LETTER TO THE EDITOR

On Phasing the Small-Angle X-Ray Diffraction Pattern from Nerve Myelin

ABSTRACT Using a method they developed, Stamatoff and Krimm (1976) have phased swelling data from nerve myelin. Although most phases agree with those I determined previously, there are a few differences. In this letter the two different phasings, theirs and my own, are used to compute the corresponding electron-density profiles, which are then closely compared. For both phasings, small differences are seen in the membrane profile at different degrees of swelling. The explanation that these differences are due simply to errors in measuring intensity is shown to be quite improbable; thus the differences indicate a real change in the profile. It follows that the assumption of a constant membrane profile appears to be invalid in the case of myelin swelling. The differences therefore are assumed to indicate a real change in the profile. It is shown that this change can be attributed consistently to interdigitation of protein molecules at the surfaces of neighboring membranes, while the membrane structure itself remains unchanged. In this case, valid phases still can be determined by swelling, but the phases determined by Stamatoff and Krimm are not valid.

Dear Sir:

In a recent paper, Stamatoff and Krimm (1976) present a method for phasing certain small-angle diffraction patterns, and apply the method to the data I recorded from nerve myelin (Blaurock, 1967). Their result is a set of phases that agree with my own result, except for a few phases. I will show that the best set of phases determined by their method has striking implications for the structure of the myelin membrane, and that it is therefore important to consider their result carefully.

In their paper, Stamatoff and Krimm (1976) present a rigorous mathematical method for phasing the diffraction patterns from a layered specimen that undergoes swelling. The individual layers are assumed to have a constant profile of electron density at all degrees of swelling; the only change allowed is in the thickness of the intercalated layers of the swelling fluid. After applying the method successfully to mathematical models, they apply it to diffraction data from frog sciatic-nerve myelin (Blaurock, 1967, 1971). Results from the latter application agree largely with my own phasing of the myelin diffraction pattern (Blaurock, 1971). The phasing differs from my own, however, for one order in each of two sets of swelling data. In particular, Stamatoff and Krimm (1976) find a negative sign for the first-order structure factor from myelin swollen to a 252-Å periodicity, in contrast to the positive sign I found (Blaurock, 1971). They also find a negative sign for the seventh-order structure factor when the period is 342 Å, in contrast to the positive sign I found. The significance of the different signs will now be considered.

Previous experiments have indicated that $F_{1,252}$ is positive. ($F_{h,D}$ is the square root of the corrected, integrated intensity of order h for the period D). Near the center of the diffraction pattern, the envelope of swelling intensities was found to behave characteristically when the swelling fluid was made more electron dense by adding sucrose to water. From this observation I have concluded that $F_{1,252}$ is positive (Blaurock, 1971). I note that Moody (1963) also found that the transform $F(X)$ is positive in the neighborhood of $X = 1/252$ Å when myelin is swollen in water. Finally, McIntosh and Worthington (1974) have since published more extensive data on myelin swollen in water and in glycerol solutions. These authors recorded several patterns from nerve myelin swollen in 6.5% glycerol, and the data indicate $F(X)$ is zero at or very near $X = 1/252$ Å (see their Fig. 3). Their phasing of the data from myelin swollen in 6.5% glycerol can be extrapolated to the case of swelling in water by allowing for the effect on the transform of changing the electron density of the swelling medium (Blaurock, 1971). Water is less electron dense than 6.5% glycerol solution, and I deduce accordingly that $F_{1,252}$ will be

