BRIEF COMMUNICATIONS

THE QUANTUM EFFICIENCY OF THE BACTERIORHODOPSIN PHOTOCYCLE

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ABSTRACT The quantum yield of the primary photoprocess in light-adapted bacteriorhodopsin (ϕ_1) was determined at room temperature with low-intensity 530 nm neodymium laser excitation, with bovine rhodopsin as a relative actinometer. The observed value of $\phi_1 = 0.25 \pm 0.05$, and the previously determined parameter $\phi_1/\phi_2 = 0.4$ [where ϕ_2 denotes the quantum efficiency of the back photoprocess from the primary species K(590)] imply that $\phi_1 + \phi_2 \cong 1$. This feature, also characterizing the photochemistry of rhodopsin, bears on the nature and mechanism of the primary event in both systems.

INTRODUCTION

The reversible photocycle of bacteriorhodopsin (BR), the purple membrane pigment of *Halobacterium halobium*, has been extensively investigated with continous or pulsed light sources (1–4). It has been shown that the light and dark reactions in the (light-adapted) system are represented by the general scheme:

$$\mathrm{BR}(570) \underset{h\nu(\phi_2)}{\overset{h\nu(\phi_1)}{\rightleftharpoons}} \mathrm{K}(590) \rightarrow \mathrm{L}(550) \rightarrow \mathrm{M}(412) \rightarrow \mathrm{BR}(570)$$

where the numbers refer to the absorption maxima (in nanometers) of the various intermediates. In analogy to visual pigments, the primary step is photoreversible and in both cases the knowledge of the quantum yields in the two directions (ϕ_1 and ϕ_2) contributes substantially to the understanding of the primary event (5). Moreover, the BR system has been proposed as a light receptor for solar to electrical energy conversion (6). It is thus of primary importance to have a precise knowledge of both parameters, ϕ_1 and ϕ_2. In a laser-pumped quasi-stationary system we have previously measured (7) the quantum yield ratio $\phi_1/\phi_2 = 0.4$, setting an upper limit of 0.4 for ϕ_1, which corrects the early estimate of 0.79 (8).

In the present work, special efforts were devoted to carry out an accurate relative

actinometry of low-intensity 530 nm laser pulses (9).[1] This method, which utilizes bovine rhodopsin as a standard under conditions of negligible back photoreactions, leads to the determination of ϕ_1.

METHODS

The condition necessary to obtain a quasi-stationary equilibrium between the original molecule (A) and its primary photoproduct (B) in a laser-excited, photo-reversible system,

$$A \underset{h\nu(\phi_2)}{\overset{h\nu(\phi_1)}{\rightleftharpoons}} B,$$

is shown to be given by (7)

$$(\phi_1\sigma_1 + \phi_2\sigma_2)I > 1. \tag{1}$$

ϕ_1, ϕ_2, σ_1, and σ_2 (in square centimeters) are the corresponding quantum yields and cross-sections for absorption of A and B, and I[photons-per square centimeter] is the excitation light intensity, integrated over a period shorter than the lifetime of B. We have shown experimentally that this relation is attainable in several systems (7, 10, 11) excited by intense laser pulses (30 ns, 100 mJ·cm^{-2}).

In the present work, experiments were carried out under exactly opposite conditions, i.e. with ~0.5 mJ·cm^{-2} pulses, yielding:

$$(\phi_1\sigma_1 + \phi_2\sigma_2)I < 1. \tag{2}$$

In such a case, the concentration of transient B is relatively small so that a back (7) or side (11) photoreaction is negligible. At very low intensities we can therefore, assume a linear dependence of the concentration $[B]$ of photoproducts upon laser excitation intensity.[1] Thus for a weakly absorbing thin solution we have:

$$[B] = [A]\phi_1\sigma_1 I \tag{3}$$

from which ϕ_1 may be estimated by the relative (9) actinometric method.

It can be shown that a better approximation (not requiring an optical thin solution), obtained under conditions of almost total absorption of the weak laser pulse, is given by:

$$D_B = \tfrac{1}{2.3}\,\phi_1\sigma_2 I(1 - 10^{-D_0}) \tag{4}$$

where D_0 and D_B are the absorbance of the nonexcited A and of the transient B, respectively. This relation is applicable in the present colinear excitation setup, where no concentration gradient restriction is imposed on D_0, as to avoid poor spatial correlation between exciting and monitoring beam (12). Therefore, almost all the energy of the low-intensity laser output is absorbed in the solution according to the Beer-Lambert law of absorption, which is no longer valid at high excitation intensities.[2] For a weakly absorbing system ($D_0 \ll 1$) expression 4 reverts to the approximation 3.

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[1]Goldschmidt, C. R. Laser flash photolysis: a dynamical actinometer at high light intensity. In preparation.

[2]Goldschmidt, C. R. The Beer-Lambert Law of absorption at high light intensity. In preparation.

RESULTS AND DISCUSSION

Experiments in rhodopsin and light-adapted bacteriorhodopsin solutions prepared as previously described (13–15) were carried out with a 530 nm laser pulse attenuated so as to excite the sample with about 0.5 mJ·cm^{-2}, far below the value of ~100 mJ·cm^{-2} used in the previously described saturation experiments (7). To keep a good geometrical correlation between the colinear exciting and monitoring beams (12), only the central part of the TEMoo laser beam cross-section was used for excitation. The photochemical changes after excitation were determined by monitoring the bleaching of the main pigment bands at 530 nm at the relatively long times corresponding to the formation of M(412 nm) for bacteriorhodopsin and of metarhodopsin II (380 nm) for bovine rhodopsin. By introducing a time constant of ~0.5 ms in the detection system, absorbance measurements down to $D = 0.001$ could be made with a reasonable signal-to-noise ratio, with no appreciable bleaching of rhodopsin during the short exposures (10^{-2} s) to the monitoring light beam (11)[1] (see Fig. 1).

