintillation counter or position-sensitive detector. The same indications for stacking disorder are seen as in previous conventional exposures which required one to two days. Thus, the stacking disorder is characteristic of myelin in a freshly dissected nerve. The present data, obtained with a more nearly monochromatic X-ray beam than in the previous study, remove one of two ambiguities which bear on the possible phasing of the higher order Bragg reflections.

Introduction

The study of the nerve myelin membrane by X-ray diffraction is complicated by the fact that the spaces between the membranes are variable [1]. The variability causes both broadening of the stacking reflections and diffuse diffraction. These two effects make it difficult to measure the intensities of the higher order reflections accurately. A lengthy analysis of the stacking disorder has improved the accuracy over past studies, but some uncertainty remains in the measured intensities [2]. In turn, these uncertainties affect the choice of phases, and consequently a unique electron-density profile could not be derived [2]. It is, therefore, an attractive possibility that the diffraction pattern be recorded free of the effects of stacking disorder.

In the past, long exposures of one to two days have been needed to record the diffraction pattern on X-ray film [1—3], and I thought it possible that the disorder was the result of the specimen deteriorating during that time. Thus, the effects might be avoided by recording the pattern quickly from a freshly dissected nerve. To shorten the exposure time, myelinated nerve has been

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exposed to the more intense, point-focused beam of X-rays provided by the Caltech monochromator [4] at the Stanford Synchrotron Radiation Project. In addition, the use of a scintillation counter or a linear position-sensitive detector [5] in place of X-ray film further shortened the exposure time. In this way the most intense reflections were recorded accurately in a few seconds, and a few minutes were needed to record all of the stacking reflections which have been seen on film. In these exposures the same effects of disorder were observed as before, and the effects are independent of dose. The stacking disorder therefore appears to be characteristic of the myelin in a freshly dissected nerve.

The Caltech monochromator also has the advantage that the X-ray beam is more nearly monochromatic [4] than in a conventional set-up of an X-ray generator and camera [2]. The previous study [2] was hampered by a small contribution from X-rays of other than the desired wavelength. Because this contribution is absent, the present data remove one ambiguity which bears on the possible phasing of the higher order reflections. However, one further ambiguity remains, and a decisive choice between four remaining profiles [2] is still not possible.

Materials and Methods

Specimens of frog (*Rana catesbeiana*) sciatic nerve were prepared as described previously [1,2]. A specimen was mounted in a cooled (approx. 4°C) brass cell, which was placed after the final set of guard slits [4]. The specimen was exposed to an X-ray flux of the order of 10^9 photons/s; the precise value depends on the energy and current of the electron beam in the storage ring, SPEAR. The main X-ray beam and the diffracted X-rays were then passed through a beryllium window into a vacuum chamber (pressure approx. 1 mm), which was used to avoid scattering by the air in the path of the main beam. The main beam and most of the parasitic scatter were absorbed by a lead stop at the further end of the chamber, and the diffracted X-rays passed through a Kapton window and out of the chamber. In early experiments, the diffraction patterns were recorded using a scintillation counter (Fig. 1). A position-sensitive detector was used later (Figs. 2 and 3).

The scintillation counter was advanced stepwise along a line at right angles to the main beam by a stepping motor which was controlled by a mini-computer. At each step counts were accumulated until a pre-set integrated flux of the main beam was reached, the latter flux being measured with an ionization chamber placed between the guard slits and the specimen. However, experience showed that the ionization-chamber reading was not strictly proportional to the flux in the main beam (Blaurock, unpublished). Since the synchrotron radiation from SPEAR decreases gradually during each 2—6 h cycle, the data recorded with the counter include a significant error for counts recorded several minutes apart. When the position-sensitive detector was introduced later, the scintillation counter was used in lieu of the ionization chamber; the counter recorded X-ray photons scattered by the air in the path of the main beam between the guard slits and the specimen. The total counts accumulated by the scintillation-counter system (approx. 10^6) during the set time that the

linear detector recorded a diffraction pattern provided an accurate value for normalizing exposures to one another. In addition a correction was needed for dead-time in the multi-channel analyzer (see below).

