REPLY TO "MYELIN MEMBRANE STRUCTURE AS REVEALED BY X-RAY DIFFRACTION" BY DAVID HARKER

C. K. AKERS *and* D. F. PARSONS

From the Biophysics Department, Roswell Park Memorial Institute, Buffalo, New York 14203

ABSTRACT The interpretation of the heavy metal-labeled data can either be accomplished with the analysis of the observed intensity differences (Akers and Parsons) or with the analysis of the observed structure amplitude changes (Harker). Both methods of analysis give essentially the same results: that two possible electron density distributions are valid, within experimental error, depending on whether there are one or two metal-labeling sites within the membrane. At present, the correct choice must rest on either the introduction of additional physical and chemical data on the position of the proteins and lipids or on an independent phasing technique such as the Hosemann-Bagchi Q-function.

In the last 9 years, three main electron density distributions for the myelin membrane have been proposed. Worthington and Blaurock (1968) fitted a five-parameter step function model to the intensities of swollen myelin (phases $-++--$, Worthington and Blaurock model). Other workers have sought to define phases more directly. Moody (1963) used the Shannon sampling theorem on the swollen myelin data to give either $-++--$ or $-++++$. Our label technique, which is the only available one for phasing intact, unswollen myelin, selects $++++++$ or $-++++$ (Akers and Parsons model) in agreement with one of Moody's two alternatives (the other agrees with Worthington and Blaurock's phases). The two models (Worthington and Blaurock and Akers and Parsons) differ somewhat in the electron density maps calculated from the two phase combinations, but agree in placing a low density (presumably lipid) region in the center of the membrane and higher density regions (presumably protein and polar heads of lipids) at the surfaces (Fig. 1). Harker's interpretation of our data (Harker, 1972) has led him to an electron density map (Fig. 1) which is the inverse of Worthington and Blaurock's and is interpreted to represent a thin layer of protein in the middle of a layer of lipid (the Worthington and Blaurock map is still admitted as a less likely possibility by Harker).

In any method of phasing the myelin data, the limited number of strong intensities (five) requires that the method involve the smallest number of assumptions and use

the greatest possible discrimination in comparing the final choice of phase combinations. In our view Moody (1963) has made the most critical use of the swelling data, and Worthington and Blaurock's step function model has too many assumptions and parameters to ensure a unique choice of electron density distribution. It is significant that Moody's method does not lead to Harker's electron density map, but to the inverse of it. A reasonable choice among Moody's analysis, Harker's analysis, and our own must depend on assessment of the assumptions and the degree of discrimination involved in each.

In our analysis of the label data (Akers and Parsons, 1970 *a,b,c*) we have only assumed that the label distribution can be described by gaussians with variable parameters. Contrary to the statements of Worthington (1970), our method does not rely at all on electron microscopy. Our analysis gives complete freedom of position to the label sites. Each of the 32 possible phase combinations is examined first with one label site and then with two. In the course of the calculations, Harker's phase combination was selected as giving an increase in all labeled intensities, but was rejected later as having a worse fit to the data in comparison with the final choice of $+++++$ or $-++++$.

Harker calculates for a single site of labeling an R factor of 3.3% using our R factor (equation 1) whereas our best fit for two guassian label sites, using the same R factor definition, is 2.7% (Akers and Parsons, 1970 *b, c*). Harker's paper does not demonstrate on the basis of R factor that our results are less consistent with the data. In fact, it is doubtful whether small differences in the R factor can be significant when only five reflections are used in the calculations. Our R factor definition is given in equation 1 and Harker's in equation 2.

$$R = \frac{\sum (\Delta I_{\text{calc}} - \Delta I_{\text{obs}})^2}{\sum (\Delta I_{\text{obs}})^2}, \tag{1}$$

$$R = \frac{\sum (|F_{\text{calc}}| - |F_{\text{obs}}|)}{\sum |F_{\text{obs}}|}. \tag{2}$$

Harker's general method of analysis represents an alternative to our own. In our analysis we used observed intensity differences directly, which include a cross-term of metal and organic amplitudes multiplied together. In his method, by taking square roots of intensities before subtracting, no such cross-term has to be considered. It is possible, however, that one approach is more discriminating in the choice of phases than the other. Harker's analysis rests on the *arbitrary* choice of a single labeling site per membrane. Although mathematically this could be considered the best solution because of its simplicity, there is considerable evidence to anticipate two labeling sites per membrane. Two sites are expected if the membrane has the lipid polar heads facing into the water environment (as in synthetic lipid bilayers), and if protein is associated with one or both of these polar surfaces (Branton, 1969; Hendler, 1971).

