Biophys. Struct. Mechanism 5, 321–330 (1979)

Biophysics
of Structure
and Mechanism

The Directional Absorption Properties of Rhodopsin and its Photoproducts*

B. D. Gupta, Anurag Sharma, and I. C. Goyal

Physics Department, Indian Institute of Technology, Delhi
New Delhi – 110029, India

Abstract. The experimental data on the absorption of a plane polarised light by a solution of cattle rhodopsin at −196° C have been theoretically analysed to model the directional absorption properties of rhodopsin and its photoproducts. It is seen that these molecules behave like planar absorbers having a ratio of about 100 : 7 between the extinction coefficients along the long axis and perpendicular to it. Using this result and the experimental observations on absorption and dichroism in the retina in situ, a model for the configuration of chromophores in the disc membranes has been derived. In this model the plane of the chromophore is perpendicular to that of the disc and the long axis of the chromophore makes an angle of 6.6° with the plane of the disc. The solution of the problem depends on the assumption that the absorption axes are the same for the rhodopsin, prelumirhodopsin and isorhodopsin.

Introduction

It is well known that the light absorptive properties of visual pigments, such as rhodopsin, are highly directional (Dartnall, 1972). This is mainly because the primary light-absorbing structure, viz., 11-*cis*-retinal (the chromophore) is not an isotropic absorber and the absorption depends on its orientation with respect to the electric vector associated with incident light. Some studies have suggested that it is a planar molecule and probably bent and twisted (Sperling and Rafferty, 1969). In general, the absorption properties of a molecule can be described by three extinction coefficients ($\alpha_{x'}$, $\alpha_{y'}$, $\alpha_{z'}$) corresponding to the three principal axes (x', y', z'). The effective extinction coefficient depends on the orientation of these axes with respect to the electric vector associated with the incident light and on the magnitude of $\alpha_{x'}$, $\alpha_{y'}$, and $\alpha_{z'}$. If a molecule behaves like a planar absorber, one of the three extinction coefficients should be zero or negligibly small compared to the other two.

---

* Work partially supported by Department of Science and Technology (India)

0340-1057/79/0005/0321/$ 02.00

Strackee (1972), in the study of photoinduced dichroism in a solution of cattle rhodopsin at −196° C, assumed that the absorption ellipsoids ($\alpha_{x'}$, $\alpha_{y'}$, $\alpha_{z'}$) of rhodopsin and its photoproducts are rotationally symmetric about their long-axis. The experimental and theoretical results were found to be in good agreement when $\alpha_{y'}/\alpha_{z'} = \alpha_{x'}/\alpha_{z'} = 0.20$ (Strackee, 1972); z' – being along the long axis. However, with these parameters it was not possible to explain the observations of Liebman (1962) and Wald et al. (1963) on the absorption of polarized light in rods [as Strackee (1972) pointed out himself] and the observations of Brown (1972) and Cone (1972) on photoinduced dichroism in the retina. Further, the assumption of a rotationally symmetric ellipsoid is not consistent with the usually accepted planar model of the chromophore as pointed by Strackee (1972) himself.

In this paper, we have studied the absorption properties of rhodopsin and its photoproducts assuming the molecules to have a non-symmetric absorption ellipsoid. We have theoretically analysed the experimental data of Yoshizawa and Horiuchi (1973) to estimate the values of the relevant parameters. In addition, using these parameters and the observations of Brown (1972), Cone (1972), Liebman (1962), and Wald et al. (1963) on photoinduced dichroism and absorption of plane polarised light by the pigment molecules in situ in the retina, a model for the configuration of chromophores in the disc membranes has been derived.

