LETTER TO THE EDITOR

Ellipsoid Models for Rotational Diffusion of Rhodopsin in a Digitonin Micelle and in the Visual Receptor Membrane

Dear Sir:

The recent development of an inversion procedure for the Perrin equations (Wright et al., 1973) coupled with the demonstration that transient birefringence is meaningfully described as a double exponential decay for particles in the form of ellipsoids of revolution[1] (Ridgeway, 1966) has led to the suggestion that, in some cases, previously reported data may be reevaluated in terms of the above results to yield additional information concerning the shape of the particle in question. The particle chosen for this discussion is the micelle of a rhodopsin-digitonin mixture.

Strackee (1971) measured the transient photodichroism of rhodopsin-digitonin micelles in a solution diluted 1:3 with glycerol. The viscosity of the solution was reported to be 172×10^3 P at $-72°C$, the temperature at which the photodichroism was measured. Strackee analyzed the data in terms of a single exponential decay function, and compared the decay time with the rotational diffusion constant for an ellipsoid of revolution according to the work of Benoit (1951). For the assumed hydration of 0.30 (grams of water bound per gram of micelle), an axial ratio of 0.7 was calculated for a prolate ellipsoid or about 2 for an oblate ellipsoid. Finally, Strackee stated it cannot be decided whether the geometrical shape of the micelle is a prolate or oblate ellipsoid.

According to the general theory of Ridgeway, ellipsoids of revolution relax in a double exponential decay with relaxation times $1/6R_1$ and $1/(2R_1 + 4R_3)$, where $R_1 = R_2 \neq R_3$ denote the elements of the rotational diffusion tensor. Therefore a double exponential decay function was fit to the data of Strackee according to the method of Foss (1970). Fig. 1 shows the fitted curve superimposed on the transient photodichroism data of Strackee, plotted according to the equation

$$y = A(12.00e^{-0.404t} - 2.39e^{-0.205t}),$$

where A denotes a scale factor. For prolate ellipsoids $R_1 < R_3$, therefore $6R_1 < 2(R_1 + 2R_3)$. Since the fast and slow relaxation times are defined such that $\tau_f < \tau_s$, $\tau_s = 1/6R_1$ and $\tau_f = 1/(2R_1 + 4R_3)$ where $\tau_s = 1/0.205$ s and $\tau_f = 1/0.404$ s. For oblate ellipsoids $R_1 > R_3$, therefore $6R_1 > 2(R_1 + 2R_3)$, and it follows that $\tau_f = 1/6R_1$ and $\tau_s = 1/(2R_1 + 4R_3)$. The relaxation times therefore yield values of the rotational diffusion constants for both prolate and oblate ellipsoids.

Reduced rotational diffusion constants were calculated for the stated experimental conditions and the particle dimensions estimated according to the numerical inversion procedure of

[1] Wright, A. K. 1973. Submitted for publication.

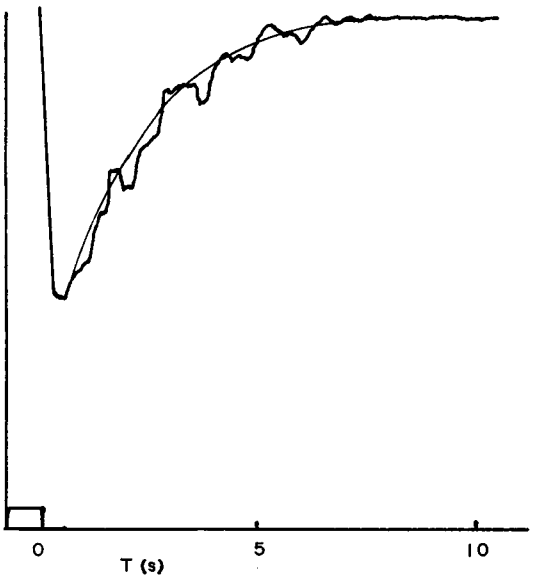

FIGURE 1 Double exponential decay curve fit to the transient photodichroism data of Strackee (1971).

Wright et al. (1973). The case of the oblate ellipsoid is ruled out since the ratio $R_1/R_3 = 3.81$ is greater than the maximum value 1.2561. The dimensions for the semiaxes of the prolate ellipsoid are calculated to be $a_3 = 85.20$ Å and $a_1 = 34.12$ Å, whose axial ratio is 0.40.

In summary, a reevaluation of the transient photodichroism of rhodopsin-digitonin micelles reported by Strackee, according to the estimation procedure of Wright et al. yields a model shape for the micelle particle as a prolate ellipsoid of length 170 Å and diameter 68.2 Å, whose axial ratio is 0.40. Additionally, these estimates were obtained independently of the necessity of assuming an arbitrary hydration.

Although a structure for the rhodopsin-digitonin micelle may be estimated from the transient photodichroism data of Strackee, it is not possible to infer the structure of rhodopsin without more knowledge of the action of digitonin on rhodopsin.

During the recent meeting of the FASEB, Stryer (Steinemann et al., 1973) reported binding three fluorescent molecules to specific sites on rhodopsin and that the largest distance calculated between the binding sites was about 75 Å. Wald et al. (1973) suggested that in order for the shape of rhodopsin to accommodate the dimension reported by Stryer, the shape of rhodopsin must be highly asymmetrical. He further suggested the shape may be a prolate ellipsoid of axial ratio 7:1 diffusing in a membrane as fluid as olive oil (Cone, 1972). Cone (1971) reported two relaxation times of 4 and 50 μs for photodichroism of rhodopsin in fresh frog retina at 10°C. If one assumes these relaxation times correspond to molecular motion, then a prolate ellipsoid may be determined from these data. Accordingly, $R_1 = \frac{1}{6}\,\tau_s$ and $R_3 = (1/4\tau_f) - (1/12\tau_s)$, where $\tau_f = 4$ μs and $\tau_s = 50$ μs. The viscosity of olive oil at 10°C is given as 1.38 P (Handbook of Chemistry and Physics, Chemical Rubber Publishing Co., Cleveland, Ohio). The linearly interpolated estimates of the corresponding semiaxes, according to the procedure of Wright et al., are $a_3 = 138$ Å and $a_1 = 14.4$ Å, whose axial ratio is 9:1. This value, under the arbitrarily assumed viscous environment, is surprisingly

close to the value suggested by Wald. Such an axial ratio is consistent with the highly asymmetric particle discussed by Wright et al. (1972).

The dimensions of the ellipsoid of revolution calculated from the two relaxation times reported by Cone (1971) are not in agreement with the currently believed structure of rhodopsin as resembling a sphere of diameter 40–46 Å (Cone, 1972). This calculation is not sufficient to distinguish the better of the two models. The basis, however, for making such a calculation based on Cone's data is the challenge of Professor Wald to seek a model for rhodopsin as a prolate ellipsoid of axial ratio 7:1 (Wald et al., 1973).

Received for publication 18 July 1973.

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