THE PHOTOCONVERSION OF LUMIRHODOPSIN AT 77°K

ESTIMATION OF THE QUANTUM EFFICIENCY

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ABSTRACT Evidence is presented that lumirhodopsin (containing all-*trans* retinal) is not directly photoconverted to bathorhodopsin (all-*trans*) at 77°K as previously suggested (Yoshizawa and Wald. 1963. *Nature (Lond.)*. **197**:1279–1286). Rather, lumirhodopsin is converted to a new species, L′ (11-*cis* and/or 9-*cis* retinal) which, on warming to room temperature, is indistinguishable from rhodopsin or isorhodopsin. The quantum efficiency for the conversion of lumirhodopsin to L′ is estimated to be 0.5 ± 0.1. This value is significantly higher than that of other all-*trans* to *cis* conversions for bovine rhodopsin intermediates, indicating that the opsin conformation has a significant effect on a pigment's quantum efficiency.

INTRODUCTION

The most widely accepted initial sequence of reactions leading to visual excitation is that rhodopsin (containing 11-*cis* retinal) photoisomerizes to bathorodopsin (all-*trans*) which when warmed to 170°K thermally decays to lumirhodopsin (all-*trans*). However, it has been reported that lumirhodopsin is directly photoconverted to bathorhodopsin when illuminated at 77°K. Specifically, Yoshizawa and Wald (1963) have reported that the irradation of lumirhodopsin at 77°K results in a "sharp isosbestic point at about 523 nm (that) indicates the transformation of a single precursor into a product, in this case lumirhodopsin into prelumirhodopsin" (bathorhodopsin). This observation poses obvious problems to the above sequence of reactions since, at 77°K, thermal reactions presumably cannot occur and only photoisomerization of retinal is possible. More recently, it has been suggested that photoconversion of lumirhodopsin (all-*trans*) to bathorhodopsin (all-*trans*) at 77°K may involve absorption of two photons (Yoshizawa, 1972). The first photon would isomerize the chromophore to the 11-*cis* configuration and residual energy would rearrange the opsin to form rhodopsin. The second photon would then convert rhodopsin to bathorhodopsin by isomerization of retinal to the all-*trans* configuration. In this paper, evidence is presented that at 77°K lumirhodopsin is not photoisomerized directly to bathorhodopsin but to a new species, L′, which contains 11-*cis* and/or 9-*cis* retinal and on warming is converted to rhodopsin and/or isorhodopsin. In addition, the quantum efficiency of lumirhodopsin conversion to L′ is estimated.

MATERIALS AND METHODS

Bovine rod outer segments were prepared according to the procedures of Papermaster and Dryer (1974) and the rhodopsin solubilized in 2% digitonin and 0.067 M potassium phosphate buffer (pH 7.0). Glycerol was then added to prevent crystallization of the samples at low temperatures (67% vol/vol final

concentration). After illumination and absorption measurements at 77°K (below), the samples were warmed to room temperature. 2 M hydroxylamine (NH_2OH titrated to pH 7.0) was added to all samples to a final concentration of 0.1 M to fully convert any bleached rhodopsin to retinaloxime and opsin. Absorption measurements were then repeated at room temperature.

A Cary 118 spectrophotometer (Varian Instruments, Palo Alto, Calif.) was used to record all absorption spectra. Low temperature spectra were recorded using a Dewar flask with flat windows on all four sides to allow uniform sample illumination and accurate absorption measurements. The samples at room temperature were injected through a syringe needle into a 0.2-cm pathlength quartz cuvette that was almost entirely immersed in liquid nitrogen. This prevented cracking of the sample on lowering the temperature to 77°K. The cell was then rapidly transferred to a Dewar filled with liquid nitrogen. Absorption measurements and illumination of the samples at 77°K followed. For measurements at 170°K, the samples were then plunged into a second Dewar filled with ethanol previously cooled with liquid nitrogen to 170°K. In some experiments the samples were recooled to 77°K by removing the cell from the ethanol bath, rapidly blowing off any ethanol remaining on the cell surface with a burst of nitrogen gas, and plunging the cell back into the Dewar filled with liquid nitrogen. This prevented excessive cracking of the sample so that irradiations were consistent. Samples were illuminated through the Dewar windows at fixed time periods using a slide projector light source (500 W) and a fixed rheostat setting. A 480-nm interference filter (12-nm bandwidth) was used in all illuminations.