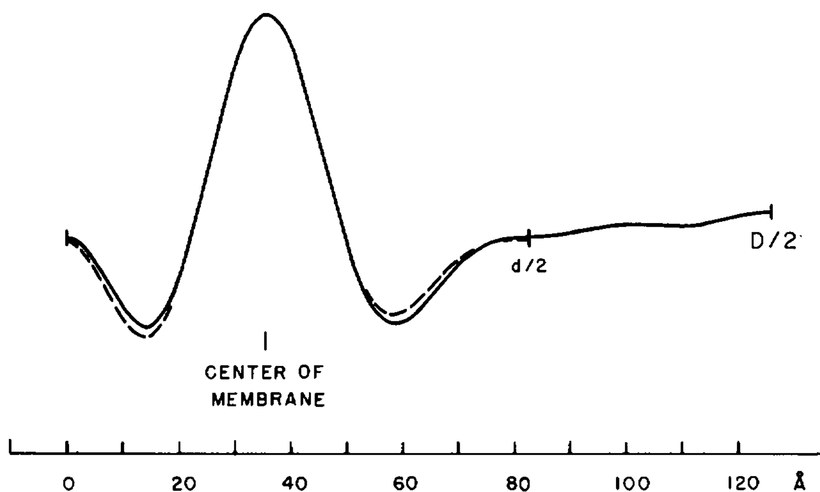


FIGURE 1 Low resolution electron-density profiles calculated by using the negative of the phasing derived by Stamatoff and Krimm (see text). Note that in Figs. 1–3 there are mirror lines at 0 Å and at the half-period ($d/2$ or $D/2$, respectively). The solid curve is a profile for myelin swollen in water, $D = 252$ Å. The intensity data are from Blaurock (1967, 1971), and the phasing (+, 0, −, −, −, +, +, +) is the negative of that found by Stamatoff and Krimm (1976). Note the greatly expanded extracellular space, which is identified as a uniform layer of water. The dashed curve is the profile of myelin first swollen in water and then made compact in 1 mM $CaCl_2$, $d = 166$ Å. Again, the phasing (+, −, −, +, +) is the negative of that found by Stamatoff and Krimm (1976). The pair of curves here and in Figs. 2 and 3 have been scaled for the best fit in each case.

positive for myelin swollen in water. I note that while McIntosh and Worthington (1974) did not phase the transform $F(X)$ for myelin swollen in water, the modulus of $F(X)$ does have an appreciable value for $X = 1/252$ Å, as expected. In view of all these results, it is reasonable to conclude that $F_{1,252}$ is positive for swelling in water. I believe, therefore, that the negative phase found by Stamatoff and Krimm (1976) for $F_{1,252}$ is invalid. The matter does not end there, however, because there remains another possibility to consider.

There is nothing in the method developed by Stamatoff and Krimm that distinguishes between a given set of phases and its negative (the negative set being the one obtained by reversing every sign in the given set). It follows that the set of phases (+, 0, −, −, −, +, +, +) for the 252-Å periodicity is just as valid as the set (−, 0, +, +, +, −, −, −). The latter set is the one actually chosen by Stamatoff and Krimm (1976), since they do not believe that the former set can be valid.[1] If, however, $F_{1,252}$ is assumed to be positive, then the former set is the only consistent choice. The consequences of choosing the former set will now be considered.

The effect of reversing all the signs is to invert the calculated profile. Thus, assuming a positive sign for $F_{1,252}$, together with Stamatoff and Krimm's method of phasing, I infer a profile that is the bilayer profile inverted (Fig. 1). The physical interpretation of the inverted bilayer profile would be quite different from the usual bilayer interpretation; for this reason it is important to consider whether the inverted profile is likely to be valid.

I have not repeated the calculations done by Stamatoff and Krimm (1976); I accept their results as given. Instead, I have calculated electron-density profiles with the negative of the Stamatoff-Krimm phasing (Fig. 1) to compare them to profiles calculated using my own phasing (Blaurock, 1971) (Fig. 2).

Fig. 1 shows the two profiles calculated with the negatives of the sets of phases given by Stamatoff and

[1]Stamatoff, J. B. Personal communication.

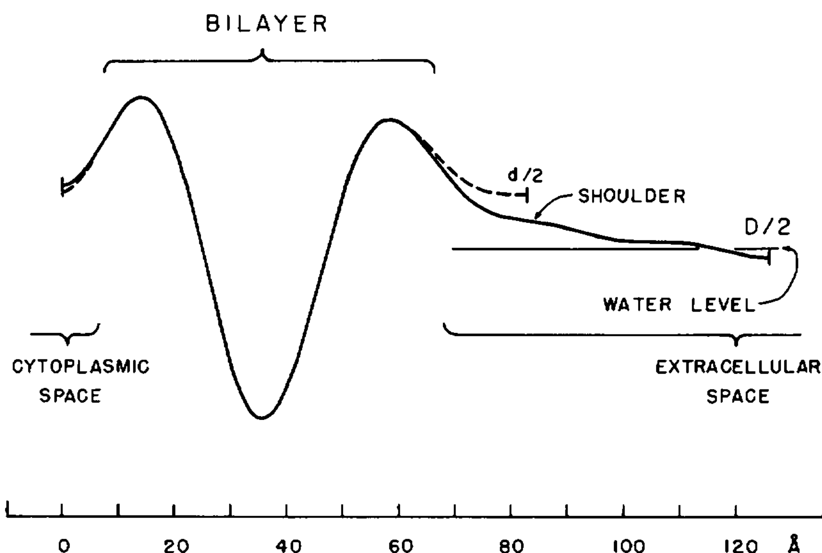


FIGURE 2 The solid curve is as in Fig. 1, except that the phasing (+, 0, +, +, +, −, −, −) is from Blaurock (1967, 1971); the same phasing has been found by several other workers (see text). Note that the dashed curve is the inverse of the dashed curve in Fig. 1, but that the solid curve is not precisely the inverse of the solid curve in Fig. 1.

