

BRIEF COMMUNICATIONS

ON THE PRIMARY QUANTUM YIELDS IN THE BACTERIORHODOPSIN PHOTOCYCLE

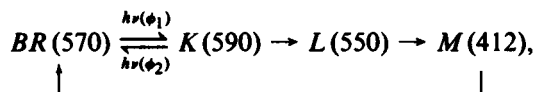
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ABSTRACT Pulsed Nd laser experiments in suspensions of the purple membrane of *Halobacterium halobium* are carried out at room temperature. At sufficiently high laser intensities, a photostationary mixture of bacteriorhodopsin (*BR*) and its red-shifted (batho) photoproduct (*K*) is obtained. The spectra of the first three intermediates in the photocycle are reported. The data yield a value of $\phi_1/\phi_2 = 0.40 \pm 0.05$ for the ratio of the quantum yields of the forward (ϕ_1) and reverse (ϕ_2) processes, setting an upper limit of ~ 0.4 for the quantum efficiency of the cycle at room temperature. This method is generally available for the determination of ϕ_2 in the case of a

photoequilibrium: $A \xrightleftharpoons[h\nu(\phi_2)]{h\nu(\phi_1)} B$, where *B* is a short-lived transient and ϕ_1 is known from low intensity measurements. Its potential application is of importance for the study of the photophysics of visual pigments at physiological temperatures.

Photochemical processes following optical excitation of bacteriorhodopsin (*BR*) in the purple membrane of *Halobacterium halobium* have been studied in fluid and rigid solutions over a wide temperature range (77–333°K), by applying continuous and pulsed (flash or laser) excitation methods (1–3).¹ The light and dark reactions in this (light-adapted) system are represented by the scheme:



where the numbers refer to the absorption maxima (nanometers) of the various intermediates. In analogy to visual pigments, the primary step is photoreversible, and in

¹Sherman, W. V., R. Korenstein, and S. R. Caplan. 1976. Energetics and chronology of phototransients in the light response of the purple membrane of *Halobacterium halobium*. Submitted for publication.

both cases the knowledge of the quantum yields in the two directions (ϕ_1 and ϕ_2) contributes substantially to the understanding of the photophysics of the primary event.²

In low temperature rigid matrices, where K is indefinitely stable, relative quantum yields (i.e., ϕ_1/ϕ_2) can be derived from the spectrum of the photostationary mixture obtained by continuous excitation with conventional light sources (4). However, in the present fluid solutions at room temperature, K (generated within less than 15 ps)³ decays with a half-life of $\sim 2 \mu\text{s}$ (1), and a photoequilibrium between BR and K is attainable only at very high excitation intensities. In the present communication we describe a pulsed laser photolysis experiment carried out under conditions allowing the establishment of a photostationary equilibrium between BR and K at room temperature, leading to the determination of the yield ratio ϕ_1/ϕ_2 . This method is of general importance and should be especially suitable in the case of visual pigments where ϕ_1 (i.e. the quantum yield for bleaching) is known, thus allowing the evaluation of ϕ_2 .

The laser flash photolysis system employed and the principles of high excitation intensity governing saturation effects have been described elsewhere (5). The TEM₀₀ output of a Q-switched Nd-glass laser, converted to a 530 nm harmonic pulse in a potassium dihydrogen phosphate (KDP) crystal, is colinearly correlated after proper filtration through Schott-Jena NG-4 filters, and slight convergence by a long focal lens ($\lambda = 530 \text{ nm}$, $\Delta t = 40 \text{ ns FWHM}$, $I = 1.5 \times 10^{17}$ to $6 \times 10^{18} \text{ photons} \cdot \text{cm}^{-2}$), with the monitoring light output of a pulsed xenon lamp, onto a 1.5 mm diameter aperture at the sample cell. Experiments were carried out with aqueous suspensions of light-adapted purple membrane fragments (*H. halobium*, M_1) with an initial concentration of $C_{BR}^0 = 8.9 \times 10^{-6}$ to $2 \times 10^{-6} \text{ M}$ ($N = 5.3 \times 10^{15}$ to $1.2 \times 10^{15} \text{ molecules} \cdot \text{cm}^{-3}$). At the laser wavelength the cross sections σ_1 and σ_2 of BR and K , respectively, are both of the order of $\sim 10^{-16} \text{ cm}^2$ ($\epsilon \sim 3 \times 10^4$) (1). For such high values and in view of the high intensities employed, we have $\sigma I \gg 1$ and $I \gg NI$, implying that every molecule along the $l = 1 \text{ cm}$ optical path is excited several times. For a photoequilibrium to be attained, the cross sections and the quantum yields of both BR and K should also be considered. Since a fraction $(1 - \phi)$ of the molecules return to their initial state after excitation, a photostationary mixture of BR and K will be obtained provided that $(\sigma_1\phi_1 + \sigma_2\phi_2)I \gg 1$. Taking, for example, a cross section of 10^{-16} cm^2 and a quantum yield of $\phi = 0.1$, the photostationary conditions will be satisfied around $I = 5 \times 10^{17} \text{ photons} \cdot \text{cm}^{-2}$. At present, no accurate dynamical actinometry of the 530 nm pulse has yet been performed. However, from Fig. 1 it is clearly seen that above 5 mJ ($7.5 \times 10^{17} \text{ photons} \cdot \text{cm}^{-2}$, estimated at the aperture of the sample cell) a photoequilibrium is actually attained.