Theoretical Analysis

In the experiment to be analysed here, a solution of cattle rhodopsin in glycerol-water (1 : 2) mixture at −196° C was bleached by a plane polarised light of wavelength 437 nm and its absorption spectrum was measured with natural light for various exposures until the steady state was reached (Yoshizawa and Horiuchi, 1973). At temperatures as low as −196° C, the molecules remain fixed in space (Strackee, 1972) and no thermal reaction takes place. The photochemical reaction at this temperature can be represented as (Yoshizawa and Wald, 1963):

$$\text{Rhodopsin} \leftrightharpoons \text{Prelumirhodopsin} \leftrightharpoons \text{Isorhodopsin} \tag{1}$$

Let $R(t)$, $P(t)$, and $I(t)$ represent the total concentrations (in number of molecules per unit area of cross-section) of rhodopsin, prelumirhodopsin and isorhodopsin molecules, respectively, at time t. Since initially (i.e., at $t = 0$) only rhodopsin molecules are present, the change in absorbance of the solution as a function of time would be given by

$$\Delta A(\lambda, t) = \alpha_1^m(\lambda)[R(t) - N_T] + \alpha_2^m(\lambda)P(t) + \alpha_3^m(\lambda)I(t) \tag{2}$$

where $\alpha_1^m(\lambda)$, $\alpha_2^m(\lambda)$, and $\alpha_3^m(\lambda)$ are the extinction coefficients of the three pigments, respectively, for the measuring light of wavelength λ and

$$N_T = R(t) + P(t) + I(t) \tag{3}$$

is the total concentration of bleachable molecules which remains the same throughout the experiment, since no molecules are lost during the reaction. The concentra-

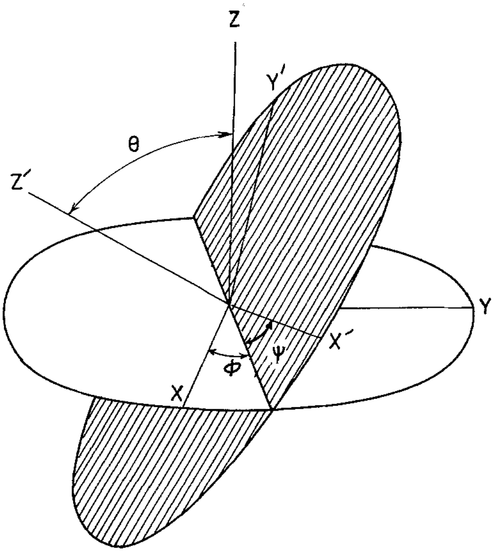

Fig. 1. Coordinate systems defining the Eulerian angles

tions of the pigments at different times would, in general, depend upon the orientations of different molecules with respect to the direction of polarisation of the bleaching light, since the molecules are not isotropic absorbers.

The light absorbing properties of a molecule can, in general, be represented by three extinction coefficients $\alpha_{x'}$, $\alpha_{y'}$, and $\alpha_{z'}$ corresponding to three mutually perpendicular axes x', y', and z' of the coordinate system fixed with the molecule. However, the effective absorption coefficient would depend upon orientation of the molecule (represented by x', y', z' axes) with respect to a coordinate system (x, y, z) in which the direction of polarisation of the bleaching light is fixed. The orientation can be expressed in terms of the three Eulerian angles θ, ϕ, and ψ defined in Figure 1 (Goldstein, 1950). If the electric vector of the plane polarised bleaching light, propagating along the x-axis is assumed to be parallel to the z-axis, the effective extinction coefficient of a molecule with orientation specified by angles θ, ϕ, and ψ would be given by

$$\alpha(\theta, \psi) = \alpha_{z'} f(\theta, \psi) = \alpha_{z'}(K_1 \sin^2\theta \sin^2\psi + K_2 \sin^2\theta \cos^2\psi + \cos^2\theta), \quad (4)$$

where $K_1 = \alpha_{x'}/\alpha_{z'}$ and $K_2 = \alpha_{y'}/\alpha_{z'}$, $\alpha(\theta, \psi)$ is independent of ϕ because of the rotational symmetry about the z-axis. Therefore, since initially all the molecules are isotropically distributed and the measurements are being made by natural (unpolarised) light, all the subsequently appearing quantities would be independent of ϕ.

