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LATERAL DIFFUSION OF VISUAL PIGMENTS IN TOAD (*BUFO MARINUS*) RODS AND IN CATFISH (*ICTALURUS PUNCTATUS*) CONES

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SUMMARY

1. The lateral diffusion coefficient, D, was determined for visual pigments in red rods, green rods and red-sensitive cones with a photon-counting microspectrophotometer.

2. A novel protocol that involved the placement on a photoreceptor of a single micromeasuring/bleaching beam permitted the determination of D of the pigment.

3. Demonstration that D for red rods compared very well with values obtained by other workers using other means validated the protocol.

4. Applied to green rods, the protocol gave a value of D that was about 80% greater than that for red rods.

5. D for cone pigment was found to be slightly less than that of red rod pigment.

6. The dichroic ratio of cones and the average orientation of the chromophore in the cone lamellae were 1·71 and 28·4 deg, respectively.

7. The photosensitivity at λ_{max} of the red-sensitive cones was found to be about 16% less than that of red rods.

INTRODUCTION

The diffusion of proteins and other molecules in bilayer membranes probably plays a crucial role in the function of cell membranes, for example in receptor-mediated processes, electron transfer and photoreception (Strittmatter & Rogers, 1975; Hanski, Rimon & Levitzki, 1979; Liebman & Pugh, 1979; Hackenbrock, 1981; Pastan & Willingham, 1981). In the case of enzymatic cascade reactions, where different types of proteins interact, the lateral diffusion of integral membrane proteins plays an important role in regulating the overall reaction rate of the process. The diffusion coefficient of these membrane components depends upon the membrane composition (protein, lipid and carbohydrate) and physical and chemical conditions (e.g. temperature, pH and ionic strength). Therefore, it is of interest to measure lateral diffusion coefficients of membrane constituents, especially membrane

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proteins, and a number of methods have been developed to measure the lateral diffusion of lipid and protein components in both natural and artificial membranes (Edidin, 1974; Cherry, 1979; Elson & Schlessinger, 1979; Schinitzky & Henkart, 1979; Poo, 1981). These include fluorescence photobleaching recovery, electrophoresis and microspectrophotometer measurements.

In toad and frog retinas two types of rod photoreceptors have been found. These are called red and green rods. The green rods are structurally distinguished from red rods on the basis of their short outer segment. Spectrally these green rods can be distinguished from the red on the basis of λ_{max} of their photosensitive pigment, which absorbs maximally at wavelengths of 430–440 nm. Because they comprise only about 8% of the rods in the retina and are difficult to isolate, much less work has been carried out on green rods than on red rods. It has been reported that the extinction coefficients and the photosensitivities of the visual pigments of red and green rods are approximately the same at their respective absorption maxima (Donner & Reuter, 1962; Reuter, 1966), but the pigment of green rods has been found to regenerate much faster after light adaptation than does rhodopsin, the pigment of red rods. Intracellular electrical recordings from green rods suggest that the absolute sensitivity of these cells is one-tenth that of red rods (Gold, 1979). The lateral diffusion coefficient of the visual pigment in the disc membrane, which may play an important role in transduction, has so far been measured only in red rods. Reports of its value for rhodopsin and porphyropsin in frog and mudpuppy rod outer segments, which have large diameters (8–13 μm), range from 2 to 6×10^{-9} cm^2 s^{-1} (Poo & Cone, 1973, 1974; Liebman & Entine, 1974; Wey & Cone, 1981).

The lateral diffusion coefficient of visual pigments for neither green rods nor any cones has been determined. Methodological problems have probably limited study of cones which are typically small and functionally different from rods.

We have made a comparative study of the lateral diffusion coefficients of the visual pigments in red and green rod disc membranes and in cone photoreceptor lamellar membranes using a single-photon-counting microspectrophotometer. Our protocol is based on the observations of Liebman & Entine (1974), who reported that the apparent photosensitivity of rhodopsin depended upon the diameter of the rod relative to the size of the bleaching microspot on the cell. They recognized that this anomaly was caused by lateral diffusion of rhodopsin into and out of the measuring/bleaching beam and that the diffusion was responsible for the slow bleaching rate in large-diameter rods. Using our new protocol, we have found that the diffusion coefficient for red rod pigment in toads is approximately equal to the value reported by others (Poo & Cone, 1973, 1974; Liebman & Entine, 1974). Finding this sort of support for our new protocol, we applied the method to the study of green rods and red-sensitive cones.

METHODS

The experiments were carried out on red and green rods of *Bufo marinus* and on the large, red-sensitive cones of catfish, *Ictalurus punctatus*. After an animal had been dark adapted overnight, it was decapitated in dim red light, the eyes were enucleated, and the eyecups were soaked in calcium-free Ringer solution for about 5 min. A square patch of *Bufo* retina, *ca* 3 mm on a side, was removed from a region of the eyecup (superior-temporal) that contained a large number of green

rods. Each patch was then dabbed onto a polylysine-coated cover-slip (Mazia, Schatten & Sale, 1975) and rod outer segments, which adhered to the polylysine, were deposited on the surface of the glass. A thin line of silicone oil was spread around the perimeter of this cover-slip and another, identical cover-slip was applied to it. Surface tension between the two cover-slips pulled them