The difference Patterson calculated by Harker shows principal vector distances of

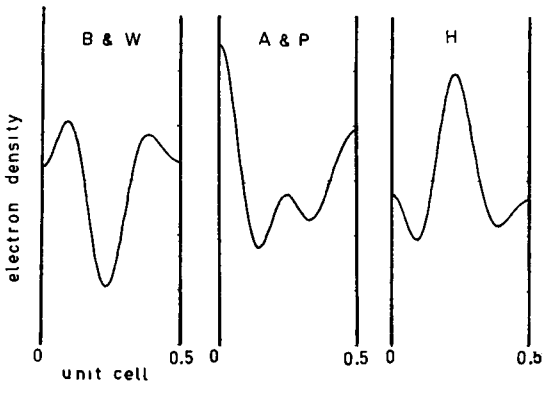

FIGURE 1

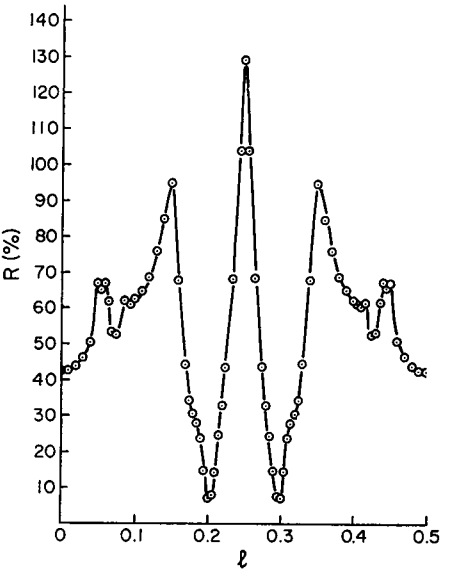

FIGURE 2

FIGURE 1 Three electron density distributions proposed for the myelin membrane. B&W, Worthington and Blaurock; A&P, Akers and Parsons; H, Harker.

FIGURE 2 The R factor (equation 2) for each single labeling site solution.

0.4 and 0.6 and a minor vector distance at 0.25 and 0.75 which can be interpreted either on the basis of his one-site label model or our two-site label model. As pointed out earlier (Worthington, 1970) our original Patterson function used noncorrected intensities; however, the Patterson function with the corrected intensities shows essentially the same features.

Using Harker's method, the single-site solution was calculated according to

$$\Delta F(h) = F' \times \cos(2\pi hl) \tag{3}$$

is the l position of the label delta function, $F' = f_0 \times C$ where f_0 is the metal scattering factor and is a constant value due to $(\sin\theta)/\lambda$ being less than 0.015 A^{-1}, and C is a normalization factor such that

$$\sum |\Delta F_{calc}| = \sum |\Delta F_{obs}|. \tag{4}$$

The R factor (equation 2) associated with each single-site solution is shown in Fig. 2. The well defined minima R for a single-site labeling occurs at $l \cong 0.200$ (or 0.300 with reversal of origin). This corresponds to a phase sequence of $+--++$ as found by Harker.

We then used Harker's technique to search for a two-site solution and computed the degree of fit with the best single-site solution. Following Harker, we assumed the

TABLE I
COMPARISON OF DEGREE OF FIT (R EQUATION 2)
FOR 1 AND 2 SITES USING HARKER'S METHOD

No. of sites	R	B	l	m	Phase
1	7.8	—	0.200	—	+−−++
2	7.6	0.6	0.095	0.465	++−−−
2	4.2	0.1	0.205	0.380	+−−++
2	2.1	0.7	0.005	0.405	+++++
2	5.0	0.3	0.185	0.360	+−−−+
1*	6.4	—	0.201	—	+−−++
2*	1.9	0.70	0.001	0.406	+++++

* Refined calculation varying l and m in small increments of 0.001 uc.

sites to be delta functions and calculated the magnitude of the change of amplitude of each reflection on double labeling using

$$\Delta F(h) = F' [\cos (2\pi hl) + B \times \cos (2\pi hm)], \quad (5)$$

where l and m are the positions of the delta sites, B is the ratio of label in site m to that in site l, and F' is defined as before. Harker's R factor (equation 2) was used to determine the degree of fit. The parameters l and m were allowed to vary. The range of l and m was 0.0–0.5 uc (uc = unit cell length, 1.0 uc = 171 A). The range of B was from 0.00 to 1.00 in steps of 0.05. The best-fitting parameters are shown in Table I (equivalent phase pairs are not included).

In order to examine the discrimination by R factor in more detail, the two-site solution with $R < 20\%$ (hatched zone) and the two-site solution with $R < 5\%$ (solid zone) are shown in Fig. 3. The hatched zone centered about $l = 0.200$ represents essentially the single labeling site with the second site being small.

Holding $l = 0.005$, a contour map of R (5, 10, 15, and $>20\%$) is shown on a plot of B vs. m (Fig. 4). This illustrates the effect of B and m on equation 5 and the degree of definition of the minimum around the best-fitting solution ($l = 0.005$, $m = 0.405$, $B = 0.7$; see Table I).