RESULTS AND DISCUSSION

Evidence of Lumirhodopsin to L' Photoconversion

In the first preliminary experiment (see Fig. 1), rhodopsin was converted to a mixture of rhodopsin (11-*cis*; λ_{max} = 505 nm), bathorhodopsin (all-*trans*; λ_{max} = 543 nm) and a small

First Preliminary Experiment

$$\underset{\substack{\text{R}\\(11\text{-}cis)}}{[2\text{-}1]} \xrightarrow[77°K]{480h\nu} \underset{(11\text{-}cis)}{\text{R}} + \underset{(9\text{-}cis)}{\text{I}} + \underset{(\text{all-}trans)}{\text{B}} \xrightarrow[170°K]{} \underset{(11\text{-}cis)}{\text{R}} + \underset{(9\text{-}cis)}{\text{I}} + \underset{(\text{all-}trans)}{\text{L}} \xrightarrow{77°K}$$

$$\underset{(11\text{-}cis)}{\text{R}} + \underset{(9\text{-}cis)}{\text{I}} + \underset{(\text{all-}trans)}{\text{L}} \xrightarrow[293°K]{NH_2OH} 45\%\{\underset{(11\text{-}cis)}{\text{R}} + \underset{(9\text{-}cis)}{\text{I}}\} + 55\%\ \text{retinaloxime (from all-}trans\text{)}$$

Second Preliminary Experiment

$$45\%\{\text{R} + \text{I}\} + 55\%\ \text{retinaloxime} \xrightarrow[77°K]{480h\nu^*} \xrightarrow[293°K]{NH_2OH} 41\%\{\text{R} + \text{I}\} + 59\%\ \text{retinaloxime}$$

Lumirhodopsin Photoconversion Experiment

$$100\%\ \text{R} \xrightarrow[77°K]{480h\nu} 45\%\{\text{R} + \text{I}\} + 55\%\ \text{B} \xrightarrow[170°K]{} 45\%\{\text{R} + \text{I}\} + 55\%\ \text{L} \xrightarrow{480h\nu^*}_{77°K}$$

$$\text{R} + \text{I} + \text{L}' + \underset{(\text{all-}trans)}{\{\text{B} + \text{L}\}} \xrightarrow[293°K]{NH_2OH} 46.5\%\{\underset{(11\text{-}cis)}{\text{R}} + \underset{(9\text{-}cis)}{\text{I}}\} + 53.5\%\ \text{retinaloxime (from all-}trans\text{)}$$

FIGURE 1. Flow diagrams of the preliminary and lumirhodopsin photoconversion experiments. R, B, I, and L are rhodopsin, bathorhodopsin, isorhodopsin, and lumirhodopsin, respectively. The assumed isomeric forms of retinal are given in parentheses. Numbers in brackets correlate the steps of the reaction to the figure and curve numbers of the recorded absorption spectra. In the lumirhodopsin photoconversion reaction, 480hν* refers to that degree of 480-nm illumination sufficient to convert 10% of the rhodopsin to bathorhodopsin. The existence of L' is postulated.