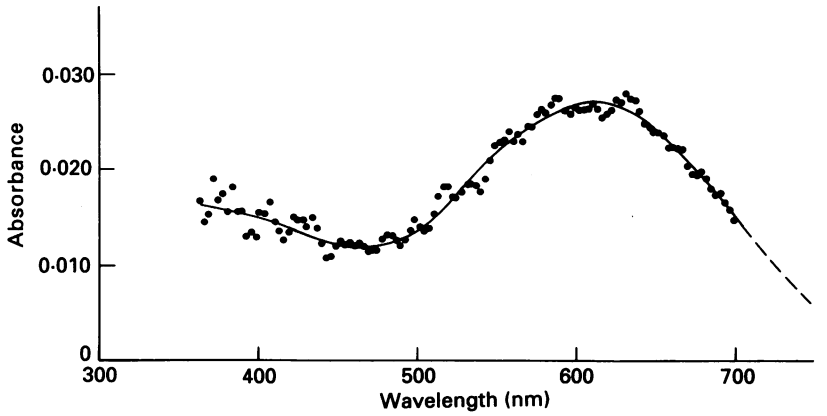

Fig. 1. Absorption spectrum of catfish cone pigment measured by microspectrophotometer.

together and created a seal that prevented evaporation of the medium between them. This preparation was then put onto the stage of the microspectrophotometer. The same procedure was followed for catfish cones.

The microspectrophotometer used in the present study is a single-photon-counting apparatus that combines infra-red full-field viewing of the rod outer segments with rapid (0·75 s) scanning of the entire visible spectrum. The instrument is basically identical to that built by MacNichol (1978), with the exception that quartz optics are sometimes used along with a piezo-electric autofocusing device. The results were not affected by the use of these quartz optics. With this microspectrophotometer it is possible to study spectral properties of small parts of single outer segments. In all the experiments we used a rectangular measuring microbeam whose dimensions depended upon the type of experiment and the photoreceptor being studied. The long axis of the rectangle was set parallel to the long axis of the photoreceptor outer segment. This beam of light was polarized with a Nicol prism in such a way that the electric vector of the beam was orthogonal to the outer segment long axis; it was, thus, roughly parallel to the transition moment of the retinal chromophore when the long axes of receptor and rectangle coincided. The receptor and the measuring beam were focused in infrared light by means of an infra-red-sensitive television system. Typically, ten scans between 350 and 700 nm were averaged together to set a baseline. Then the measuring spot was imposed upon a receptor and a measurement on the visual pigment was made by averaging ten scans through the outer segment. A computer automatically calculated the absorbance at each wavelength, displayed spectra on a video screen and stored the spectra on magnetic disc. (Although spectra were stored, baselines were not saved as separate entities.) The red and green rods were distinguished from each other by their absorption spectra and cones were distinguished from rods by their conical shapes and absorption spectra. The diameter of the catfish cone outer segment was about 4–4·5 μm at the base and about 2 μm at the tip. A typical absorption spectrum of the cone pigment is shown in Fig. 1. The λ_{max} of the fifteen cones measured was 609 ± 4 nm (S.D.).

The method used to study lateral diffusion coefficient of photopigments of green and red rods was similar to that used by Williams (1984) for the estimation of relative lateral diffusion and photoconversion rates of red rods. A single beam of high intensity was chosen to bleach visual pigments in a small portion of the disc membranes. The same beam was also used to measure simultaneously the absorbance changes in that particular region. The intensity of the beam was

chosen to cause bleaching as it traversed the outer segment, but its intensity was subsequently reduced at the photon-counting tube (to avoid coincident photons) by means of a neutral-density filter. The wavelength of this measuring/bleaching beam was 550 nm for red rods, 475 or 480 nm for green rods and 650 nm for red-sensitive cones. The wavelengths were chosen because they are strongly absorbed by their corresponding visual pigments and are capable of causing rapid bleaching, but are far enough from the absorption spectra of their photoproducts to prevent overlapping spectra. Therefore, absorbance changes at these wavelengths reflect only the decrease in the concentration of the native visual pigments in the measuring beam region. Williams (1984) found that, when the measuring/bleaching beam was intense (e.g. photon rate *ca* 10^7 s^{-1} with a spot whose dimensions are $1{\cdot}5 \times 5$ μm), the absorbance loss occurred in two stages, one fast and one slow. The fast loss he ascribed to simple photoconversion of rhodopsin in the irradiated region of the rod. The slow absorbance loss appeared to be controlled by the rate of lateral diffusion of bleached and unbleached molecules. These hypotheses were confirmed when the rod outer segment was treated with glutaraldehyde, which prevents the diffusion of rhodopsin, and a single first-order process (the photoconversion of rhodopsin) was observed that was found to be indistinguishable from the fast component of the absorbance change observed in the presence of lateral diffusion (Williams, 1984).

To calculate the lateral diffusion coefficient from this type of experiment, we consider a co-ordinate system (x, y, z) associated with a rod outer segment of radius r (Fig. 2*A*). The z-axis is perpendicular to the plane of the disc membrane, whereas the light beam is assumed to be incident along the y-axis. The diffusion of molecules, which affects the absorbance, occurs along the x-axis. Figure 2*B* shows a cross-section of the rod outer segment which has been divided into two idealized compartments, I and II. The hatched portion is the area traversed by the measuring/bleaching beam (A_1), and the remaining portion is the unilluminated area (A_2). Therefore, we can write

$$A_1 = \left(\frac{\pi r^2}{2}\right) - (h' - r)(2h'r - h'^2)^{\frac{1}{2}} - r^2 \sin^{-1}\left(\frac{h'-r}{r}\right)$$

and

$$A_2 = \pi r^2 - A_1,$$

where h' is the maximum width of the unilluminated portion of the rod outer segment along the x-axis.