As a further check we calculated for another possible type of single site using Harker's method. A wedge function (minimum width at half-height of 3.4 A) was used which could be expanded to a gaussian (maximum width at half-height of 34 A). No fit could be obtained with R (equation 2) less than 25%.

It should be emphasized that the original assumption of delta function site is unrealistic because of the broad reactivity of osmium tetroxide to protein and lipid functional groups (Hendler, 1971). For this reason, our analogue varies the shape of the label site from a narrow gaussian to a broad one.

In spite of the deficiency in describing the label sites, Harker's method appears to confirm our original choice of phases (++++++) as a solution and that two reac-

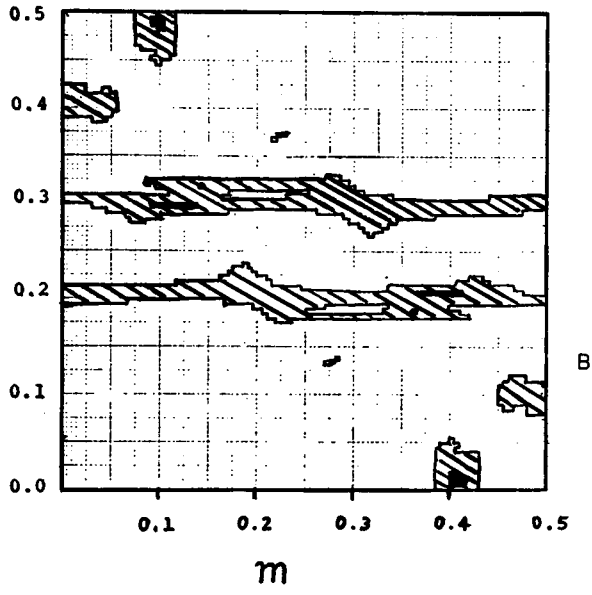

FIGURE 3

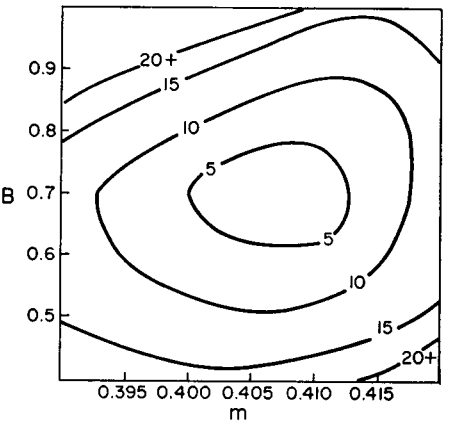

FIGURE 4

FIGURE 3 An R factor (equation 2) contour map on an l vs. m plot. The lined zone represents two-site solutions with $R < 20\%$. The solid zone represents two-site solutions with $R < 5\%$.

FIGURE 4 An R factor (equation 2) contour map on a B vs. m plot with $l = 0.005$. Contour lines at 5, 10, 15, and >20% are shown illustrating the well-defined minima of the best two-site solution.

tive label sites may exist rather than one; however, increasing the number of label sites from one to two (four variables instead of two) can itself be expected to give a better fit to the labeled data (decreased R factor). We are attempting to use the three additional reflections (total of eight reflections) to establish whether a definite choice can be made, on crystallographic grounds, between Harker's model and our own. At present, it appears that a final choice will rest on the introduction of other kinds of evidence about the localization of the protein and lipid in the myelin membrane. Such evidence may be obtained from a reassessment of surface tension estimates of the surface of isolated myelin pellets in relation to that of protein and lipid interfaces. Recent work in our laboratory in collaboration with Doctors Peterson and Pease shows that myelin can be embedded in a urea-glutaraldehyde water-miscible resin without significant change in the X-ray diffraction pattern. Enzyme digestion of thin sections of this material may reveal the sites of lipid and protein in the electron microscope.

This work was supported by National Science Foundation grant GB-15389.

Received for publication 23 April 1971 and in revised form 14 October 1971.

REFERENCES

AKERS, C. K., and D. F. PARSONS. 1970 *a*. *Biophys. J.* **10**:101.
AKERS, C. K., and D. F. PARSONS. 1970 *b*. *Biophys. J.* **10**:116.
AKERS, C. K., and D. F. PARSONS. 1970 *c*. *Biophys. J.* **10**:1122.
BRANTON, D. 1969. *Annu. Rev. Plant Physiol.* **20**:209.
HARKER, D. 1972. *Biophys. J.* **12**:1285.
HENDELER, R. W. 1971. *Physiol. Rev.* **51**:66.
MOODY, M. F. 1963. *Science* (*Wash. D.C.*). **142**:1173.
WORTHINGTON, C. R. 1970. *Biophys. J.* **10**:675.
WORTHINGTON, C. R. 1970. *Biophys. J.* **10**:675.
WORTHINGTON, C. R., and A. E. BLAUROCK. 1968. *Nature* (*Lond.*). **218**:87.