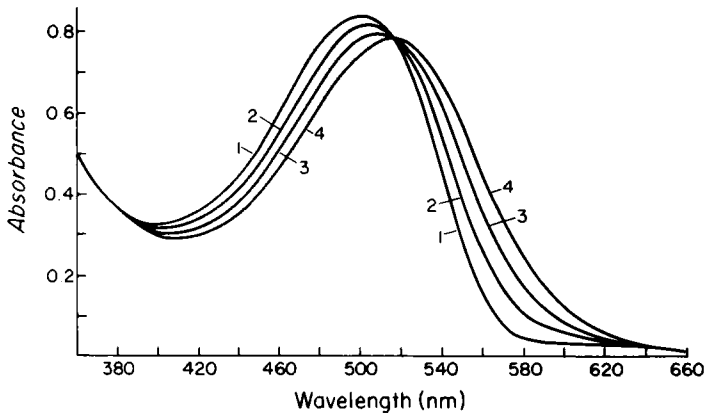

FIGURE 2. Absorption spectra of the partial conversion of rhodopsin to bathorhodopsin and isorhodopsin at 77°K with 480-nm light. Curve 1 is the spectrum of 100% rhodopsin. Curves 2, 3, and 4 are spectra of the stepwise photoconversion to bathorhodopsin and isorhodopsin. It was calculated that curve 4 is the spectrum of a mixture of 55% bathorhodopsin, 43% rhodopsin, and 2% isorhodopsin (see text). The apparent isosbestic point at 516 nm is indicative of the relatively low concentration of isorhodopsin in the mixture and the similarity of its absorption spectrum to that of rhodopsin.

amount of isorhodopsin (9-*cis*; λ_{max} = 491 nm) at 77°K with 480 nm light (Fig. 2, curves 1–4). (λ_{max} are from Yoshizawa and Wald [1963].) The mixture was then warmed to 170°K converting the bathorhodopsin to lumirhodopsin. The resulting lumirhodopsin (Fig. 3, curve 2) was recooled to 77°K (Fig. 4, curve 1). This mixture of rhodopsin, lumirhodopsin, and isorhododpsin was then warmed to room temperature (293°K) in the presence of hydroxylamine. The resulting mixture consisted of rhodopsin and isorhodopsin which are stable at room temperature and retinaloxime which resulted from the interaction of hydroxylamine and the thermal products of lumirhodopsin (Fig. 5, curve 3). The room temperature mixture had a band at 498 nm with 45% of the intensity of the original rhodopsin sample at room temperature. Therefore, the initial mixture after illumination at 77°K (Fig. 2, curve 4) was

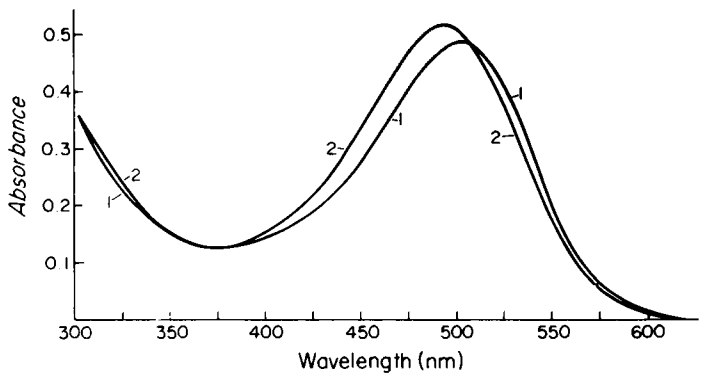

FIGURE 3. Curve 2 is the absorption spectrum of a mixture of lumirhodopsin, rhodopsin, and isorhodopsin at 170°K formed by thermally converting the bathorhodopsin of Fig. 2, curve 4 into lumirhodopsin. It was calculated that curve 2 is the spectrum of 55% lumirhodopsin, 43% rhodopsin, and 2% isorhodopsin (see text). Curve 1 is a spectrum of 100% rhodopsin shown for comparison.