Krimm (1976). I note that both curves have the shape of an inverted bilayer profile (cf. Figs. 2 and 3). The profile calculated with data from frog sciatic-nerve myelin swollen in water, $D = 252$ Å, is shown by the solid curve. The dashed curve shows the profile after the swelling has been reversed by placing the nerve in 1 mM $CaCl_2$, $d = 166$ Å. The two curves superimpose nearly, but not quite, perfectly.

In comparison, Fig. 2 shows the two profiles calculated with the same intensity data, but now using my own phases (Blaurock, 1971). In this case, the two curves superimpose better over part of the profile, but they separate for $x > 60$ Å. Thus the superposition is somewhat imperfect in both Figs. 1 and 2.

The question arises as to whether the differences between the two profiles in Fig. 1 are significant. If the answer is yes, then the same will be true of Fig. 2 and all other possible pairs of profiles since, according to Stamatoff and Krimm (1976), the pair in Fig. 1 (or, equally, the pair that is inverse to Fig. 1) shows the best fit of all. To answer the question, it is necessary to examine the relative errors in measuring the intensity data.

In all, I recorded five sets of intensity data from the myelin swollen in water or dilute solutions (Blaurock, 1971). Of these, three sets ($D = 166$, 252, and 342 Å) appear to be consistent with the assumption of a constant profile.[2] (The other two sets of data [$D = 288$ and 294 Å] clearly are not consistent with the assumption, and the systematic differences with the first three sets have been attributed to the differing methods of preparing the specimens [Blaurock, 1967].) One of the three sets of data ($D = 252$ Å) was used to calculate a continuous transform (Blaurock, 1971). Using the data shown in Fig. 1 *a* of Blaurock (1971), I calculate that the other two sets of data (14 independent points in all) conform to the continuous curve with a SD of 0.027. I note that this value corresponds to an error of 7% in a structure factor of average magnitude. In comparison, the data of Caspar and Kirschner (1971) for rabbit and frog sciatic-nerve myelins show an average mean uncertainty that is 3–5% of an average magnitude.

[2]The period 166 Å occurs for nerve in 1 mM $CaCl_2$ (Blaurock, 1971). The electron density of this medium (0.33366 $e/Å^3$; Blaurock, unpublished) differs by very little from the value for pure water (0.33364 $e/Å^3$). Hence, although in principal the transform in the case of 1 mM $CaCl_2$ is different from the transform in the case of water, the difference will not in fact be detectable.

The largest differences in Fig. 1 are at 15 and 59 Å. Assuming a Gaussian distribution of errors (Bevington, 1969) and taking for its SD the above value of 0.027, I have considered whether these differences are likely to be due simply to errors in measuring intensity. As the coefficient of a cosine term, each structure factor contributes to the two points in question. Based on these contributions, the highest probability of accounting for the differences in terms of error of measurement occurs when the error is assumed to be exclusively in $F_{1,166}$ and in $F_{2,252}$, and the errors in all other structure factors are assumed to be zero. In this case, errors each sufficient to account for half of the difference at either 15 or 59 Å have probabilities of 3.6 and 6.4%, respectively. It follows that the probability of both errors occurring simultaneously is 0.2%. If half the difference at 15 or 59 Å is assumed to be due to errors distributed equally among all the $F_{h,166}$, for $h = 1–5$, then the probability is 0.6%, with a similarly small probability that the other half of the difference is due to error distributed equally among the $F_{h,252}$, for $h = 1–8$. Again, the probability that errors of this magnitude occur is well below 1%. Thus, it is quite improbable that the differences at 15 and 59 Å in Fig. 1 are due simply to errors in measuring intensity. As noted above, all other possible pairs of profiles, including the pair in Fig. 2, would show larger differences. It follows that no pair can be found in which the profiles differ by insignificant amounts. I conclude that the assumption of a constant membrane profile is invalid.