To develop a tractable, if simplistic, mathematical model we assume that a rod outer segment has a square cross-section of area equal to that of the actual rod outer segment (see Fig. 2*C*); compartments I and II in the new geometry are also assigned the same areas they had in the actual cross-section (i.e. in Fig. 2*B*). In the new geometry the distance h' becomes h which is equal to

$$h = \frac{\sqrt{\pi} r}{2} + \frac{1}{\sqrt{\pi} r}\left((h'^{-r})(2h'r - h'^2)^{\frac{1}{2}} + r^2 \sin^{-1}\left(\frac{h'-r}{r}\right)\right).$$

This h is, in essence, the average width of the unilluminated portion of the rod outer segment along the x-axis. In the actual (disc) geometry, this distance varies along the y-axis, making the mathematical analysis very complicated. The assumption that the rod outer segment has a square cross-section gives the same results (see Results) on lateral diffusion of rhodopsin as have been reported earlier (Poo & Cone, 1973, 1974). Furthermore, this assumption has been found to yield results in good agreement with those obtained on the assumption of a circular cross-section, if the bleaching rate is high compared to the lateral diffusion rate of the pigment in the bleaching compartment (Gupta, 1981). In the case of cones, the values of r and h' vary along the z-axis. Their mean values in the illuminated region of the outer segment have been used to calculate h.

As mentioned above, the diffusion of molecules that affects the absorbance occurs along the x-axis, and the rate of bleaching in compartment I is, in the beginning, many times faster than the rate of diffusion. Under these conditions and for time sufficiently large, the slope of the second branch of the $\ln((A_t - A_\infty)/(A_0 - A_\infty))$ *vs.* time curve will be equal to $\pi^2 D/4h^2$ (Jost, 1960); A_t is the absorbance of the visual pigments in compartment I at time t while A_0 and A_∞ are its values at $t = 0$ and $t = \infty$, respectively; D is the lateral diffusion coefficient of the visual pigment in the disc membrane or lamellar membrane. Thus the slope of the diffusion-controlled branch of the above-mentioned plot will give the value of the lateral diffusion coefficient of the visual pigment. Therefore,

$$D = 4mh^2/\pi^2,$$

where m is the slope of the second (slow) branch of the plot and has units of $(1/t)$.

RESULTS

Red rods

To test the validity of our method for the determination of the diffusion coefficient of photopigments in general, we first carried out experiments on red rods because

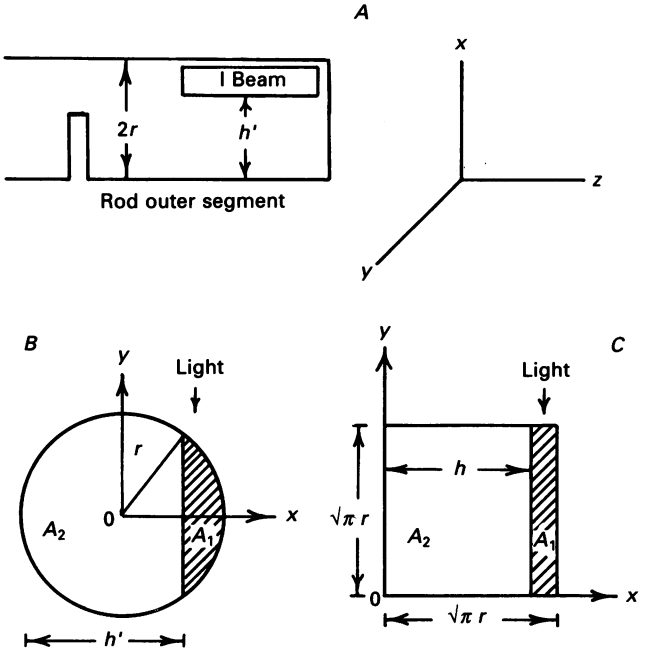

Fig. 2. *A*, geometry and expected results on absorption changes of a single-beam, high-intensity experiment. The measuring/bleaching beam is subtended at the edge of the discs and its width along the transverse direction is $(2r-h')$; r is the radius of the rod outer segments. (x, y, z) is a co-ordinate system associated with the rod outer segment; the z-axis is perpendicular to the plane of the disc membrane and the direction of incidence of the beam is along the y-axis. The diffusion that affects the absorbance occurs along the x-axis. In the case of cone, r and h' vary along the z-axis. Their mean values in the illuminated region of the outer segment have been used to calculate h. *B*, end-on view of rod outer segment; the z-axis is perpendicular to the plane of the paper. The hatched portion is the area covered by the measuring/bleaching beam, i.e. the compartment I. *C*, idealized, rectilinear cross-section of rod outer segment.