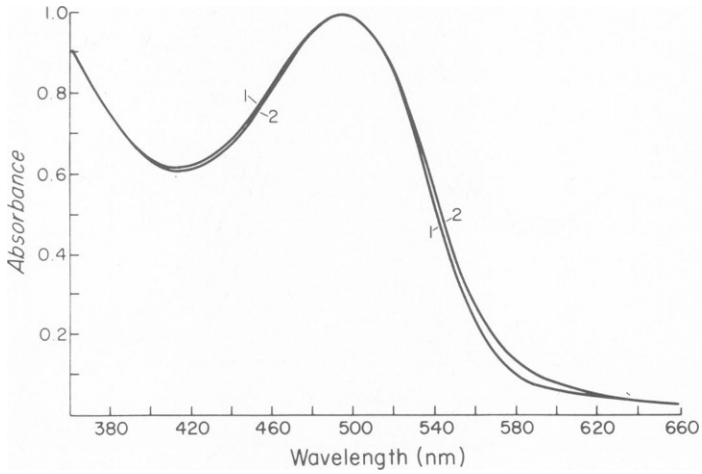

FIGURE 4. Lumirhodopsin photoconversion to L′ at 77°K. Curve 1 is a mixture calculated to be 55% lumirhodopsin, and 45% rhodopsin and isorhodopsin. Curve 2 is the same mixture after exposure to that amount of 480-nm light which converts 10% of the rhodopsin to bathorhodopsin. It was determined that the same irradiation also results in partial conversion of the lumirhodopsin to 5.5% L′.

45% rhodopsin and isorhodopsin and 55% bathorhodopsin. (Isorhodopsin constituted only ~2% of the mixtures [see below] and has an absorption spectrum similar to that of rhodopsin. As a result, it was determined to within the accuracy of the experiment that the room temperature mixture [and therefore the initial mixture] contained 45% rhodopsin and isorhodopsin.)

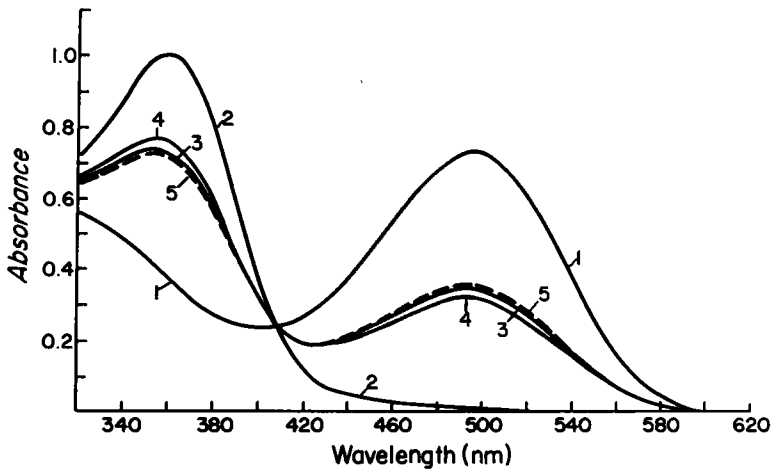

FIGURE 5. Room temperature (293°K) absorption spectra (refer to Fig. 1). Curve 1, 100% rhodopsin; curve 2, after complete conversion to retinaloxime (baseline); curve 3, 45% rhodopsin and isorhodopsin plus 55% retinaloxime (first preliminary experiment); curve 4, 41% rhodopsin plus isorhodopsin and 59% retinaloxime resulting from 77°K irradiation and warming of the mixture in curve 3 (second preliminary experiment); curve 5, 46.5% rhodopsin and isorhodopsin plus 53.5% retinaloxime (final result of lumirhodopsin photoconversion to L′ experiment).