If the membrane profile is not constant, then there is no basis for choosing either the phasing in Fig. 1, or its negative, as Stamatoff and Krimm (1976) have done. Nonetheless, Fig. 1 does show a best fit of a kind, and it is interesting to ask whether the pair of profiles can be valid. There are two lines of evidence indicating that these two profiles in fact are not valid. First, a pair of profiles has been calculated by using data from a frog sciatic nerve in which first LiCl, and then CsCl, were substituted for the NaCl in Ringer's solution (Blaurock, 1971). If one assumes there is an isomorphous replacement, the differences between the two profiles will show where the Cs is located. Given my phasing, the profile for the Cs-containing myelin lies above the other profile outside the bilayer (see Fig. 4 *d* in Blaurock, 1971). This result is reasonable in view of the greater electron density of the CsCl solution, and the difference is accounted for quantitatively (Blaurock, 1971). If the phasing of Fig. 1 is used, then the Li- and Cs-containing profiles are inverted and the difference between them is not readily interpretable. Second, neutron scattering-length densities tend to be in proportion to the x-ray electron densities (Kirschner and Caspar, 1977). If the profiles in Fig. 1 were valid, then one would expect the neutron scattering-length profile to show a peak of density near the center of the membrane similar to that seen in Fig. 1. In fact, the density there is lowest of all (Kirschner and Caspar, 1977). Since the phasing of the neutron profile has been confirmed by isomorphous replacement of deuterium for hydrogen, the profiles in Fig. 1 and a similar profile proposed by Harker (1972) are doubtful.

If the principal of a constant membrane profile is invalid, can the difference data be phased reliably? I suggest that this can be done by assuming that the membrane structure is constant. In this case, the membrane profile will remain constant so long as the membranes are well separated from one another by thick layers of the swelling fluid. This would be the case, for example, for $D = 252$ and 342 Å. However, when two membranes come close together, material on the surfaces may interdigitate, causing a change in the *profile* of the single membrane, even though there need be no change in the *structure* of the membrane. This would be the case for $d = 166$ Å. Fig. 2 supports this picture, as follows.

When the water level (horizontal line in Fig. 2) is extrapolated to the left, a shoulder more electron dense than water is evident just outside the bilayer. I note that a similar shoulder has been seen consistently when the electron density of the extracellular fluid was varied, and it was suggested that the shoulder is due to protein lying at the surface of the bilayer (Blaurock, 1971). Referring to the solid curve in Fig. 2, I note that the neighboring membrane to the right (not shown) would be some distance away, and that the intervening space is filled with water. This water can be removed by immersing the nerve in 1 mM $CaCl_2$ (Blaurock, 1971), and then the neighboring membranes come together. It is evident from the solid and dashed curves in Fig. 2 that when the membranes come together, neighboring shoulders must overlap. As a result, there will be an increase in electron density in the region of overlap. In this way, it is possible to account for the dashed curve in Fig. 2 lying above the solid curve between 60 and 83 Å without assuming a change in the membrane structure.

The increase in electron density would occur, for example, if globular protein molecules projected out from the extracellular surface of a membrane, and if they were separated from one another by fluid

spaces large enough to allow the protein molecules projecting from the surface of the neighboring membrane to interdigitate. Thus, the higher density in the dashed curve in Fig. 2 can be interpreted in a way that is physically reasonable and is consistent with other observations.

Stamatoff and Krimm (1976) also found different phasing for the third set of data from myelin swollen in water (D = 342 Å). It should be noted at the outset that I avoided working with this set of data because the first order was not recorded: the swollen period of 342 Å was unexpectedly large, and the small-angle diffraction camera was not set to record the first order[3] (Blaurock, 1967). Thus it is difficult to phase this set of data as it exists. The matter having been raised, however, I feel obliged to consider it.

Fig. 3 *a* shows profiles calculated for D = 342 Å (solid curve) and for d = 166 Å (dashed curve) using the phases found by Stamatoff and Krimm (1976). The dashed curve superimposes fairly well on the solid curve. In comparison, the solid curve in Fig. 3 *b*, calculated with the phasing indicated by my results (Blaurock, 1971), does not fit as well. However, to the right of the bilayer, in the region identified with the fluid layer, the solid curve in (*a*) shows larger ripples than in (*b*); in this respect (*b*) clearly is a better choice.