they have been well studied. As mentioned in the last section, a single, plane-polarized, high-intensity beam of 550 nm wavelength is used as the measuring/bleaching beam. The results of an individual run are shown in Fig. 3. The ● represents the experimental points. It can be seen that absorbance is lost in two stages as observed earlier by Williams (1984). If the loss were simple first-order (i.e. bleaching only) the function in Fig. 3 would be a single straight line (Williams, 1984). The first stage occurs immediately upon exposure of the rod outer segment and is due to the photoconversion of rhodopsin, whereas the second stage is due to the lateral

diffusion of rhodopsin. From the slope of the second stage we obtain $D = 4{\cdot}3 \times 10^{-9}$ cm^2 s^{-1} as the lateral diffusion coefficient of rhodopsin in disc membrane at room temperature. The average value ($\pm$S.D.) of D for ten red rods was $(3{\cdot}93 \pm 0{\cdot}86) \times 10^{-9}$ cm^2 s^{-1}, very close to the values reported by Poo & Cone (1973,

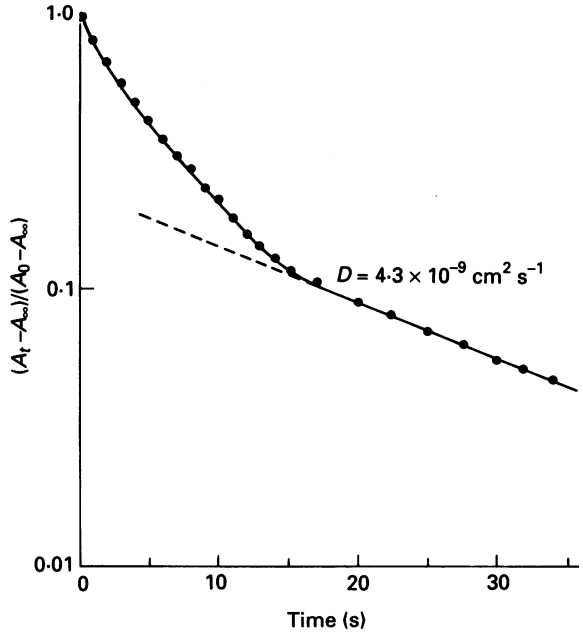

Fig. 3. Results of a single-beam, high-intensity experiment on fresh red rod outer segment. The wavelength of the measuring/bleaching beam was 550 nm. The beam dimensions were 2×5 μm and $h = 4{\cdot}74$ μm. Double-branched loss of absorbance is evident and conforms to expectations. The first branch is photoconversion of rhodopsin and the second is caused by lateral diffusion of rhodopsin as observed by Williams (1984). ● represent the experimental points.

1974); they reported $(3{\cdot}9 \pm 1{\cdot}5) \times 10^{-9}$, $(3{\cdot}5 \pm 1{\cdot}5) \times 10^{-9}$ and $(3{\cdot}9 \pm 1{\cdot}2) \times 10^{-9}$ cm^2 s^{-1} as the values of D for *Necturus*, frog and mudpuppy rods, respectively. This close agreement shows that our single-beam method can be used to obtain the lateral diffusion coefficient of visual pigment in disc membranes.

Green rods

Figure 4 shows results obtained for green rods at 475 nm and different intensities of the bleaching beam. It can be seen that, as for red rods, absorbance is low in two stages. The value of D obtained from the slope of the second stage of each individual run is shown in the figures. The mean ($\pm$S.D.) of the lateral diffusion coefficient of the visual pigment in green rod disc membrane obtained from these results is $(7{\cdot}3 \pm 1{\cdot}2) \times 10^{-9}$ cm^2 s^{-1}, about 1·8 times higher than the lateral diffusion coefficient of rhodopsin in red rod disc membrane.

This value of D is quite large and prompted us to explore possible reasons for its magnitude. For example, are the absorbance changes anomalous? Do photoproducts

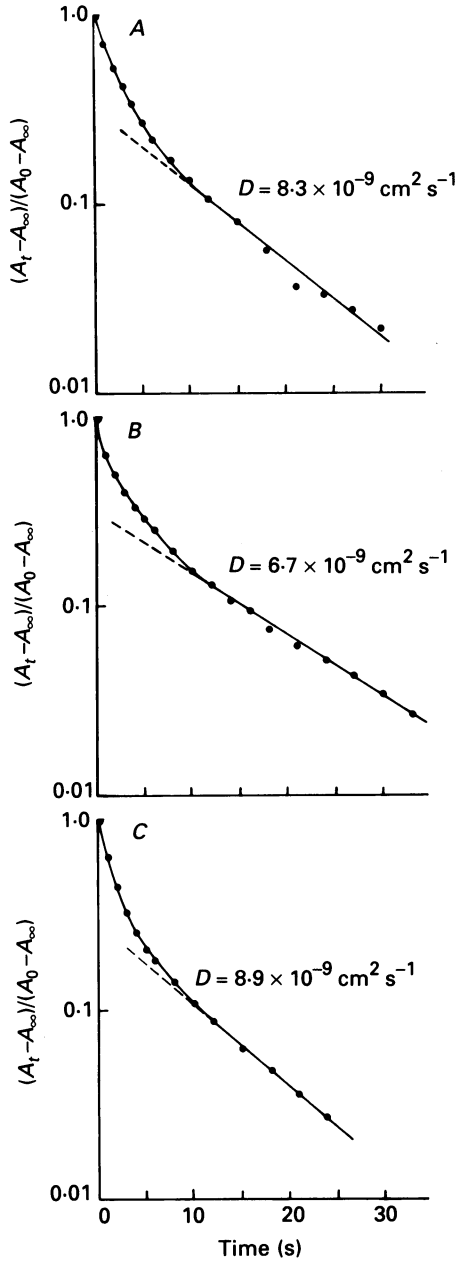

Fig. 4. Results of a single-beam, high-intensity experiment on fresh green rod outer segment at three different intensities. The wavelength of the measuring/bleaching beam was 475 nm and the beam dimensions were the same as for red rods. ● represent the experimental points. As for the red rod, double-branched loss of absorbance can be seen in all these results.