The saturation value of the light-induced absorbance change in the above system should be considered in terms of the expression:

²Rosenfeld, T., B. Honig, M. Ottolenghi, J. Hurley, and T. Ebrey. 1976. Cis-trans isomerization in the photochemistry of vision. Submitted for publication.

³Kaufmann, K. J., P. M. Rentzepis, W. Stoeckenius, and A. Lewis. 1976. Primary photochemical processes in bacteriorhodopsin. Submitted for publication.

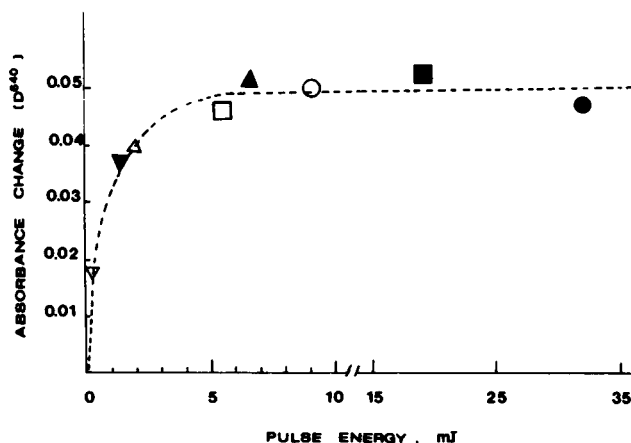


FIGURE 1 Dependence of the amount of *K*, present after laser excitation of light-adapted *BR* at 20°C, on the laser pulse intensity. D^{640} is the absorbance change at the 640 nm maximum of the *BR/K* difference spectrum (see Fig. 2A, I), measured 300 ns from the origin of the laser pulse. $[BR] = 5.7 \times 10^{-6}$ M, \circ , \square , Δ , ∇ , energy variations obtained by varying the laser amplification in the presence of a neutral density filter transmitting 18% of the laser light. \bullet , \blacksquare , \blacktriangle , \blacktriangledown , points corresponding to laser amplifications identical to the above, with the 18% filter being replaced by one with 63% transmittance. (A behavior similar to that of D^{640} is also observed for the intensity dependence of the absorbance of *M* measured in the millisecond range).

$$\phi_1/\phi_2 = (\epsilon_K^{530}/\epsilon_{BR}^{530})(C_K/C_{BR}) = (\epsilon_K^{530}/\epsilon_{BR}^{530})(C_K/C_{BR}^0 - C_K),$$

where ϵ_{BR}^{530} , ϵ_K^{530} , C_K , and C_{BR} are the extinction coefficients (at the 530 nm excitation wavelength) and the photostationary concentrations of *BR* and *K*, correspondingly. It should be pointed out that the above expression is strictly valid only if the lifetime of any excited state intermediate between *BR* and *K* is very short compared with the length of the laser pulse. Since the generation of *K* occurs within a few picoseconds (footnote 3) this condition is fulfilled in the case of the present experiments. A more complicated situation may prevail when exciting with ultrashort picosecond pulses.

For the determination of ϕ_1/ϕ_2 we have evaluated ϵ_K^{530} and C_K from an analysis of the difference spectra shown in Fig. 2A, obtained under saturating conditions. Following the procedure of Lozier et al. (1) the spectra of the first three transients (*K*, *L*, *M*) were calculated from I, II, and III correspondingly, using the known spectrum of *BR* (Fig. 2B) and assuming that *M* contributes only negligibly to curve III in the range above 550 nm. This assumption is based on the observation that in this range the band shape of curve III is essentially identical to that corresponding to the absorption spectrum of *BR* (Fig. 2B). Except for a smaller relative intensity of the secondary (520 nm) band of *M*, the spectra are in close agreement with those reported by Lozier et al. at 1°C (1). It is thus implied that in curve III the bleaching at $\lambda > 550$ nm represents the amount of *BR* cycling, which is equal to C_K . The data in Fig. 2B indicate that $C_K/C_{BR}^0 = 0.37$, leading to the ratio $\phi_1/\phi_2 = 0.4$. The same result (i.e., $\phi_1/\phi_2 = 0.40 \pm 0.05$) was obtained for several values of C_{BR}^0 in the 2×10^{-6} to 9×10^{-6} M range. It