The specific percentages of rhodopsin and isorhodopsin in the initial mixture after illumination cannot be accurately determined from the absorption spectrum of the mixture because of the similarity of the spectra of the two species. However, their percentages can be calculated. The quantum efficiencies of the reaction are:

$$\text{rhodopsin} \underset{0.30}{\overset{0.67}{\rightleftharpoons}} \text{bathorhodopsin} \underset{0.14}{\overset{0.06}{\rightleftharpoons}} \text{isorhodopsin.}$$

The quantum efficiency of bathorhodopsin to isorhodopsin conversion was estimated (Mao et al., 1980) from the relative concentrations of isorhodopsin and bathorhodopsin in steady state mixtures (Oseroff and Callender, 1974), the absorption spectra of isorhodopsin (Mao et al., 1980) and bathorhodopsin (Yoshizawa and Wald, 1963), and the quantum efficiency of isorhodopsin conversion to bathorhodopsin (Hurley et al., 1977). The remaining quantum efficiencies were taken from Dartnall (1972), Collins et al. (1952), and Rosenfeld et al. (1977). By solving the set of simultaneous differential equations for the concentrations of the three species in the above reaction, it was numerically determined that on converting 100% rhodopsin to 55% bathorhodopsin at 77°K, ~2% isorhodopsin is also produced. Therefore, the initial mixture in the control experiment was 43% rhodopsin, 2% isorhodopsin, and 55% lumirhodopsin. Although the specific percentages of rhodopsin and isorhodopsin in the 45% mixture are not required for evidence of lumirhodopsin to L′ conversion (see below), they are required for the quantum efficiency estimation discussed in the next section.

In a second preliminary experiment (see Fig. 1), the mixture of 45% rhodopsin plus isorhodopsin and 55% retinaloxime from the first preliminary experiment was recooled to 77°K and exposed to that amount of 480-nm light which was previously determined to convert ~10% of a pure rhodopsin sample to bathorhodopsin under identical conditions. This small degree of illumination essentially prevents the pigments from absorbing more than one photon. Consequently, "back reactions" from any photoproducts formed during this irridation are not expected to occur to a significant degree. After warming to 293°K in the presence of hydroxylamine, the mixture contained 41% rhodopsin plus isorhodopsin and 59% retinal-oximine as expected (Fig. 5, curve 4).

In the first lumirhodopsin photoconversion experiment (Fig. 1), rhodopsin was first converted to a mixture of 45% rhodopsin and isorhodopsin, and 55% lumirhodopsin at 77°K (Fig. 4, curve 1) exactly as in the first preliminary experiment. The mixture was then exposed to the same amount of 480-nm light which converts ~10% of the rhodopsin sample to bathorhodopsin as in the second preliminary experiment. If lumirhodopsin (all-*trans*) were converted to bathorhodopsin (all-*trans*) by this illumination, it is clear that no change in the eventual retinaloxime (all-*trans*) concentration would result from this photoreaction. Nevertheless, the illumination would be expected to result in an overall increase of ~4% in the retinaloxime concentration due to the 10% conversion of the 43% rhodopsin as seen in the second preliminary experiment (Fig. 5, curve 4). However, when the resulting mixture (Fig. 4, curve 2) was warmed to 293°K in the presence of hydroxylamine, it contained 46.5% rhodopsin plus isorhodopsin and 53.5% retinaloxime—a *1.5% decrease* in retinaloxime (Fig. 5, curve 5). (In six experiments, the mean decrease was 1.5% ± 0.5% (mean + standard error of the mean]). This result demonstrates that lumirhodopsin (all-*trans*) is not directly photoconverted to bathorhodopsin (all-*trans*) (see Fig. 1). Rather, 5.5% lumirhodopsin must

be photoconverted to some species L′ (not all-*trans*) which, on warming, goes on to a stable species, probably rhodopsin or isorhodopsin. The specific reations involved are given below.[1]

$$43\%\ R + 2\%\ I + 55\%\ L \xrightarrow[77°K]{\text{“10\% h}\nu\text{”}} 39\%\ R + 2\%\ I + L + 4\%\ B + L'$$

$$\begin{matrix} 39\%\ R + 2\%\ I + L' \\ + \\ 4\%\ B + L \end{matrix} \xrightarrow[293°K]{NH_2OH} \begin{matrix} 46.5\%\ (R + I) \\ + \\ 53.5\%\ \text{retinaloxime} \\ \text{(from all-}trans\text{ retinal)} \end{matrix}$$

(As in the preliminary experiments, a set of six experiments gave a standard error of the mean of ±0.5% for the percentage concentrations of R + I and retinaloxime.)