interfere in this protocol? Bowmaker (1977) studied the long-lived photoproducts of the green rod pigment of the frog and reported that the first detectable photoproduct is metarhodopsin II (MR II) (λ_{max} = 390 nm), which is followed by a metarhodopsin II (MR III) (λ_{max} about 445 nm), along with retinal (λ_{max} = 380–390 nm) and retinol

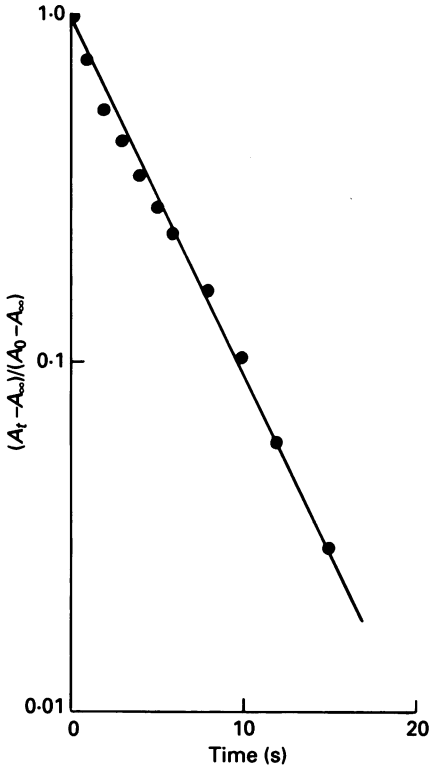

Fig. 5. Results of a single-beam, high-intensity experiment on fresh green rod. The wavelength of the measuring/bleaching beam was 475 nm and its width was about twice the diameter of the rod outer segment. The absorbance loss with time in this case is a simple exponential function.

(λ_{max} = 325 nm). The photoproducts, retinal and retinol, do not participate in the absorbance changes given in Fig. 4 because at wavelength 475 nm their absorption is virtually zero. Furthermore, according to the results of Bowmaker (1977), the appearance of MR III should not contaminate our diffusion measurements for two reasons: (i) MR III appearance is measured in hundreds of seconds, whereas our diffusions are measured in tens and (ii) MR III is rotated out of the plane of the disc and would be virtually transparent in our protocol.

To verify that the deviation from linearity in Fig. 4 is due to the diffusion of visual pigments, we carried out measuring/bleaching experiments with the microbeam width about twice the diameter of the rod outer segment. This protocol bleaches the discs uniformly, and, although diffusion occurs, it cannot contribute to absorbance changes. The results so obtained for λ = 475 nm are shown in Fig. 5. It can be seen that the experimental points are fitted well by a straight line, which indicates that