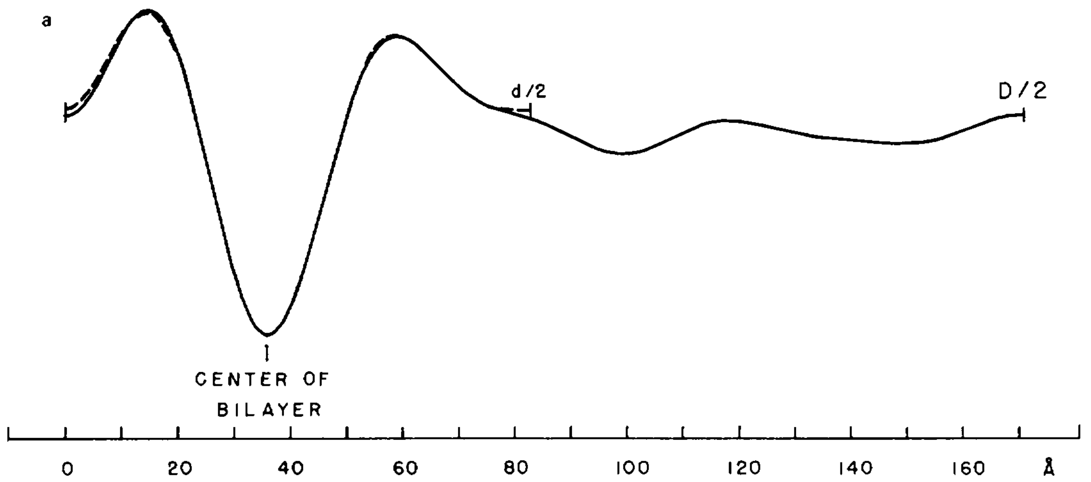
Lacking the first-order intensity, perhaps one can draw no firm conclusion as to the phasing of the data for D = 342 Å. Nonetheless, two observations are relevant. First, assuming that order 6 is positive ($F_{6,342} = +0.562$) while order 8 is negative ($F_{8,342} = -0.400$) (choices of phase on which I agree with Stamatoff and Krimm), the transform necessarily goes through zero between these orders. If one assumes that the transform behaves linearly around the zero (Blaurock, 1967), then order 7 can be approximated by the arithmetic average of orders 6 and 8: the interpolated value for order 7 is +0.081. Since the magnitude observed for order 7 is 0.135, a positive phase (Fig. 3 *b*) would be the better choice ($F_{7,342} = +0.135$). In this case, the transform varies nearly linearly (Blaurock, 1971). In contrast, the choice of a negative sign ($F_{7,342} = -0.135$) implies a distinct bump in the transform. There is, however, no indication of a bump in the other swelling data (Blaurock, 1971; McIntosh and Worthington, 1974). Based on the SD of 0.027 (see above), the Gaussian probability that $|F_{8,342}|$ is in fact greater than $|F_{6,342}|$, and that the reverse has been found because of errors in measuring intensity, is less than 0.002%. Hence there is little doubt that the better choice for $F_{7,342}$ is positive. Second, a value for the first-order structure factor can be estimated from the transform determined by other swelling data (Blaurock, 1971): $F_{1,342} = +0.26$. With this estimate, the solid curve in Fig. 3 *b* has been recalculated (Fig. 3 *c*). In Fig. 3 *c* it can be seen that adding the nonzero estimate for $F_{1,342}$ largely corrects the discrepancy between the solid and dashed curves out to 60 Å. Moreover, the water layer remains flat, as in Fig. 3 *b*. In contrast, when I used the value $F_{1,342} = +0.26$ to recalculate Fig. 3 *a*, the large ripple was still present in the water layer, and the fit between the solid and dashed curves was made worse. These results all indicate that the positive sign for $F_{7,342}$ is the better choice.

I note that in Fig. 3 *c* there is, with respect to the extrapolated water level, a shoulder in the solid curve similar to the one in Fig. 2. This shoulder would be accounted for in the same way, by protein molecules projecting from the bilayer surface. Moreover, the discrepancy between the solid and dashed curves in the interval from 60 to 83 Å is similar to that in Fig. 2, and again I suggest that the shoulders sum up to account for the dashed curve.

