THE QUANTUM EFFICIENCY FOR THE PHOTOCHEMICAL CONVERSION OF THE PURPLE MEMBRANE PROTEIN

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ABSTRACT The quantum efficiency for the formation of M(412), an intermediate product in the photoconversion of the purple membrane protein of *Halobacterium halobium*, was determined to be 0.30 ± 0.03 at −40°C. This photochemical reaction was photoreversible to the original pigment and the ratio of the quantum efficiencies $\gamma_{PM(568) \rightarrow M(412)}/\gamma_{M(412) \rightarrow PM(568)}$ was 0.39 ± 0.02. No change was seen in either value when exciton interaction between chromophores was eliminated. The sum of $\gamma_{PM(568) \rightarrow M(412)}$ plus $\gamma_{M(412) \rightarrow PM(568)}$ was 1.07 ± 0.10, approximately 1, suggesting that the pigment and its primary photoproduct share a common excited state.

INTRODUCTION

Light can be utilized as an energy source by the pigment in the purple membrane of *Halobacterium halobium* (Oesterhelt and Stoeckenius, 1973). The chromophore of this pigment, the purple membrane protein (also called bacteriorhodopsin), is retinal (Oesterhelt and Stoeckenius, 1971). When a photon is absorbed by purple membrane in its light-adapted form (λ_{max} = 568 nm), a photochemical reaction cycle through a set of intermediates is initiated, ending with the pigment returning to its original form (Lozier et al., 1975). The first intermediate, the primary photochemical product, is called the bathoproduct; we will be particularly interested in an intermediate absorbing at ca. 412 nm, called the "M(412)" or "M" intermediate. Here we report on two fundamental photochemical properties of the purple membrane protein, its quantum efficiency to form the M(412) intermediate, and the quantum efficiency of the photoreversion of M(412) to the original pigment.

METHODS

Purple membrane was prepared as previously described (Becher and Cassim, 1975). Three types of purple membrane samples were used. In addition to the native purple membrane preparation, we bleached all the chromophores in the purple membrane protein and then added all-*trans* retinal either to regenerate the membrane entirely (100% regenerated purple membrane) or to regenerate only 15% of the original chromophore sites (15% regenerated purple membrane). We had previously shown (Becher and Ebrey, 1976) that in the native or 100% regenerated membrane there is an exciton interaction among a set of three chromophores that is absent in the 15% regenerated membrane, presumably because the chromophores occupying the sites are too far apart to interact.

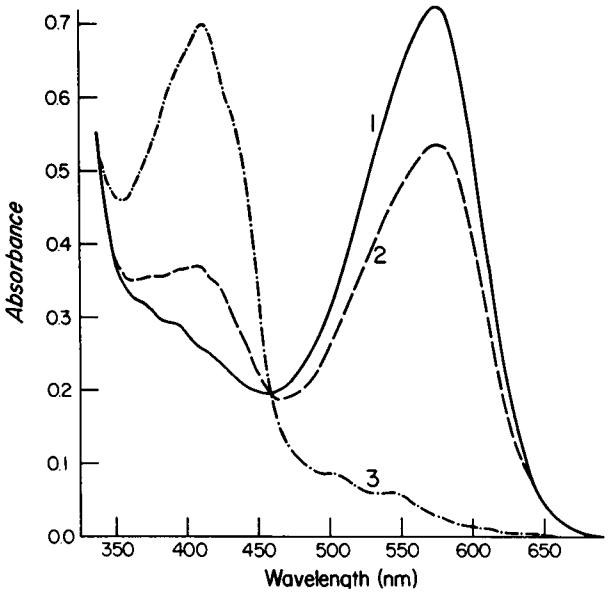

FIGURE 1 Photochemical conversion of PM(568). 1) Absorption spectrum at −40°C; 2) after irradiation at 460 nm until a photosteady state is achieved; 3) after irradiation at 580 nm converting all the PM(568) to M(412). The residual absorption is due to light scattering and carotenoid.

The principal problem in doing a simple quantum efficiency experiment like those performed on visual pigments is the need for a bleaching product that is stable and does not absorb appreciably at wavelengths where the initial pigment absorbs. Although M(412) fulfills the second requirement, it is a short-lived intermediate in the photoreaction cycle of purple membrane. Goldschmidt et al. (1976) have used a rapid kinetic technique to detect the transient disappearance of the pigment to the 412 intermediate. Oesterhelt and Hess (1973) have used the addition of ether, which somehow increases the lifetime of M(412). We have found a method to increase the lifetime of the 412 intermediate greatly without the use of ether. Purple membrane is suspended in a 25% NaCl solution adjusted to pH 10. Irradiation at 580 nm (interference filter, 12 nm bandwidth) of the purple membrane suspension results in the formation of the M(412) intermediate (Fig. 1). At room temperature M(412) eventually decays to the next intermediate, which absorbs in the visible, but at −40°C M(412) is stable in the dark. The M(412) intermediate converts back to the 568-nm form with 420 nm irradiation (interference filter, 12 nm bandwidth). Since 580 nm light is not absorbed by M(412), it can completely convert the 568 nm pigment to the 412 intermediate (Fig. 1).