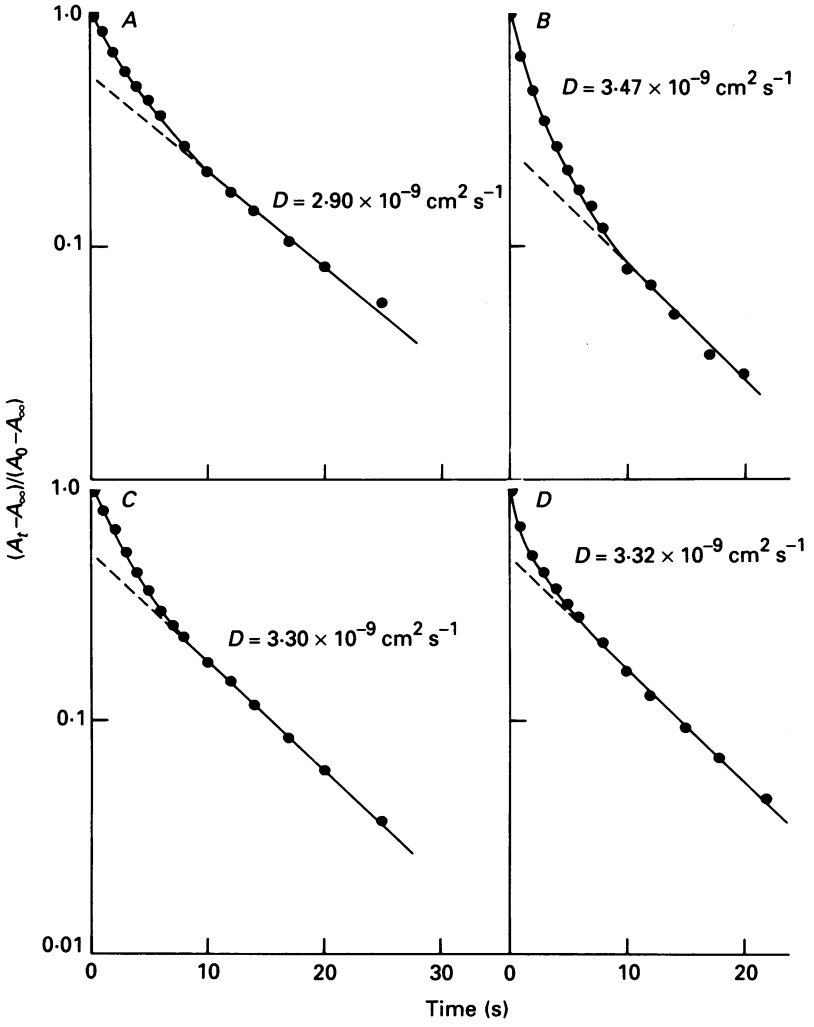

Fig. 6. Results of a single-beam, high-intensity experiment on fresh catfish cone outer segments. The wavelength of the beam was 650 nm. The experiment was carried out at four different rates of bleaching. The beam dimensions were $1{\cdot}5 \times 4$ μm and $h = 2{\cdot}74$ μm. Double-branched loss of absorbance is evident and conforms to expectations. ● represent the experimental points.

only one process, the first-order photoconversion of the visual pigments, is affecting the absorbance. This result ensures that the non-linearity in absorbance decrease in Fig. 4 is due to the lateral diffusion of the visual pigments of the green rod in its disc membranes. By other means (fixation of the rod outer segment with glutaraldehyde) Williams (1984) obtained a similar result.

Cones

Figure 6 shows the results obtained for catfish cones for different intensities of the measuring/bleaching beam. The filled circles represent the experimental points. As

in red and green rods, absorbance in cones is lost in two stages. The values of D obtained from the slope of the second stage of each individual run are shown in the figure. To make a fair comparison with the values obtained from red and green rods, we examined a total of eight cones and found that the average value ($\pm$S.D.) of D for these cells was $(3{\cdot}17 \pm 0{\cdot}28) \times 10^{-9}$ cm^2 s^{-1}, close to the value obtained for rhodopsin in red rod disc membranes.

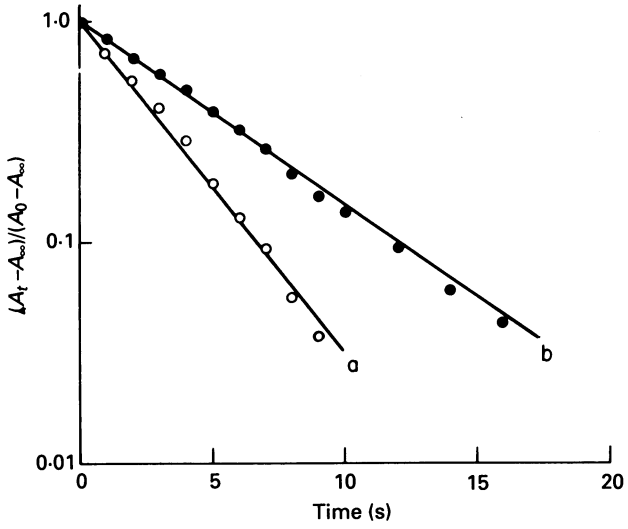

Fig. 7. Results of a single-beam, high-intensity experiment on fresh catfish cones for two different rates of bleaching. The beam dimensions were 7×2 μm while its wavelength was 650 nm. The absorbance loss with time in both cases is a simple exponential function.

To verify that the deviation from simple linearity in Fig. 6 is due to the diffusion of visual pigments, we carried out a control experiment similar to the one for green rods (Fig. 5). The results obtained for two rates of bleaching are shown in Fig. 7. The experimental points are fitted well by a single straight line, which indicates that only one process is affecting the absorbance, namely the photoconversion of the visual pigments; no photoproduct is contributing to absorbance at 650 nm during the course of this experiment.

We have not observed any longitudinal diffusion of cone pigment, although lamellar membranes are connected to each other at the edges. This result is further assurance that the non-linearity in absorbance decrease in Fig. 6 is due to the lateral diffusion of the cone visual pigment in its membranes.

In addition to lateral diffusion, we have also measured the photosensitivity of the cone pigment from similar experiments. The only difference was that the width of the microbeam was made equal to the diameter of the cone at the position where the outer segment was subtended by the beam. The experiment was carried out on nine different cones with various intensities (I) of the measuring bleaching beam of wavelength 650 nm. The results were plotted as $\ln((A_t - A_\infty)/(A_0 - A_\infty))$ *vs.* time. In

all cases the variation was linear. From the slopes of these plots we calculated the values of the bleaching-rate constant (k_b) for all the cones. Mathematically, it is given by

$$k_b = \Gamma \alpha_t(\lambda) I \cos^2 \Theta, \tag{1}$$

where Γ represents the quantum efficiency of bleaching of the visual pigment; $\alpha_t(\lambda)$ is the extinction coefficient of the visual pigment for the transverse incidence of the plane-polarized beam with electric vector parallel to the plane of lamellar membrane at wavelength λ. Θ is the angle of the dipole axis of the chromophore of the visual pigment makes with the plane of the lamellar membrane. On substituting $\alpha_t(\lambda) = \frac{3}{2}$ $\alpha_{sol}(\lambda)$ (where $\alpha_{sol}(\lambda)$ is the extinction coefficient of the visual pigment in solution at wavelength λ) into eqn (1), we obtain the following relation

$$\Phi(\lambda) = \Gamma \alpha_{sol}(\lambda) = 2k_b/(3I \cos^2 \Theta); \tag{2}$$

here Φ is the photosensitivity of the visual pigment in solution at wavelength λ. In order to determine $\Phi(\lambda)$ for catfish cone pigment, we calculated I and Θ a follows.