The linear position-sensitive detector was similar to that described by Gabriel and DuPont [5]. A larger wire (50 μm) was used to increase the maximum counting rate at the expense of resolution (Gamble, R.C., Trentacosti, F. and Blaurock, A.E., unpublished). In use, the detector system was found to give peaks which were appreciably broader than the minimum 0.4 mm (for an active length of 100 mm) at rates over 30000 cps and this was taken as maximum rate in these experiments. Detector counts were analyzed and accumulated in a multi-channel analyzer interfaced to the computer. The accumulated pattern was recorded on floppy disc and typed out later.

Simple tests showed that the diffraction patterns were not strictly linear with distance along the detector and that the sensitivity of the detector varied from point to point. A correction for the latter variation was made by recording the fluorescent X-rays (K_α, K_β, etc.) from an iron foil placed where the specimen had been. The foil was a point source of X-rays which were isotropic over the diffraction angles of interest. The specimen pattern was divided by the foil pattern point for point. The number of channels per unit distance varied along the detector, there being fewer channels/mm near the middle and more at the ends. This broad non-linearity caused no difficulty in the present experiments.

In order to normalize two diffraction patterns for comparison, the "dead time" of the multi-channel analyzer must be considered. Although the dead-time losses do not distort the accumulated diffraction pattern, they do depend on the counting rate of the detector as a whole and a correction is needed if the counting rate is not the same for the two patterns. For the present experiments the diffraction patterns were recorded at different rates (Figs. 2 and 3), and a correction for the dead-time losses was included. The correction was made using a curve of total multi-channel analyzer counts vs. total counts registered by the detector in 1 s (Blaurock, unpublished).

To avoid overloading the detector with the intense lower order reflections, the front of the detector was masked by sheet aluminum with a vee-shaped opening in it. The vee (angular aperture approx. 0.1 rad) opened along the detector axis, and the point of the vee was in line with the main beam. The reflections seen on film are arcs [1,2], and the mask reduces the geometric broadening when the arcs are projected onto the detector axis. By passing a constant fraction of each arc, the aperture also would make it simple to correct the observed intensities. In the case where the arc length is comparable to the size of the focused beam, an extra correction would be needed [6].

Results

Fig. 1 shows the diffraction pattern recorded in 10 min from a sciatic nerve. The intense lower order reflections appear as discrete peaks with deep minima between them (arrows between orders 2—6). The width of these peaks, approx. 1 mm FWHM, is determined by the detector aperture. In contrast, the minima between the higher order reflections to the right are barely visible (arrows

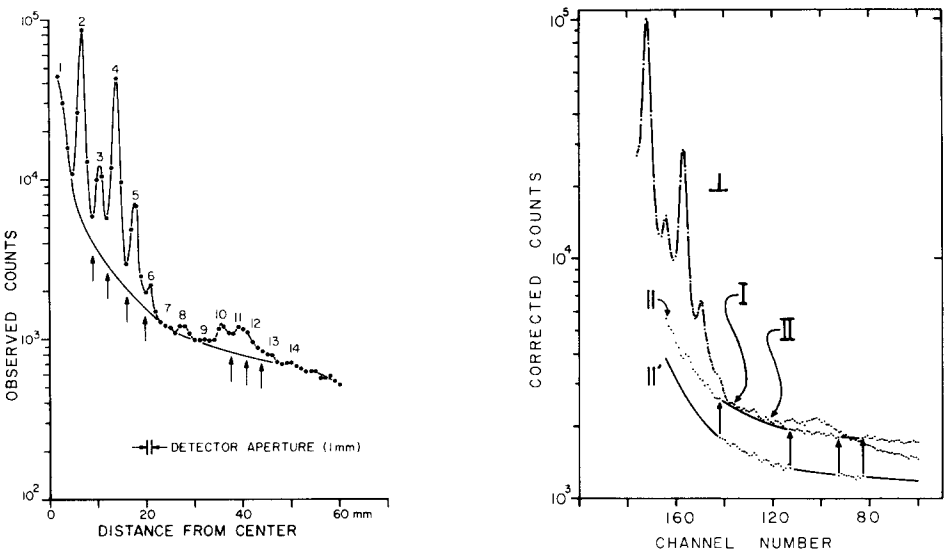

Fig. 1. Diffraction pattern (●———●) recorded at right angles to the frog sciatic-nerve axis. The nerve was in Ringer's solution. Positions of the stacking reflections are indicated by order numbers. Each point was recorded in 6—7 s. The background (———) was not measured but was sketched on the basis of past observations (see ref. 1 and Fig. 2). The counter aperture was 1 mm wide by 5 mm at right angles to the scan direction and the counter was stepped by 1 mm. The X-ray wavelength was 1.74 Å and SPEAR was operating at 3.7 GeV and a maximum electron current of 60 mA.

