The Choice between the Positive and Negative Structures for Nerve Myelin

Dear Sir:

In the October issue of this journal Harker (1972) describes an interpretation of the Akers and Parsons (1970) X-ray data from frog sciatic nerve after treatment with heavy atoms. We use the convention that the PLP (protein-lipid-protein) model is the positive structure and has low-order phases $(-++--)$ whereas the LPL (lipid-protein-lipid) model is the negative structure and has $(+--++)$ phases. Harker (1972) finds that either the positive or the negative structure is a valid solution but he prefers the LPL model. Earlier, Worthington (1970) pointed out that the PLP model could account for the Akers and Parsons (1970) data but the LPL possibility was not mentioned. The choice of either the PLP model or the LPL model is now considered. In the discussion here, only the diffraction evidence is treated and we do not draw upon the considerable information provided by the many other different techniques, information which strongly supports the PLP model. In this letter, we point out that the PLP and LPL models can be distinguished on the basis of diffraction evidence. We agree, however, with Harker (1972) that either the PLP or the LPL model suffices to account for the X-ray data of Akers and Parsons (1970).

The diffraction evidence is obtained by changing the fluid medium between adjacent membrane pairs in the unit cell. Thus, the nerve fiber is placed in solutions of different electron densities. The nerve may swell, that is, the size of the unit cell increases, but this is not necessary as live nerve has an accessible extracellular fluid layer. The X-ray data contains information relating to the electron density of the extracellular fluid layer. The analysis can be carried out using model-building considerations or by computing Fourier syntheses. When the electron density of the fluid medium is increased, one set of phases will show an increase in the electron density level of the fluid layer whereas the other set of phases will show a decrease and hence a choice can be made.

The choice between the PLP and LPL models could have been made from a study of the X-ray data recorded from frog sciatic nerve in sucrose solutions (Worthington and Blaurock, 1968). Slight changes in the membrane pair, however, occurred on swelling and this tended to complicate the analysis. A new series of swelling experiments using glycerol solutions enables a choice to be directly made for the membrane pair structure does not change as a result of swelling in glycerol solutions. Many sets of X-ray data from frog sciatic nerve swollen in glycerol solutions have been recorded. The swelling in glycerol solutions is reversible. The effect to be demonstrated is clearly present at glycerol concentrations of 0–30%. Two typical sets of X-ray data at 0% glycerol and at 20% glycerol are chosen for demonstration. Fourier series representations of frog sciatic nerve in 0% glycerol, d = 237 Å and frog sciatic nerve in 20% glycerol, d = 206 Å are shown in Fig. 1. Phases corresponding to the $(-++--)$ choice for live nerve are used in the computation. The bilayer profile is about 70–75 Å wide and is the same for the two glycerol concentrations. The fluid layers have different electron density levels; the electron density of the fluid layer is higher for 20% glycerol and lower for the 0% glycerol. Thus, the $(-++--)$ set of phases is correct.

A few remarks on the Akers and Parsons (1970) analysis are in order for the idea of finding the low-order phases from heavy atom labeling is sound and it forms the basis for studying protein crystals. The Akers and Parsons (1970) observation that all orders of chemically treated nerve increase in intensity, but at different rates, was puzzling, and Worthington (1970) deduced that the membrane pair had shrunk as the result of the chemical treatment.

It is of interest to compute Fourier series representations of frog sciatic nerve and frog sciatic nerve chemically treated with osmium tetroxide for 10 min duration using Akers and Parsons's (1970) published intensities for the first five orders of diffraction. The Fourier syntheses computed using the phases $(-++--)$ are shown in Fig. 2. It can be seen that the membrane profiles do not superimpose. The molecular structure of the membrane pair has undergone some change as a result of chemical treatment; the membrane pair has shrunk by about 9 Å. This change in structure as a result of the chemical treatment therefore complicates the interpretation of the heavy atom labeling. The heavy atom labeling of nerve myelin differs from that of protein crystals where the heavy atom attachment is an isomorphous replacement. Thus, the usual methods of protein crystallography do not rigorously apply to chemically treated nerve.

The actual location of the osmium atoms within the unit cell of frog sciatic nerve after staining is an important consideration. The Fourier synthesis in Fig. 2, however, does not provide explicit information on the uptake of the osmium atoms although it does contradict the idea of there being specific attachment sites on the nerve membrane. As a result of staining the shrinkage of the membrane pair has a marked effect on the X-ray diffraction intensities whereas the X-ray intensities are little affected by the uptake of the osmium atoms. Thus, the Fourier synthesis of chemically treated nerve indicates that the membrane pair has

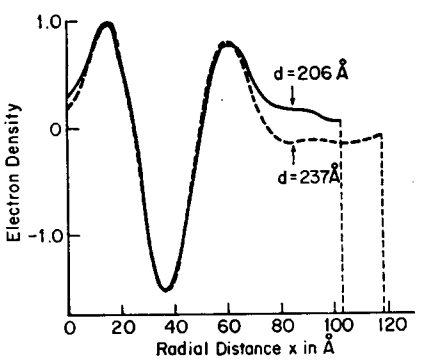

FIGURE 1

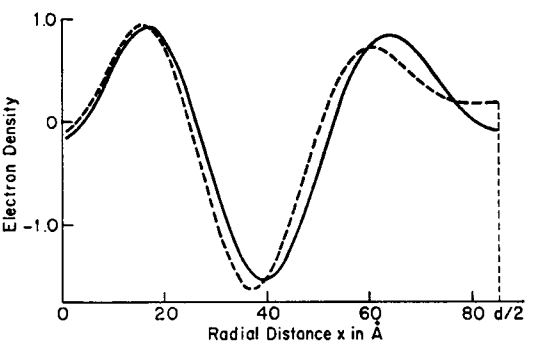

FIGURE 2

FIGURE 1 Fourier series representations for the myelin layers of frog sciatic nerve swollen in two different glycerol solutions. Only half the unit cell from $x = 0$ to $x = d/2$ is shown. The Fourier syntheses have a resolution of 17 Å and correspond to the Fourier synthesis of live nerve computed using the first five orders. The Fourier syntheses are on the same relative scale and the phases correspond to the $(-++--)$ phases of live nerve. The continuous curve refers to frog sciatic nerve swollen in 20% glycerol whereas the dotted curve refers to frog sciatic nerve swollen in 0% glycerol. It is seen that the electron density level of the 20% glycerol profile is above the electron density level of the 0% glycerol profile in the region of the extracellular fluid layer.

FIGURE 2 Fourier series representations of the myelin layers of frog sciatic nerve and frog sciatic nerve chemically treated with osmium tetroxide for 10 min duration. The Fourier syntheses were computed using the $(-++--)$ phases and the Fouriers are on the same relative scale. The continuous curve refers to frog sciatic nerve and the dotted curve refers to chemically treated frog sciatic nerve. It is seen that the nerve membrane profile of the chemically treated nerve has moved closer towards the origin ($x = 0$); the membrane pair thickness of the chemically treated nerve is less than the membrane pair thickness of live nerve.

shrunk by about 9 Å. Although the resolution of the Fourier synthesis is 17 Å, this observed shift in the membrane profile is significant as the X-ray diffraction intensities are sensitive to shifts as small as 1 or 2 Å.

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