Let $2\pi\, N_i(\theta, \psi, t) \sin\theta\, d\theta\, d\psi$ represent the concentration of molecules of the *ith* type having the orientations between θ and $\theta + d\theta$ and ψ and $\psi + d\psi$ at time t ($i =$ 1, 2, 3 would correspond to rhodopsin, prelumirhodopsin and isorhodopsin, respectively). The total concentration of molecules of the *ith* type can be obtained by integrating $2\pi\, N_i(\theta, \psi, t) \sin\theta\, d\theta\, d\psi$ over all possible orientations; thus for example,

$$R(t) = 2\pi \int_0^{\pi} d\theta \sin\theta \int_0^{2\pi} d\psi\, N_1(\theta, \psi, t)\,. \quad (5)$$

Since it is assumed that, initially, the angular distribution of molecules is isotropic and only rhodopsin molecules are present, we can write

$$N_1(\theta, \psi, 0) = \frac{N_T}{8\pi^2}\,; \quad N_2(\theta, \psi, 0) = 0 = N_3(\theta, \psi, 0)\,. \tag{6}$$

The quantities N_1, N_2, and N_3 are given by three simultaneous linear differential equations describing the photochemical reaction (1):

$$\begin{aligned} \frac{dN_1}{d\tau} &= -\,a\,f(\theta, \psi)N_1 + b\,f(\theta, \psi)N_2 \\ \frac{dN_2}{d\tau} &= a\,f(\theta, \psi)N_1 - (b + c)\,f(\theta, \psi)N_2 + d\,f(\theta, \psi)N_3 \\ \frac{dN_3}{d\tau} &= c\,f(\theta, \psi)N_2 - d\,f(\theta, \psi)N_3\,, \end{aligned} \tag{7}$$

where $\tau = I_{\text{inc}}\,t$; I_{inc} being the incident bleaching intensity and

$$a = \alpha_{z',1}(\lambda)\,\gamma_{1,2} \qquad b = \alpha_{z',2}(\lambda)\,\gamma_{2,1}$$

$$c = \alpha_{z',2}(\lambda)\,\gamma_{2,3} \qquad d = \alpha_{z',3}(\lambda)\,\gamma_{3,2}$$

$\alpha_{z',i}(\lambda)$ represents the extinction coefficient of the molecules of the *ith* type corresponding to its long-axis (the z'-axis) for the bleaching light of wavelength λ; γ_{ij} represents the quantum efficiency of photoconversion from *ith* type molecules to *jth* type molecules and $f(\theta, \psi)$ is defined by Eq. (4). In writing Eqs. (7) we have made the critical assumption, for simplicity in computations, that K_1 and K_2 are the same for the three pigments, respectively, and that the concentration (N) of pigments is sufficiently small so as to allow the approximation:

$$1 - e^{-\alpha N} \simeq \alpha N\,,$$

α being the extinction coefficient. Equation (7) can be solved easily subject to the initial conditions given by Eq. (6) to yield

$$N_1(\theta, \psi, t) = \frac{N_T}{8\pi^2}\left[-\frac{b(B + C)}{a} + \frac{bB}{a - m_1}\,e^{-m_1 f(\theta, \psi)\tau} + \frac{bC}{a - m_2}\,e^{-m_2 f(\theta, \psi)\tau}\right] \tag{8}$$

where m_1 and m_2 are the roots of the equation

$$m^2 - (a + b + c + d)m + a\,c + a\,d + b\,d = 0\,;$$

$$B = \frac{a}{m_1}\left(\frac{a + b + c - m_2}{m_2 - m_1}\right)$$

and C is obtained by interchanging m_1 and m_2 in the expression for B. Similar expressions are obtained for N_2 and N_3. Using these expressions for N_1, N_2 and N_3 in

Eqs. (5) and (2), the change in absorbance as a function of time can be obtained. The integration over ψ can be carried out analytically and the final expression is

$$\Delta A(\lambda, \tau) = N_T[A_0(\lambda) + A_1(\lambda, \tau) + A_2(\lambda, \tau)] \tag{9}$$

where

$$A_0(\lambda) = \left[1 + \frac{b(B+C)}{a}\right] [\alpha_3^m(\lambda) - \alpha_1^m(\lambda)] + (B + C)\, [\alpha_3^m(\lambda) - \alpha_2^m(\lambda)]$$

$$A_1(\lambda) = \frac{B}{2}\left[\left\{ \alpha_1^m(\lambda) - \alpha_3^m(\lambda)\right\} \frac{b}{a - m_1} + \alpha_2^m(\lambda) - \alpha_3^m(\lambda)\right] e^{-m_1\tau}$$