To prevent crystalization at −40°C, the purple membrane samples (in 25% NaCl and at pH 10) were mixed with glycerol (1:2). The sample, in a stoppered 1-cm cuvette, was then lowered into a Dewar flask filled with acetone previously cooled with dry ice until the temperature was −40 ± 4°C. The Dewar flask had flat windows on four sides to allow uniform sample illumination and then accurate absorption measurements.

We first determined the ratio of the quantum efficiencies of the PM(568) → M(412) and M(412) → PM(568) reactions for purple membrane ($\gamma_{PM(568)}$ and $\gamma_{M(412)}$) by measuring the concentration of each species in a photosteady-state mixture obtained by irradiating at 460 nm (interference filter, 12 nm bandwidth). At this wavelength, the 568 nm and 412 nm forms

have the same extinction coefficient, as shown by the 460 nm isosbestic point (Fig. 1). Irradiating at the isosbestic point conveniently minimizes possible artifacts due to changing pigment absorbances and allows the extinctions of the two pigments to be cancelled in the photoequilibrium equation

$$\gamma_{PM(568)}\epsilon_{PM(568)}[PM(568)] = \gamma_{M(412)}\epsilon_{M(412)}[M(412)] \quad (1)$$

(see Kropf and Hubbard, 1958). It is easily seen that in a 460 nm photosteady state, the ratio of concentrations of M(412) to PM(568) is equal to the ratio of $\gamma_{PM(568)}$ to $\gamma_{M(412)}$. The relative concentrations of the 568 nm and 412 nm pigments in the photosteady state were directly determined by the relative decrease in the absorbance at 568 nm.

The values of $\gamma_{PM(568)}$ and $\gamma_{M(412)}$ were then determined by the technique of Dartnall et al. (1936). Their Eq. 10 can be expressed, in absorbance units, in the form: $-\ell n(10^{A_t-A_f}-1) = \phi[\epsilon(\lambda)\gamma I/a]t +$ constant, where I is the intensity of light incident on the solution (in quanta per second) (the incident light and measuring light must be the same wavelength), A_t is the absorbance of the solution at time t, A_f is the absorbance of the solution after complete bleaching, $\epsilon(\lambda)$ is the molar extinction coefficient at the wavelength of the illuminating light, γ is the quantum efficiency, t is the time from the initial exposure of the solution to light, a is the exposed area of the solution, and ϕ is a correction factor (see Dartnall et al., 1936) due to the presence of "inert", nonphotolabile absorbing substances other than rhodopsin or purple membrane: $\phi = (1-10^{-A_t+A_f})^{-1}(1-10^{-A_t})[(A_t-A_f)/A_t]$. (Although light scattering is also detected as a residual nonphotolabile optical density, it cannot be treated by using the correction factor ϕ. Therefore, light scattering was kept to a minimum by matching the index of refraction of the membrane to that of the suspending medium, using low sample concentrations, and irradiating at longer, less scattering wavelengths.)

By plotting $\ell n(10^{A_t-A_f}-1)$ versus time, a straight line is obtained with slope

$$m = [\phi\epsilon(\lambda)\gamma I]/a, \quad (2)$$

the rate of bleaching. To determine the photosensitivity, $\epsilon_{PM}(\lambda)\gamma_{PM(568)}$, the absolute light intensity is determined by measuring, at room temperature, the slope for bovine rhodopsin (m_{Rh}). Then from Eq. 2:

$$\gamma_{PM(568)} = \frac{m_{PM}}{m_{Rh}}\,\frac{\epsilon_{Rh}}{\epsilon_{PM}}\,\frac{\phi_{Rh}}{\phi_{PM}}\,\gamma_{Rh} \quad (3)$$

Since γ_{Rh}, $\epsilon_{Rh}(\lambda)$, and $\epsilon_{PM}(\lambda)$ are known and ϕ_{Rh} and ϕ_{PM} are easily calculated, then by determining the slopes m_{PM} and m_{Rh}, γ_{PM} can be determined.

Samples of bovine rhodopsin, native, and 100% regenerated purple membrane were irradiated with either 540 or 580 nm light for 30–60s intervals, photoconverting 1–2% of the samples with each exposure to a product which did not absorb at the λ_{max} of the pigments. The absorbances of the samples were recorded and after 10% of the samples were converted, the samples were then totally bleached to obtain the nonphotolabile absorbance.