It should be pointed out that in experiments in which the period of 480-nm illumination of the mixture was greatly increased, the concentration of rhodopsin plus isorhodopsin eventually decreased as expected (a photostationary state at 77°K with 476-nm light contains only ~37% rhodopsin plus isorhodopsin [Oseroff and Callender, 1974]). With respect to the identity of the L′ isomer, Maeda et al. (1978) found that quasi-photostationary states of rhodopsin at −75°C (a temperature at which lumirhodopsin is found) contain small amounts of 7-*cis* and 13-*cis* retinal. However, these experiments were performed under conditions (degree of illumination, temperature) considerably different than in the present study. In addition, warming the 5.5% L′ reported here results in a spectrum indistinguishable from that of rhodopsin or isorhodopsin whereas the 7-*cis* pigment, for example, has a maximum of 40–50 nm below these species (DeGrip et al., 1976). Therefore, it is most likely that 11-*cis* and/or 9-*cis* retinal constitute the major isomeric species in L′.

In summary, during the lumirhodopsin photoconversion sequence, rhodopsin must be photoconverted to some species (L′) at 77°K (see Fig. 1). On warming, L′ undergoes thermally induced protein changes resulting in rhodopsin and/or isorhodopsin (or to a species with a similar absorption spectrum). The chromophore of L′ is most likely the 11-*cis* and/or 9-*cis* isomer.

Estimation of the Quantum Efficiency of Lumirhodopsin to L′ Conversion

From the results and reactions given above, it was estimated that 5.5 ± 0.7% (1.5 ± 0.5% + 4.0 ± 0.5%) of the total pigment was converted to L′.

The following equation can be written for the (partial) photoconversion of the rhodopsin and lumirhodopsin in the first reaction:

$$\frac{\epsilon_L}{\epsilon_R} \times \frac{\gamma_{LL'}}{\gamma_{R\to B}} = \frac{\text{fraction of L converted to L′}}{\text{fraction of R converted to B}},$$

where ϵ_L/ϵ_R is the ratio of the extinction coefficients of lumirhodopsin and rhodopsin at 480 nm at 77°K which is equal to 1.35 ± 0.05 (calculated from Fig. 2, curve 1 and Fig. 4, curve 1). The fraction of L converted is (5.5 ± 0.7)/(55 ± 0.5), the fraction of R converted is (4.0 ± 0.5)/(43 ± 0.5), $\gamma_{R\to B}$ is the quantum efficiency of rhodopsin to bathorhodopsin conversion,

[1] < 0.04% isorhodopsin is converted to bathorhodopsin.

0.67, and $\gamma_{L \rightarrow L'}$ is the quantum efficiency of lumirhodopsin to L′ conversion at 77°K with 480-nm light. By solving the above equation it was estimated that $\gamma_{L \rightarrow L'} = 0.5 \pm 0.1$.

In conclusion, the results presented here demonstrate that lumirhodopsin (all-*trans*) is not directly photoconverted to bathorhodopsin (all-*trans*) at 77°K but rather to an intermediate L′ (probably 11-*cis* and/or 9-*cis*). L′, in turn, can be photoconverted to bathorhodopsin. (L′ could be rhodopsin and/or isorhodopsin or a precursor to these species.) In addition, the quantum efficiency of lumirhodopsin to L′ conversion (a *trans* to *cis* isomerization) is estimated to be 0.5 ± 0.1. This is in contrast to bathorhodopsin (all-*trans*) to rhodopsin (11-*cis*) or isorhodopsin (9-*cis*) conversions which have quantum efficiencies of 0.30 and 0.06, respectively. This indicates that the quantum efficiencies of the visual pigment and its intermediates are not simply a function of the isomeric state of retinal but are very much a function of opsin conformation and interaction with the chromophore.

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