Effect of a Change in the Profile on the Fourier Transform of the Membrane Pair

It is interesting to consider what effect the proposed overlap will have on the Fourier transform of the pair of membranes, which is the immediate object of the swelling experiments. At large degrees of swelling there will be no overlap between neighboring profiles across the swollen extracellular space, and the transform of the profile of the isolated membrane pair will remain constant, provided only that the structure of the pair does not change. Thus the continuous transform that was calculated with the $F_{h,252}$, h = 1–8 (Blaurock, 1971) will be valid for this and all larger degrees of swelling. The good fit of the $F_{h,342}$, h = 2–11 to the continuous transform calculated with the $F_{h,252}$ (Blaurock, 1971) confirms that

[3]The data given on p. 212 of my thesis (Blaurock, 1967) show a dash for the first-order intensity when D = 342 Å. Stamatoff and Krimm (1976) substituted a zero ($F_{1,342} = 0$) to use the data in their calculations.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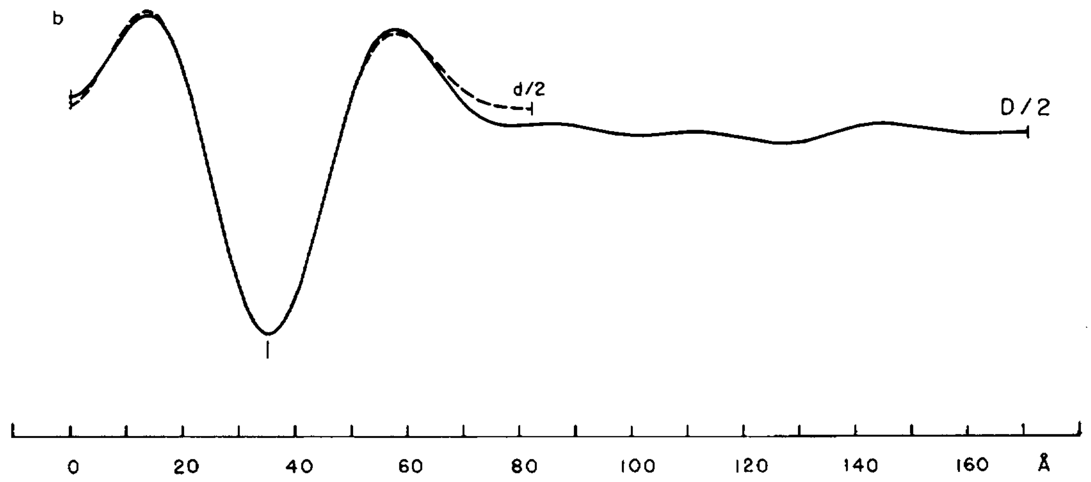
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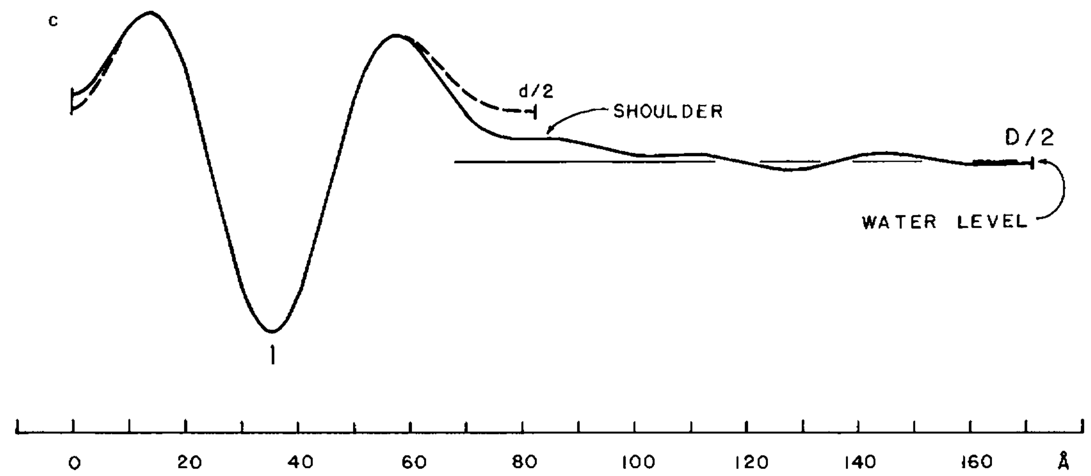
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the profile has remained constant, as predicted when assuming constant structure. It remains to show the effect on the transform of the difference between the two profiles in Fig. 2.