The values of ϕ_{PM} and ϕ_{Rh} were not constant during an experiment but the value changes were less than 1% and so the mean values of ϕ were used as constants in the calculations.

Because glycerol:water is still fluid at −40°C (Strackee, 1971), no polarization artifacts in the measurements were expected (Ebrey and Yoshizawa, 1973). Their absence was confirmed by our finding no change in the quantum efficiency after changing the direction of the actinic light by 90°, so that it was parallel to the measuring light.

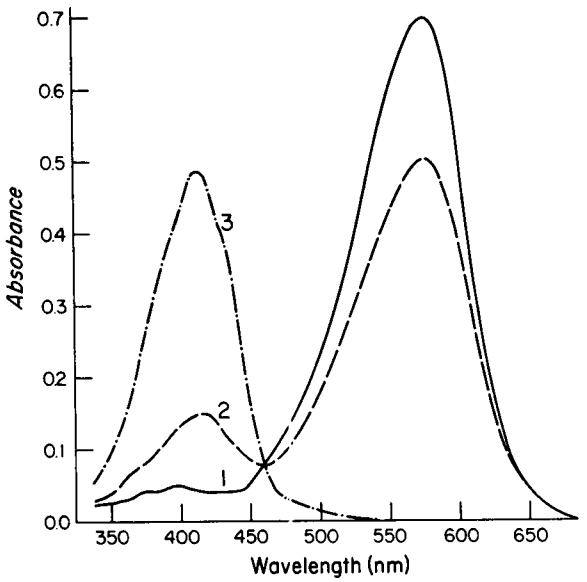

FIGURE 2 Corrected absorption spectra of the photochemical conversion of PM(568) at −40°C. A base line having no free or bound chromophore absorption from 320 nm to 750 nm was obtained by totally bleaching a purple membrane sample with white light in hydroxylamine. (The retinaloxime initially formed on bleaching is destroyed by the white light.) This base line, when subtracted from the three spectra in Fig. 1, gives curves 1–3.

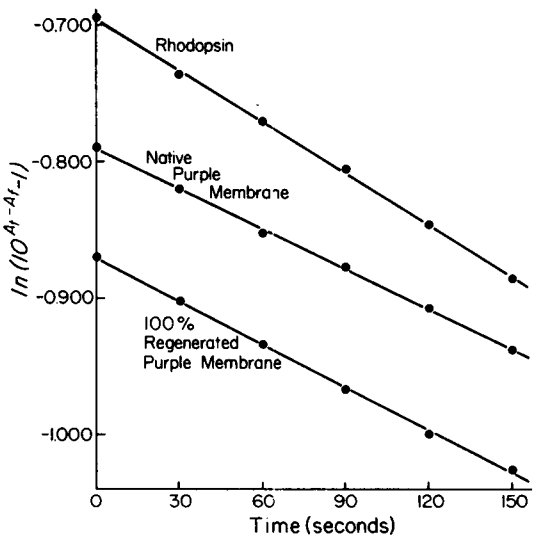

FIGURE 3 The relative photosensitivities of rhodopsin, native purple membrane, and 100% regenerated purple membrane. See text for details.

RESULTS

Fig. 1 shows the absorption spectra of 100% regenerated purple membrane in the 568 nm form (curve 1), after attaining a photosteady state with 460 nm light (curve 2), and after complete conversion to the 412 nm form with red light (curve 3). The corrected absorption spectra are shown in Fig. 2. The ratio $\gamma_{PM(568)}/\gamma_{PM(412)}$ is determined from the decrease in the 568 nm band in this photosteady state. In 12 different experiments the average decrease was 28 ± 1%. From Eq. 1, this gives $\gamma_{PM(568)}/\gamma_{PM(412)} = 0.28/0.72 = 0.39 \pm 0.02$.

A sample determination of the photosensitivity, $\gamma_{PM(568)}$, for both the native purple membrane and 100% regenerated membrane compared to that of rhodopsin is shown in Fig. 3. The values of the slopes are m(100% regenerated membrane) = $1.04 \times 10^{-3}\ s^{-1}$, m(native membrane) = $0.99 \times 10^{-3}\ s^{-1}$, and m(rhodopsin) = $1.27 \times 10^{-3}\ s^{-1}$. The values of the corresponding correction factors are ϕ(100% regenerated membrane) = 0.94, ϕ(native membrane) = 0.95, and ϕ(rhodopsin) = 0.98. Substituting these values into Eq. 3 with ϵ_{Rh} = 22,000 and $\epsilon_{100\%\ PM} = \epsilon_{native\ PM}$ = 42,000 $cm^{-1}\ M^{-1}$ at 540 nm [based on the values of ϵ_{Rh} = 40,000 (Crouch et al., 1975) and ϵ_{PM} = 55,000 ± 2,000 $cm^{-1}\ M^{-1}$ (Powers, Becher, and Ebrey, unpublished observations) at their absorption maxima], then $\gamma_{PM(568)}/\gamma_{Rh}$ can be calculated (exp. 4, Table I). These values for nine different experiments are given in Table I and the average values are 0.43 ± 0.02 for the 100% regenerated and the native purple membrane samples (Table I). Based on a quantum efficiency of 0.70 ± 0.02 for bovine rhodopsin in 2% Ammonyx LO (Hurley and Ebrey, unpublished), $\gamma_{PM(568)}$ = 0.30 ± 0.03. The efficiency was the same with either 540 or 580 nm light.