The intensity of the bleaching beam in our experiments is given by

$$I = \frac{\text{Photon rate measured from photomultiplier tube output}}{\text{Area of cross-section of the beam} \times \text{quantum efficiency of photomultiplier tube}}. \tag{3}$$

In our microspectrophotometer, photon rate and area of cross-section of the beam are directly measurable quantities, and the quantum efficiency of the photomultiplier tube was given in its manual for different wavelengths. At $\lambda = 650$ nm it was equal to 0·06. Dividing the measured photon rate by the quantum efficiency of the photomultiplier tube converts the *measured* rate into *actual* photon rate. Thus, the intensity of the bleaching beam was determined from eqn (3) for all the cones after substitution of the corresponding values of photon rate and area of cross-section. The angle Θ was calculated from the dichroic ratio of the cone. We define it as follows

$$\text{dichroic ratio} = \frac{A_\parallel}{A_\perp},$$

where $A_\parallel$ is the absorbance measured when the electric vector of the plane-polarized measuring beam is parallel to the plane of the lamellar membrane and $A_\perp$ is the absorbance when the electric vector is perpendicular to the plane of the lamellar membrane, i.e. along the cone outer segment axis. These absorbances are given by

$$A_\parallel(\lambda) = 1/2[\alpha_1(\lambda) N_0 \cos^2 \Theta] \tag{4}$$

and

$$A_\perp(\lambda) = \alpha_1(\lambda) N_0 \sin^2 \Theta, \tag{5}$$

where $\alpha_1(\lambda)$ is the extinction coefficient of the visual pigment when the electric vector is coincident with the dipole axis of the visual pigment and N_0 is the concentration of the visual pigment in cones. Equations (4) and (5) give

$$\Theta = \sin^{-1}[1/(1+2 \text{ dichroic ratio})]^{\frac{1}{2}}. \tag{6}$$

We measured the dichroic ratio of nine different catfish cones and found it equal to $1{\cdot}71 \pm 0{\cdot}26$. This value gives, after substitution in eqn (6), $\Theta = 28{\cdot}4$ deg. On

substituting the values of k_b (determined from the slope of the plot), I (for corresponding k_b and determined from eqn (3)), and Θ in eqn (2), we obtain $(8{\cdot}9 \pm 2{\cdot}2) \times 10^{-17}$ cm^2 chromophore^{-1} as the photosensitivity of the catfish cone pigment at λ_{max} *in vitro*. This is slightly lower than the value reported for rhodopsin at λ_{max}, $10{\cdot}5 \times 10^{-17}$ cm^2 chromophore^{-1} (Dartnall, 1972).

DISCUSSION

We have developed a single-beam protocol for determining the lateral diffusion coefficients of visual pigments. The protocol, based on the observations of Liebman & Entine (1974), gives values of D for red rods that are in good agreement with those published by others. In addition, earlier work showed that computer simulation of this protocol is consistent with the observations of double-branched absorbance loss (Williams & Penn, 1985). Hence, it appears that the protocol gives accurate results. It also gives precise results; the standard deviations in our measurements of D range from 6 to 16%, less than half those found in the literature for comparable results. The precision of our method may derive from its single beam which reduces focusing errors of the sort that can result when separate beams are used for measuring and for bleaching.

It is interesting that such a simple method, with its idealized, rectilinear model, fits as well as it does. For example, we gave no consideration either to disc incisures or to a more realistic geometry of the light beam as it subtends the outer segment. Poo & Cone (1973) concluded that disc incisures probably have little effect on the rate of lateral diffusion across the disc, but green rod incisures are shallower than those in red rods (Papermaster, Reilly & Schneider, 1982). This could be the major reason for the apparently very rapid diffusion of green rod pigment: the deep incisures in red rods might impede lateral diffusion across the disc.

We have been developing mathematical and physical models of the intersection of the measuring/bleaching beam with an outer segment (side-on). This work is still in progress but shows, at present, that the geometric average light path of a 7 μm (diameter) rod outer segment is effectively greater than the diameter by (about) 16% because the beam traverses the rod outer segment obliquely. Because this is such a slight effect, it can be ignored, at least until a full model has been developed. Finally, we have surely overestimated the value of h because our mathematical model is simple and because it does not take into account the complex geometry of the outer segment–beam intersection. In the actual experiment, the beam intersects more of the outer segment than is evident from the video image. Thus, the illuminated compartment is larger than it appears, making the unilluminated region smaller. Consequently, the real h is smaller than the one we used to calculate D. Because D is proportional to h, a smaller, more realistic h will yield a smaller value of D, but a definitive value of h awaits the full development of our mathematical model. For the present, good agreement with other workers, as in the case of red rods, justifies our new protocol.