Fig. 2. Pair of corrected diffraction patterns from sciatic nerve in Ringer's solution. The difference between the upper and middle curves is largely the lamellar diffraction. The upper curve (⊥) was recorded at 5000 cps with the detector perpendicular to the nerve and the middle curve (||), parallel to the nerve at 2300 cps. The lower curve (||′) shows data points which, for clarity, were omitted from the middle curve. The maximum in the upper curve ($2.9 \cdot 10^5$ counts before correcting by the foil pattern) was arbitrarily set at $1 \cdot 10^5$ counts, and the middle curve was scaled as in Materials and Methods. Including the foil pattern, the maximum Poisson uncertainty in the upper curve is 1.6% (between orders 2 and 3). In the lower curve the maximum uncertainty is 1.7%. In regions I and II every point in the upper curve lies above the corresponding point in the middle curve; the rms difference, point for point, is 2—3 times the rms deviation of points in either curve from best-fit straight lines. The X-ray wavelength was 1.74 Å, the exposure time was 900 s and the distance from the specimen to the mid-plane of the detector was about 40 cm. SPEAR was operating at 3.7 GeV and a maximum current of 30 mA.

between orders 10—13). In these respects the pattern is similar to exposures recorded on film with a conventional set-up [1,2].

As noted above, the conventional exposures required from one to two days. In this case, the spaces between membranes have been shown to be variable [1,2]. The diffraction pattern in Fig. 1 was recorded within 30 min after beginning the dissection, and yet it shows the same attenuation of the higher order reflections, with shallow minima between them, as for conventional exposures. A pattern recorded subsequently with 0.5 mm steps of the counter was the same as the initial pattern. It follows that the variability is not due simply to aging for one or two days, nor is there any evidence of dose-dependent radiation damage. Thus, the spaces between the myelin membranes probably are variable in the freshly dissected nerve.

Assuming Poisson counting statistics for the data in Fig. 1, the uncertainty in the integrated intensity of order 2 is 0.3%. The exposure time could be

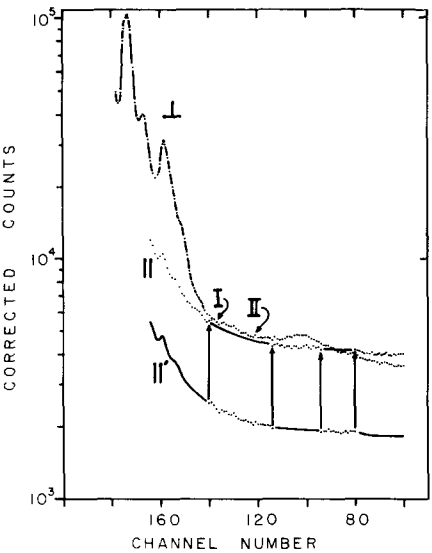

Fig. 3. Pair of corrected diffraction patterns from sciatic nerve soaked for two days in tap water. The maximum count in the upper curve ($1.8 \cdot 10^5$ counts before correcting) has been re-scaled arbitrarily to $1 \cdot 10^5$ counts after the foil correction; the maximum statistical uncertainty is 1.3%. The maximum uncertainty in the lower curve is 1.5%. Experimental conditions, corrections and scaling as for Fig. 2 except that the counting rates were 6000 cps (upper curve) and 3300 cps (middle curve). The specimen-to-detector distance was 39 cm, and SPEAR current was 21 mA. The stacking reflections are broader than in Fig. 2, indicating increased stacking disorder [2]. This disorder may be related to the much greater motions, seen by Singer and Bryant [7], in swollen myelin than in normal myelin.

reduced 10-fold while holding the uncertainty to 1%, i.e., less than 1 s would be needed per point.

Fig. 2 shows a pair of corrected, normalized 15 min exposures recorded with the position-sensitive detector. The upper curve (⊥) shows the diffraction at right angles to the nerve axis. The width of the lower orders is approx. 1 mm FWHM. Since the detector can resolve 0.4 mm, this width evidently is due to the size of the focused beam. As in Fig. 1, the higher order reflections show the effects of stacking disorder: attenuated reflections and proportionately more diffuse diffraction between them than between the lower order reflections.