$$\int_0^\pi e^{-\frac{m_1\tau}{2}(K_1 + K_2 - 2)\sin^2\theta}\, I_0\left[\frac{m_1\tau}{2}(K_1 - K_2)\sin^2\theta\right] \sin\theta\, d\theta$$

and $A_2(\lambda)$ is obtained by interchanging m_1 and m_2 in $A_1(\lambda)$ and I_0 is the modified Bessel function of first kind and of order zero. The remaining integrals in Eq. (9) have to be evaluated numerically. The $\alpha_{z', i}$ component of the absorption ellipsoid can be calculated by assuming that the measured extinction coefficient is an average of $\alpha_{x', i}$, $\alpha_{y', i}$, and $\alpha_{z', i}$, i.e.,

$$\alpha_i = \frac{1}{3}(\alpha_{x', i} + \alpha_{y', i} + \alpha_{z', i}) = \text{trace}\ \alpha_1 .$$

Then,

$$\alpha_{z', i} = 3\, \alpha_i/(K_1 + K_2 + 1) .$$

However, since only ratios of $\alpha_{z', i}$ occur in the final expressions, this constant multiplicative factor is not important.

Results and Discussion

I. Selection of Parameters

In order to compare our results with the experimental results of Yoshizawa and Horiuchi (1973) we have plotted the change in absorbance with time for three randomly chosen wavelengths of the measuring light (Figs. 2, 3, and 4). The extinction coefficients of rhodopsin and prelumirhodopsin at −196° C are taken from the same paper of Yoshizawa and Horiuchi (1973). The extinction coefficient of isorhodopsin is estimated by assuming that the shape of its spectrum at −196° C is similar to that of rhodopsin at −196° C and the maximum value of extinction coefficient ($\alpha_{\max}$) and the corresponding wavelength ($\lambda_{\max}$) are the same as given by Yoshizawa (1972). The values of various extinction coefficients used in the calculations are summarised in Table 1.

Since the initial concentration (N_T) of rhodopsin molecules and the incident intensity (I_{inc}) are not available with the experimental results, we have normalized the horizontal and vertical scales of the plot for one wavelength and have used the

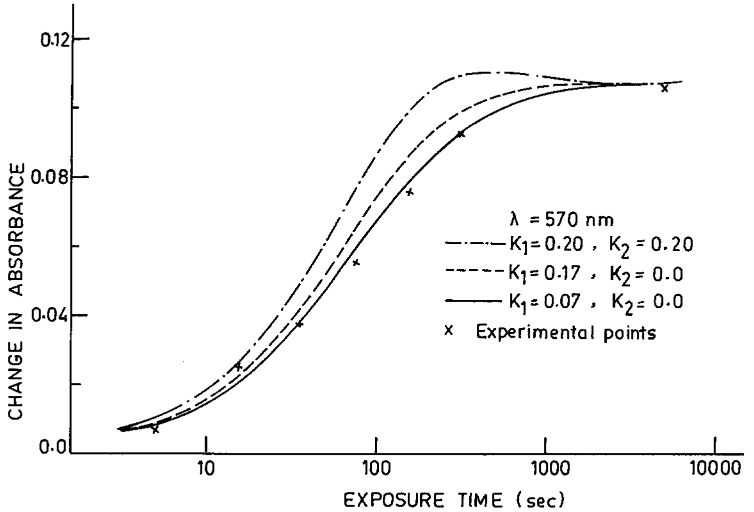

Fig. 2. The variation of change in absorbance (ΔA) of a solution of cattle rhodopsin in glycerol-water (1 : 2) mixture at −196° C as a function of the light exposure. The solution was bleached with a plane polarised light of wavelength 437 nm. The wavelength of the measuring light (natural) was 570 nm. The crosses show the experimental points taken from the paper of Yoshizawa and Horiuchi (1973); the curves are theoretical with different sets of values of K_1 and K_2