The main difference between the two curves in Fig. 2 is the excess area between the dashed and solid curves from 60 to 83 Å. The dashed curve can be corrected for the presumed overlap by imagining the case that the neighboring membrane to the right (not shown) moves far to the right: in this case the excess area needs to be relocated by reflecting it in a vertical "mirror line" at $d/2$ (83 Å) and then adding it onto the average water level. When this is done, the dashed curve becomes nearly congruent with the solid curve. It is clear that, because of the excess area between the two curves in Fig. 2, the Fourier transform of the dashed curve will not be the same as that of the solid curve. Nonetheless, the predicted effect of shifting the excess area as described, by reflection in the mirror line at $d/2$, is to leave the $F_{h,166}$ all unchanged (Blaurock, unpublished calculations): the shift will change the transform of the dashed curve only at points between the $F_{h,166}$. Thus the $F_{h,166}$ will lie on the transform of the isolated membrane pair. The good fit of the $F_{h,166}$ to the continuous transform calculated with the $F_{h,252}$ (Blaurock, 1971) confirms the prediction. It follows that the overlap cannot be detected by plotting the $F_{h,D}$ vs. h/D, for there can be no observable discrepancy to indicate that overlap has occurred.

This striking conclusion may be illuminated by the following considerations. Assume a one-dimensional electron-density profile of width W; outside the width W the electron density is assumed to be zero. The Fourier transform of the profile can always be calculated; the transform is denoted $F(X)$. If a periodic array of the profiles occurs, with the period $D \geq W$, then the Fourier transform of the array is a set of narrow peaks located at $X = h/D$, $h = 0, \pm 1, \pm 2, \ldots$, i.e. $F(X)$ is sampled at $X = h/D$. Thus given the transform $F(X)$, it is possible to compute the original profile by sampling F at $X = h/D$ and by using these values as the coefficients of a Fourier series. All this is well known from diffraction theory (see e.g. James, 1965).

It is interesting to ask, what is the result of sampling $F(X)$ at $X = h/d$, where $d < W$? In this case the values of $F(h/d)$ can be used in the same way to calculate an electron-density profile. A periodic array of these profiles clearly must sample $F(X)$ in the same way as before. However, when $d < W$, the original profiles must overlap one another, and the overlapped profile of a single membrane in general will differ from the original profile. This result demonstrates that there can exist two distinct profiles that, when they occur in periodic arrays, sample the same transform $F(X)$. The transforms of the distinct profiles may indeed differ from each other, but clearly they must intersect at $X = h/d$, $h = 0, \pm 1, \pm 2, \ldots$. This example confirms that the overlap cannot be detected by plotting the $F_{h,D}$ for various values of D, as is done generally in the swelling experiment (Finean and Burge, 1963; Moody, 1963; Blaurock, 1971; McIntosh and Worthington, 1974). Instead, one must compare the calculated profiles to detect changes of this kind.

CONCLUSIONS

Stamatoff and Krim (1976) have applied their method to myelin swelling data to find those phases that will minimize the integrated, squared differences between profiles calculated for the different degrees of swelling. Most of their best-fitting phases are the same as those widely accepted. Two phases are not the same, however. These two phases have been shown to have important consequences for the choice of a

FIGURE 3 (*a*) The solid curve approximates the low-resolution profile of myelin swollen in water, D = 342 Å. The intensity data are from Blaurock (1967, 1971) and the phasing (0, −, +, +, +, +, −, −, −, −, −) is from Stamatoff and Krimm (1976). Lack of an observed value for the first order intensity (see text) makes this an inaccurate profile. The dashed curve is the same as in Fig. 2. Note that the ripple in the region beyond 95 Å, which is identified with water, is larger than in (*b*). (*b*) As for (*a*), except that the phasing (0, −, +, +, +, +, +, −, −, −, −) is from Blaurock (1967, 1971). (*c*) As for (*b*), except that an estimate of $F_{1,342} = +0.26$ (see text) has been used in place of zero. The solid curve shows much the same features as the solid curve in Fig. 2, including the shoulder to the right of the bilayer.

profile that is consistent with all of the swelling data. When the phases chosen by Stamatoff and Krimm (1976) are used to calculate profiles for the different degrees of swelling, there are differences between profiles that cannot reasonably be explained by errors in the measuring of the intensities. This finding invalidates the assumption that the profile is constant, an assumption that is essential if their results are to be valid.

My explanation of the differences in the profiles is that, when neighboring membranes come near one another, adjacent shoulders overlap. My interpretation is that constant membrane structure remains valid; however, the membrane profile changes because material projecting from neighboring bilayers interdigitates. Thus the assumption of a constant profile that underlies the swelling analysis (Boyes-Watson and Perutz, 1943) is not valid at the smallest degrees of swelling. I suggest that Stamatoff and Krimm were misled for this reason.