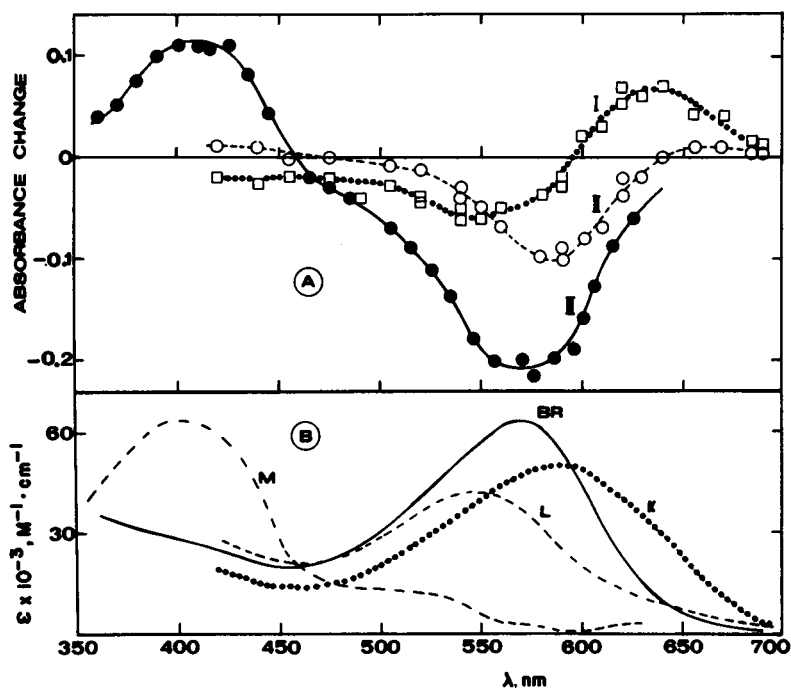


FIGURE 2 Difference and transient spectra in the laser photolysis of light-adapted bacteriorhodopsin at 20°C. $[BR] = 8.8 \times 10^{-6}$ M. The laser pulse energy employed was ~ 20 mJ (see Fig. 1). (A) Difference spectra recorded 300 ns (I), $10 \mu\text{s}$ (II), and 1 ms (III) after the pulse. The spectra are proportional (see text and ref. 1) to the extinction coefficient differences $\epsilon_K - \epsilon_{BR}$, $\epsilon_L - \epsilon_{BR}$, and $\epsilon_M - \epsilon_{BR}$, respectively. (B) Spectra of K, L, and M obtained from A(I, II, III) as described in the text, along with that of BR recorded on a Cary 14 spectrophotometer (Cary Instruments, Monrovia, Calif.). (Spectra are uncorrected for light scattering at their short wavelength side.)

should be recalled that the values of C_{BR}^0 , $\epsilon_K^{530} (= 3.2 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1})$ and $\epsilon_{BR}^{530} (= 4.6 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1})$ used in the above calculation, were estimated from the data of Fig. 2 which are uncorrected for a small amount of light scattering ($\sim 10\%$ at 530 nm). Since this introduces an error in ϕ_1/ϕ_2 which is within the limits of our experimental accuracy, no corrections for light scattering were made in the calculation of the yield ratio. Within the limits of our experimental accuracy we found this parameter to be unaffected (in a 1.1×10^{-6} M BR solution) by the presence of 4 M NaCl. Since $\phi_2 < 1$, this sets an upper limit of ~ 0.4 for ϕ_1 , a value which is lower than the early estimate of $\phi_1 = 0.79$ (6). It has recently been argued that for rhodopsin and its primary (batho) photoproduct (prelumirhodopsin) at 77°K, $\phi_1 + \phi_2 = 1$, implying that upon excitation, a common thermally relaxed excited state is populated in both cases.² Adopting this model for the pair BR and K, and using the value $\phi_1/\phi_2 = 0.4$, it would be predicted that $\phi_1 = 0.28$ and $\phi_2 = 0.72$. Work is in progress in this laboratory aiming to overcome the difficulties associated with a direct accurate determination of the absolute values of ϕ_1 and ϕ_2 .

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