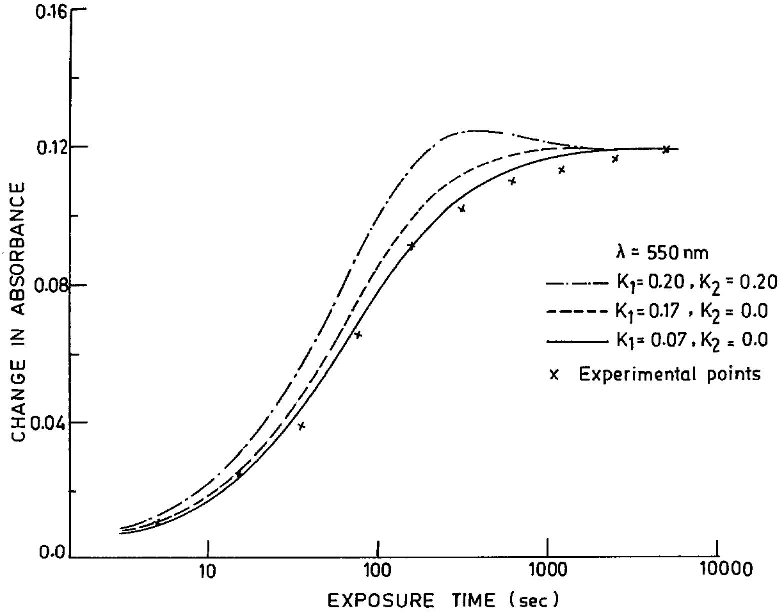

Fig. 3. Same as Figure 2 except that in this case the wavelength of measuring light was 550 nm

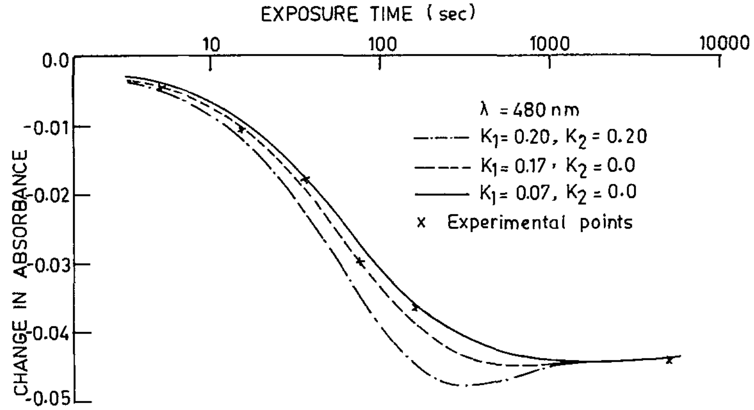

Fig. 4. Same as Figure 2 except that in this case the wavelength of measuring light was 480 nm

Table 1

Extinction coefficients					Quantum efficiencies	Absorption ratios
Wavelengths (λ)	437 nm	480 nm	550 nm	570 nm		
$\alpha_1(\lambda)/\alpha_1(\lambda_{max})$	0.425	0.842	0.315	0.087	$\gamma_{1,2}/\gamma_{1,2} = 1.0$	$K_1 = \frac{\alpha_x'}{\alpha_z'} = 0.00$
$\alpha_2(\lambda)/\alpha_1(\lambda_{max})$	0.311	0.560	1.009	0.687	$\gamma_{2,1}/\gamma_{1,2} = 0.65$ $\gamma_{2,3}/\gamma_{1,2} = 0.10$	$K_2 = \frac{\alpha_y'}{\alpha_z'} = 0.07$
$\alpha_3(\lambda)/\alpha_1(\lambda_{max})$	0.560	1.030	0.128	0.041	$\gamma_{3,2}/\gamma_{1,2} = 0.25$	

same normalizing factor for the other two wavelengths. Other parameters, viz., the four quantum efficiencies and K_1 and K_2 are chosen by fitting our results to the experimental data of Yoshizawa and Horiuchi (1973). To start with, we have used the values of these parameters as given by Strackee (1972):

$$\gamma_{1,2} = 1.0\ ; \quad \gamma_{2,1} = 0.65\ ; \quad \gamma_{2,3} = 0.10\ ; \quad \gamma_{3,2} = 0.25\ ;$$

$$K_1 = 0.20\ ; \quad K_2 = 0.20\ .$$

With these values it is not possible to match theoretical results with the experimental data. Further, it is found that the experimental results can not be explained unless K_1 or K_2 is zero (or negligibly small). As can be seen from Figure 2, the theoretical curve shows a decrease after reaching a maximum if both K_1 and K_2 are non-zero, such a behaviour is totally absent in the experimental results. Therefore, next we have set $K_1 = 0$ and tried for a best fit with different sets of values for remaining parameters starting with $K_2 = 0.17$ (as given by Strackee, 1970). However, the best fit for the three wavelengths (chosen at random) of measuring light is obtained with $K_2 = 0.07$ and the quantum efficiencies given above (see Figs. 2, 3, and 4). A variation (of about ± 10%) in each of the various quantum efficiencies has also been tried, but it only worsens the fitting. The final parameters used are summarised in Table 1.