Applying relation 3 to both rhodopsin (R) and bacteriorhodopsin (BR) systems, we obtain:

$$\phi_1^{BR} = \phi_1^{RH} \frac{\Delta\sigma^{RH}}{\Delta\sigma^{BR}} \cdot \frac{\Delta D^{BR}_{(M412)}}{\Delta D^{RH}_{(M II)}} \cdot \frac{1 - 10^{-D_0^{RH}}}{1 - 10^{-D_0^{BR}}}$$

where ϕ_1 and D_0 are as previously defined, with the superscripts RH and BR denoting rhodopsin and bacteriorhodopsin, respectively. $\Delta D_{(M412)}$ and $\Delta D_{(M II)}$ denote the absorbance drop at 530 nm associated correspondingly with the generation of the M(412) BR phototransient and of metarhodopsin II. $\Delta\sigma^{BR}$ and $\Delta\sigma^{RH}$ are the corresponding differences between the cross-sections of BR and M(412) and between RH and M II at the monitoring 530 nm wavelength.

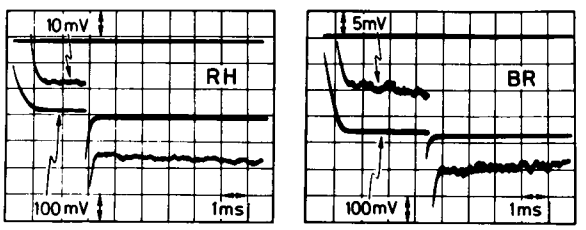

FIGURE 1 Characteristics oscillograms used for the determination of the absorbance changes at 530 nm associated with photoconversion of: bacteriorhodopsin (BR) to M(412) and bovine rhodopsin (RH) to metarhodopsin II. The upper trace in each oscillogram was recorded in the absence of the monitoring light beam (shutter closed). Both lower traces are in the presence of the monitoring source. The low-sensitivity trace (100 mV/div) was recorded under oscilloscope conditions identical to the upper one. In the high-sensitivity experiments (10 or 5 mV/div) the shutter-closed trace is out of scale. The break in the curves corresponds to the laser pulse. Absorbance changes were determined with the expression $\Delta D = \log [V_0/(V_0 + x)]$, where V_0 is the light-to-dark deflection before the pulse (250–360 mV) and x is the voltage difference before and after pulsing (15–30 mV) in the presence of the monitoring beam.

TABLE I

PRINCIPAL PARAMETERS IN THE DETERMINATION OF THE QUANTUM EFFICIENCY OF THE BACTERIORHODOPSIN PHOTOCYCLE AT ROOM TEMPERATURE. (SYMBOLS ARE DEFINED IN THE TEXT.) λ = 530 nm.

	ϵ_A	$\epsilon_{M\,II}$ or $\epsilon_{M(412)}$	$\Delta\sigma$	$\Delta D(M\,II)$ or ΔD(M412)	D_0	ϕ_1
	$M^{-1} \cdot lit^{-1} \cdot cm^{-1}$	$M^{-1} \cdot lit^{-1} \cdot cm^{-1}$	cm^2			
Rhodopsin	32,000 (ref. 17)	<1,000 (ref. 17)	1.20×10^{-16}	0.038*	0.92	0.67 (ref. 16)
Bacteriorhodopsin	47,000 (ref. 1)	15,000 (ref. 7)	1.22×10^{-16}	0.015*	1.12	0.25 ± 0.05

*Measured by pulsed laser photolysis (e.g. see Fig. 1).

Characteristic data, representative of eight sets of experiments, are shown in Fig. 1 and Table I, yielding the value of $\phi_1 = 0.25 \pm 0.05$.

It has recently been argued that for rhodopsin and its primary photoproduct (bathorhodopsin) $\phi_1 + \phi_2 \cong 1$, implying that a common, thermally relaxed, excited state is populated upon excitation of both species (5). By adapting this model for bacteriorhodopsin and its primary K(590) photoproduct, and using the previously determined ratio $\phi_1/\phi_2 = 0.4$, it would be predicted that $\phi_1 = 0.28$ ($\phi_2 = 0.72$), in close agreement with the presently determined value of $\phi_1 = 0.25 \pm 0.05$. The analogy between the photochemical behavior of rhodopsin and bacteriorhodopsin may be further extended in view of the recent observation of Becher and Ebrey (18), who at −40°C, reported the values $\phi_1 = 0.27 \pm 0.02$ (in close agreement with the present result), and $\phi_2^{M(412)} = 0.69 \pm 0.08$, with $\phi_2^{M(412)}$ referring to the M(412) $\xrightarrow{h\nu}$ BR back photoreaction. It therefore appears that ϕ_1 is temperature independent (5) and that the yield of the back photoprocess is essentially identical for different intermediates in the photocycle, as it is the case for bovine rhodopsin (up to the stage of metarhodopsin II). Such conclusions are in keeping with the suggestion (5) that isomerization (around a double bound) is associated with the primary event in both photocycles.

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