I also was misled, but I have shown here that the overlap does not invalidate my own results. This is so because the overlap does not produce a detectable discrepancy with the transform inferred from large degrees of swelling. It is my conclusion that my own phasing (Fig. 2) remains the most satisfactory, even though the phasing found by Stamatoff and Krimm (1976) best satisfies their criterion for a constant profile.

Truncation, i.e., the use of a limited number of terms in the Fourier series rather than the infinite number of terms needed to compute a perfectly faithful profile, clearly affects the calculated profiles. In Fig. 2, for example, the region identified with water is not flat, but instead shows a ripple characteristic of truncation. While the level of the dashed curve at $d/2$ in Fig. 2 may be affected by truncation, the shoulder in the solid curve cannot, in the light of my experience, be dismissed as an artifact of truncation. Moreover, if there were material superficial to the bilayer but extending only to $d/2$, then I would expect that the height of the shoulder, as measured from the water level, would fall to half its maximum value at $d/2$. In fact, the shoulder falls to half its maximum height at a point 10 Å beyond $d/2$. I conclude that there is material projecting from the surfaces of the bilayers and that it overlaps when the periodicity is 166 Å or the normal (Blaurock, 1971) value of 171 Å. The shoulder appears to be due, at least in part, to the P_0 protein of PNS myelin (Blaurock and Nelander, 1979).

In conclusion, I believe it is possible to draw valid conclusions from the swelling data, despite having to give up the assumption of a constant profile. The differences between the profiles in Fig. 2 are not large, and there is a reasonable physical interpretation of them. I note that Franks (1976) has derived a unique profile for a lipid bilayer, despite changes that were seen in the profile as the hydration varied. In view of these results, some caution is needed in using an automatic method of phasing: one clearly would do well to consider all the relevant data before accepting the single best-fitting profile as the correct choice.

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REFERENCES

BEVINGTON, P. R. 1969. Data Reduction and Error Analysis for the Physical Sciences. McGraw-Hill Book Company, New York. 1–26.

BLAUROCK, A. E. 1967. X-Ray diffraction studies of the myelin sheath of nerve. Ph.D. thesis. University of Michigan, Ann Arbor, Mich.

BLAUROCK, A. E. 1971. Structure of the nerve myelin membrane: proof of the low resolution profile. *J. Mol. Biol.* 56:35.

BLAUROCK, A. E., and J. C. NELANDER. 1979. Locating the P_0 protein in the X-ray profile of frog sciatic-nerve myelin. *J. Neurochem.* In press.

BOYES-WATSON, J., and M. F. PERUTZ. 1943. X-ray analysis of haemoglobin. *Nature (Lond.).* **151**:714.

CASPAR, D. L. D., and D. P. KIRSCHNER. 1971. Myelin membrane structure at 10 Å resolution. *Nat. New Biol.* **231**:46.

FINEAN, J. B., and R. E. BURGE. 1963. The determination of the Fourier transform of the myelin layer from a study of swelling phenomena. *J. Mol. Biol.* **7**:672.

FRANKS, N. P. 1976. Structural analysis of hydrated egg lecithin and cholesterol bilayers. I. X-ray diffraction. *J. Mol. Biol.* **100**:359.

HARKER, D. 1972. Myelin membrane structure as revealed by X-ray diffraction. *Biophys. J.* **12**:1285.

JAMES, R. W. 1965. The Optical Principles of the Diffraction of X-Rays. Cornell University Press. Ithaca, N.Y. 342–412.

KIRSCHNER, D. A., and D. L. D. CASPAR. 1977. Diffraction studies of molecular organization in myelin. *In* Myelin. P. Morell, editor. Plenum Publishing Corporation, New York. 51–89.

MCINTOSH, T. J., and C. R. Worthington. 1974. Direct determination of the lamellar structure of peripheral nerve myelin at low resolution (17 Å). *Biophys. J.* **14**:363.

MOODY, M. F. 1963. X-ray diffraction pattern of nerve myelin: a method for determining the phases. *Science (Wash., D.C.)* **142**:1173.

STAMATOFF, J. B. and S. KRIMM. 1976. Phase determination of X-ray reflections for membrane-type systems with constant fluid density. *Biophys. J. 16:503.*

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