II. Configuration of Chromophores in the Rod Outer Segment

Having selected the various parameters by way of explaining the results of an experiment on the rhodopsin in solution, we use our results to explain the observations on absorption properties of the rhodopsin in the retina. This is a test for the validity of our results and also provides the configuration of chromophores in the rod outer segment.

Brown (1972) concluded from his experiments on photoinduced dichroism in glutaraldehyde fixed frog-retina that the rhodopsin chromophore is essentially a linear absorber for light passing down the rod axis. Cone (1972) also confirmed these observations. It can be shown that the maximum dichroic ratio in the retina is given by (Cone, 1972)

$$DR_{\max} = \frac{2 + f}{2 - f}$$

where $f (\leqslant 1)$ is an empirical factor which accounts for the loss in polarisation due to the optics and the scattering. This expression is derived under the assumption that the chromophore is linear in the plane of the disc membrane. However, if the chromophore were not linear, the above expression would take the form

$$DR_{\max} = \frac{2(1 + K)^2 + f(1 - K)^2}{2(1 + K)^2 - f(1 - K)^2}$$

where K is the ratio of absorptions in the two directions (in the plane of disc membranes). When ideal conditions exist (i.e., when $f = 1$) and $K = 0$, $DR_{\max} = 3$. Brown (1972) observed a maximum value 2.8. The small discrepancy may be either due to f being less than unity or due to K being greater than zero. For $f \leqslant 1$, $DR_{\max} = 2.8$ would correspond to $K \leqslant 0.013$, in practical cases since f is less than unity, K would be less than 1%. This is a negligibly small value and hence for light passing down the rod axis the chromophore can safely be assumed to be linear (Brown, 1972). Strackee's (1970, 1972) parameters are not compatible with these observations.

In the light of above discussion we can now propose the following configuration for the chromophore in the disc membrane: the x'-axis of the chromophore ($\alpha_{x'} = K_1\alpha_{z'} = 0$) lies in the plane of the disc while z'-axis (the long-axis of the absorption ellipsoid) makes an angle θ with the plane of the disc, and the y'-axis ($\alpha_{y'} = K_2\alpha_{z'} = 0.07\ \alpha_{z'}$) then evidently, makes an angle θ with the rod axis (see Fig. 5). This is a general configuration compatible with the observation of Brown (1972) and Cone (1972). The angle θ is estimated from the observations of Liebman (1962) and Wald et al. (1963). These authors observed that there was a ratio of about 6 between absorptions of rod outer segments when viewed sidewise with light polarised perpen-

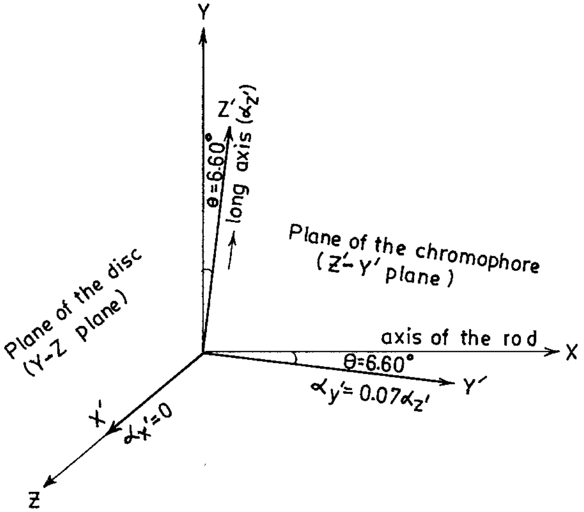

Fig. 5. The configuration of chromophore in the disc membrane. The plane of the chromophore ($Z'-Y'$) is normal to that of the disc ($Y-Z$)

dicularly ($A_\perp$) and parallel ($A_\parallel$) to their long-axes (the axes of rods). It can be shown that

$$\frac{A_\perp}{A_\parallel} = \frac{1 + K_1 \sec^2 \theta + K_2 \tan^2 \theta}{2\,(\tan^2 \theta + K_2)}\,. \tag{10}$$