To our knowledge the value of Φ for cone pigment has not been reported previously. Our value, about 15% less than that for rhodopsin, should be considered tentative. Not only is it subject to the uncertainties of beam geometry described

above, it is also based on the assumption that our microbeam is perfectly polarized and on the correctness of the manufacturer's values for the quantum efficiency of our photomultiplier tube (RCA 8550). The latter is only known to single-digit significance (e.g. 0·06 at 650 nm, the irradiation wavelength for cones). Obviously such an inadequate value contributes to the tentativeness of our value of Φ.

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REFERENCES

BOWMAKER, J. K. (1977). Long-lived photoproducts of the green-rod pigment of the frog, *Rana temporaria*. *Vision Research* **17**, 17–23.

CHERRY, R. J. (1979). Rotational and lateral diffusion of membrane proteins. *Biochimica et biophysica acta* **559**, 289–327.

DARTNALL, H. J. A. (1972). Photosensitivity. In *Handbook of Sensory Physiology*, vol. VII/1, ed. DARTNALL, H. J. A., pp. 122–145. Springer-Verlag, Berlin, Heidelberg, New York.

DONNER, K. O. & REUTER, T. (1962). The spectral sensitivity and photopigment of the green rods in the frog's retina. *Vision Research* **2**, 357–372.

EDIDIN, M. (1974). Rotational and translational diffusion in membranes. *Annual Review of Biophysics and Bioengineering* **3**, 179–201.

ELSON, E. L. & SCHLESSINGER, J. (1979). Long-range motions on cell surfaces. In *The Neurosciences: Fourth Study Program*, ed. SCHMITT, F. O. & WORDEN, F. G., pp. 691–70. MIT Press, Cambridge, MA, USA.

GOLD, G. H. (1979). Photoreceptor coupling in retina of the toad, *Bufo marinus*. II. Physiology. *Journal of Neurophysiology* **42**, 311–328.

GUPTA, B. D. (1981). Adsorption of light in photoreceptors: transverse incidence. *Biophysics of Structure and Mechanism* **8**, 35–43.

HACKENBROCK, C. R. (1981). Lateral diffusion and electron transfer in the mitochondrial inner membrane. *Trends in Biochemical Sciences* **6**, 151–154.

HANSKI, E., RIMON, G. & LEVITKI, A. (1979). Adenylate cyclase activation by the beta-adrenergic receptors as a diffusion controlled process. *Biochemistry* **18**, 846–853.

JOST, W. (1960). *Diffusion*. Academic Press, New York.

LIEBMAN, P. A. & ENTINE, G. (1974). Lateral diffusion of visual pigment in photoreceptor disk membranes. *Science* **185**, 457–459.

LIEBMAN, P. A. & PUGH, E. N. JR (1979). The control of phosphodiesterase in rod disk membranes: kinetics, possible mechanisms and significance for vision. *Vision Research* **19**, 375–380.

MACNICHOL, E. F. JR (1978). A photon-counting MSP for the study of single vertebrate photoreceptor cells. In *Frontiers of Visual Science*, ed. COOL, S. J. & SMITH, E. L., pp. 194–208. Springer Verlag, Berlin.

MAZIA, D., SCHATTEN, G. & SALE, W. (1975). Adhesion of cells to surfaces coated with polylysine. *Journal of Cell Biology* **66**, 198–200.

PAPERMASTER, D. S., REILLY, P. & SCHNEIDER, B. G. (1982). Cone lamellae and red and green rod outersegment disks contain a large intrinsic membrane protein on their margins: an ultrastructural immunocytochemical study of frog retinas. *Vision Research* **23**, 1417–1428.

PASTAN, I. H. & WILLINGHAM, M. C. (1981). Journey to the center of the cell: role of the receptosome. *Science* **214**, 504–509.

POO, M. (1981). *In situ* electrophoresis of membrane components. *Annual Review of Biophysics and Bioengineering* **10**, 245–276.

POO, M. & CONE, R. A. (19730. Lateral diffusion of rhodopsin in *Necturus* rods. *Experimental Eye Research* **17**, 503–510.

POO, M. & CONE, R. A. (1974). Lateral diffusion of rhodopsin in the photoreceptor membrane. *Nature* **247**, 438–441.

REUTER, T. (1966). The synthesis of photosensitive pigments in the rods of the frog's retina. *Vision Research* **6**, 15–38.

SCHINITZKY, M. & HENKART, P. (1979). Fluidity of cell membranes: current concept and trends. *International Review of Cytology* **60**, 121–147.

STRITTMATTER, W. J. & ROGERS, M. J. (1975). Apparent dependence of interactions between cytochrome b_5 and cytochrome b_5 reductase upon translational diffusion in dimyristoyl lecithin liposomes. *Proceedings of the National Academy of Sciences of the USA* **72**, 2658–2661.

WEY, C. L. & CONE, R. A. (1981). Lateral diffusion of rhodopsin in photoreceptor cells measured by fluorescence photobleaching and recovery. *Biophysics Journal* **33**, 225–232.

WILLIAMS, T. P. (1984). Some properties of old and new rhodopsin in single *Bufo* rods. *Journal of General Physiology* **83**, 841–852.

WILLIAMS, T. P. & PENN, J. S. (1985). Some attempts to discover molecular aging in rhodopsin of single *Bufo* rods. *Atti della Fondazione Giorgio Ronchi* **40**, 417–428.