Because the 15% regenerated samples are turbid, light scattering at the 540 and 580 nm irradiating wavelengths leads to erroneous values of the Dartnall correction factor ϕ. These complications were avoided by reducing sample concentration and by

TABLE I

QUANTUM EFFICIENCY FOR THE PM(568) → M(412) REACTION RELATIVE TO THAT FOR BLEACHING RHODOPSIN

Experiment	Illuminating wavelength	$\gamma_{PM(568)}/\gamma_{Rh}$	
		Native PM	100% regenerated PM
	nm		
1	540	0.41	—
2	540	0.46	—
3	540	0.39	—
4	540	0.42	0.44
5	540	0.47	0.46
6	540	0.45	0.40
7	580	0.41	0.43
8	580	0.47	0.47
9	580	0.43	0.40

irradiating at the less scattered 620 nm wavelength. Since rhodopsin cannot be reliably used as a quantum counter at this wavelength, we directly compared the rate of bleaching (photosensitivity) of the 15% regenerated samples to that of the 100% regenerated samples. [The extinction coefficients of the two samples are identical at 620 nm (Becher and Ebrey, unpublished.)] We determined that the photosensitivity and quantum efficiency of the 15% regenerated sample is 0.99 ± 0.05 times that of the 100% regenerated membrane. In a separate experiment like that shown in Fig. 1, we found that $\gamma_{PM(568)}/\gamma_{M(412)}$ was the same for the 15% regenerated membrane as the 100% regenerated and native purple membrane. These results indicate that all three preparations have the same average value of $\gamma_{PM(568)}$.

DISCUSSION

We have found conditions in which purple membrane can be completely converted by light to an intermediate, M(412), which has little absorption at the λ_{max} of the original pigment. Using this property, we determined that the wavelength-independent absolute quantum efficiency for forming M(412) at −40°C is 0.30 ± 0.03. Oesterhelt and Hess (1973) reported a value of 0.79; there is no obvious reason for the discrepancy except that perhaps the ether present in their purple membrane preparations raised the quantum efficiency. We have also found that the M(412) intermediate is photoreversible to the original pigment. We have measured the ratio of the quantum efficiencies for the forward-to-back reactions, $\gamma_{PM(568)}/\gamma_{M(412)} = 0.39 \pm 0.02$. Goldschmidt et al. (1976) have found a similar value of 0.40 ± 0.05 for $\gamma_{PM(568)}/\gamma_{Batho}$ where γ_{Batho} represents the quantum efficiency for the back reaction of the bathoproduct. With our value of $\gamma_{PM(568)}/\gamma_{M(412)}$, $\gamma_{M(412)} = \gamma_{Batho} = 0.77 \pm 0.08$. Therefore, the sum of the quantum efficiencies for the forward and back reactions is $\gamma_{PM(568)} + \gamma_{Batho} = \gamma_{PM(568)} + \gamma_{M(412)} = 1.07 \pm 0.10$. Rosenfeld et al. (1976) have presented a detailed argument that a value near 1 for the sum of the quantum efficiencies of the forward and back reactions strongly suggests that the pigment and its primary batho photoproduct share a common excited state. In that paper it was proposed that rhodopsin and its primary photoproduct, bathorhodopsin, share such a common excited state; the results presented here suggest that this is also true for the purple membrane protein and its bathoproduct and that the role of the protein in modifying the excited states of the retinal bound to these two pigments may be quite similar. Rosenfeld et al. (1976) already have suggested that the primary photochemistry (a geometrical change in the chromophore) is the same for both pigments. The nonequivalence of the fluorescence from the pigment and its bathoproduct (Alfano et al., 1976) suggests that the pigment's very weak fluorescence is not coming from the thermalized common excited state proposed here.

We also measured the quantum efficiencies of the forward and back reaction for the 15% regenerated membrane, in which exciton interaction between the chromophores is essentially eliminated and found no difference from the native membrane. Previously, Alfano et al. (1976) had seen no variation in the lifetime of the fluorescence

from interacting and noninteracting chromophores. Therefore, there seem to be no discernible effects on the photochemistry due to the exciton interaction of the purple membrane protein.

The temperature dependence of the photochemical reactions of purple membrane is of special interest and will be the subject of another publication.

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