(In deriving the above result it was assumed that, the chromophores are distributed randomly in the disc.) It can be easily verified that $A_\perp/A_\parallel = 6$ and $K_1 = K_2 = 0.20$ (Strackee, 1972) are not compatible with the above equation, as noted by Strackee himself. In fact, it is seen that, for real values of θ and $A_\perp/A_\parallel = 6$, K_1 and K_2 should satisfy the following condition

$$1 + K_1 \geqslant 12\,K_2\,.$$

If $K_1 = K_2$, this condition requires $K_1 \leqslant 0.09$, a value which is less than half the value obtained by Strackee (1972). On the other hand, if $K_1 = 0$, as in our case, the above condition restricts the value of K_2 to be less than 0.0833; thus the value $K_2 = 0.07$ satisfies this criterion. Using Eq. (10) it is seen that $A_\perp/A_\parallel = 6$, when $K_1 = 0$ and $K_2 = 0.07$ (for $\theta = 6.6°$). Thus, we arrive at a configuration of chromophore in the disc membrane which is consistent with the observations of Brown (1972), Cone (1972), Liebman (1962), and Wald et al. (1963) in the retina and with the observations of Yoshizawa and Horiuchi (1973) in solutions. The structure can be summarised as follows: the chromophore is a planar absorber having an absorption ratio of about 100 : 7 between two absorption axes; in the disc membranes its plane ($Y'-Z'$-plane) is normal to the plane of the disc with its long axis (Z'-axis) making an angle about 6.6° with the plane of the disc.

Acknowledgements. The authors are grateful to Prof. M. S. Sodha and Prof. A. K. Ghatak for stimulating discussions.

References

Brown, P. K.: Rhodopsin rotates in the visual receptor membrane. Nature (New Biol.) **236,** 35–38 (1972)

Cone, R. A.: Rotational diffusion of rhodopsin in the visual receptor membrane. Nature (New Biol.) **236,** 39–43 (1972)

Dartnall, H. J. A.: Photosensitivity. In: Handbook of sensory physiology. Dartnall, H. J. A. (ed.), Vol. VII/1, pp. 122–145. Berlin, Heidelberg, New York: Springer 1972

Goldstein, H.: Classical mechanics. Reading, MA: Addison-Wesley 1950

Liebman, P. A.: In situ microspectrophotometric studies on the pigments of single retinal rods. Biophys. J. **2,** 161–178 (1962)

Sperling, W., Rafferty, C. N.: Relationship between absorption spectrum and molecular conformations of 11-cis retinal. Nature (Lond.) **224,** 591–594 (1969)

Strackee, L.: Dichroism in the retina at –196° C. Vision Res. **10,** 925–938 (1970)

Strackee, L.: Photodichroism of rhodopsin solutions at –196° C. Photochem. Photobiol. **15,** 253–268 (1972)

Wald, G., Brown, P. K., Gibbons, I. R.: The problem of visual excitation. J. Opt. Soc. Am. **53,** 20–35 (1963)

Yoshizawa, T.: The behaviour of visual pigments at low temperatures. In: Handbook of sensory physiology. Dartnall, H. J. A. (ed.), Vol. VII/1, pp. 146–179. Berlin, Heidelberg, New York: Springer 1972

Yoshizawa, T., Horiuchi, S.: Studies on intermediates of visual pigments by absorption spectra at liquid helium temperature and circular dichroism at low temperatures. In: Biochemistry and physiology of visual pigments. Langer, H. (ed.), pp. 69–81. Berlin, Heidelberg, New York: Springer 1973

Yoshizawa, T., Wald, G.: Prelumirhodopsin and the bleaching of visual pigments. Nature (Lond.) **197,** 1279–1286 (1963)

Received December 13, 1978/Accepted December 22, 1978