The middle curve in Fig. 2 (||) shows the diffraction pattern parallel to the nerve axis. The difference between the upper and middle curves (not shown) is the oriented diffraction. This diffraction is mainly the lamellar diffraction from the myelin membranes, but small contributions from other structures in the nerve cannot be ruled out [1,2].

The exposure time for Fig. 2 is longer than the minimum one can hope for, partly because the electron current in SPEAR was lower than for Fig. 1 and partly because of the vee-shaped aperture covering the detector.

Fig. 3 shows a pair of exposures made from sciatic nerve swollen in tap water. (Distilled water would be preferred, but it was not available at the Stanford Synchrotron Radiation Project.) The upper curve (⊥) shows greater broadening of the lower order reflections than Fig. 2., and the higher order reflections are entirely replaced by diffuse scatter. These observations indicate that the spaces between membranes are even more variable than normal. A similar

observation has been made for myelin swollen in distilled water [2]. The middle curve in Fig. 3 (||) is similar to the middle curve in Fig. 2.

Discussion

The results indicate that the spaces between the myelin membranes are variable in a freshly dissected nerve, just as they are in a nerve exposed over a two-day period. Although the lower-order reflections are artificially broadened, Figs. 1 and 2 resemble the conventional patterns [1,2], particularly beyond order 10. Thus, it appears that the effects of disorder are similar in the two cases and that they predominate at larger diffraction angles.

In the previous analysis [2], we found that the cytoplasmic space varied by ±9.5 Å (FWHM) and that the extracellular space varied by ±7.7 Å. The similarities of Figs. 1 and 2 to the previous patterns indicate similar variabilities of the cytoplasmic and extracellular spaces in the two cases. Moreover, there is no indication that the variabilities will be greatly different in vivo.

The variability in the cytoplasmic and extracellular spaces may be related to the movements observed in nerve myelin by light microscopy [7]. In agreement with those observations, successive X-ray patterns have shown no indication of a change in the average variability over a few hours. As to the clefts specifically [7], the openings seen between neighboring membranes by electron microscopy [8] are much too large to account for the variability determined by X-ray diffraction, and the clefts are of limited extent in the myelin, whereas the X-ray diffraction averages over the whole sheath. However, motions in the clefts might disturb the inter-membrane spaces throughout the myelin and hence be a source of the general variability seen by X-ray diffraction.

It is of some interest to inspect the two curves in Fig. 2 at the minima between orders 6 and 8 (region I) and again between orders 8 and 10 (region II). In a previous paper, it has been shown that out of all the possible phase combinations for orders 7—15, only four are consistent with the oriented diffuse scatter between these reflections [2]. Further, it was shown that the choice could be reduced to two by determining whether the oriented scatter goes to zero in regions I and II. One was unable to choose, however, because of a small contribution from X-rays of wavelengths other than the desired K_α of Cu and because of a possible contribution from other oriented structures in the sciatic nerve [2]. A more nearly monochromatic beam has been used in the present experiments with the same observation of non-zero lamellar intensity in regions I and II (Figs. 2 and 3). The same observation was made for all five pairs of exposures recorded from normal and swollen nerves.

In all five pairs of patterns recorded, the parallel diffraction curve (||) lies above the perpendicular curve (⊥) at the highest angles, as seen on the right side of Figs. 2 and 3. The likely explanation for this observation is that scatter from the air between the guard slits and the specimen cell has come through the oval-shaped opening in the cell. Since the opening is elongated along the nerve axis, the air scatter will extend to higher angles in the parallel curve, as observed. A second possibility is that the parallel curve is showing the faint, diffuse diffraction maximum which has been observed on X-ray films in the direction parallel to the nerve axis (Blaurock, unpublished), at a Bragg spacing of about 12 Å. In

either case, any allowance for the effect would raise the perpendicular curve and increase the separation of the two curves in regions I and II.

It remains possible that there is a contribution from oriented structures in the nerve other than the myelin [1,2]. An assessment of the diffraction to be expected from the non-myelin structures in the sciatic nerve might settle the question. In the meantime, there remain four possible profiles.

Acknowledgments

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