Volume 263, number 2, 269–273 FEBS 08347 April 1990

# The quantum yield of bacteriorhodopsin

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Received 6 February 1990

The conflicting reports on the quantum yield of the primary reaction in the photocycle of bacteriorhodopsin led us to reinvestigate the quantum yield under various experimental conditions. Quantitation of the molecules in the photocycle was done by measuring the number of molecules being in the M intermediate state 1 ms after laser flash excitation via determination of the absorption change at 602 nm. It was shown that this procedure provides to the lower limit of the quantum yield. A value of 0.64 ± 0.04 at pH 7.0 and room temperature (19°C) was obtained, independent from the wavelength of excitation in the range 500–600 nm and the ionic strength between 1 mM and 2 M salt.

Bacteriorhodopsin; Quantum yield; Photocycle

## 1. INTRODUCTION

Bacteriorhodopsin (BR) is the only protein found in the purple membrane (PM) of halobacterial cells (for reviews see [1,2]). With retinal as a chromophoric group, this protein, upon illumination, transports protons across the plasma membrane, thereby creating an electrochemical proton gradient which is used for ATP production. The retinal is linked via a Schiff base to lysine-216 of the protein and appears in its light-adapted form as nearly 100% in the all-*trans* conformation [3]. Upon capture of a photon, retinal is isomerized around the 13,14 double bond and thermally reverts to its initial all-*trans* state by passing through several spectroscopically distinct states. This series of reactions is called photocycle and coupled to its occurrence a proton is transferred across the membrane.

A generally accepted scheme for the photocycle at pH 7.0 and room temperature is the linear sequence:

$$BR \longrightarrow J \longrightarrow K \longrightarrow L \longrightarrow M \longrightarrow N \longrightarrow O \longrightarrow BR$$

Still under discussion are branched pathways occurring under certain experimental conditions such as different pH, steady-state illumination, changing membrane potential, etc. [4].

Following the first report on the quantum efficiency of the photoreaction of BR, numerous conflicting reports on the quantum yield appeared in the literature. Oesterhelt and Hess [5] initially reported a value of 0.79 for the quantum efficiency of formation of the M intermediate in a basal salt/ether suspension of PMs under stationary illumination using an Ulbricht sphere to eliminate the large light scattering in the basal salt/ether system. This value was redetermined a couple of years later with a different experimental setup [6]; due to the light scattering a lower limit of 0.6 was found. Several research groups determined the quantum efficiency for the step BR ⟶ K at −196°C as 0.28 [7], 0.5 [8] and 0.33 [9]. For measurements of the quantum efficiency of M formation, the temperature was kept at −30°C and values of 0.30 [10] and 0.25 (measured at 530 nm 1 ms after a flash [11]) were found for M formation but 0.43 for proton release [12]. In 1980 Bogomolni et al. stated that no difference in the quantum yield of K formation in basal salt/ether and distilled water was observed [13]. This result was confirmed by measuring the yield of the primary reaction of K formation under various conditions [14]. A value of >0.6 was determined again for M formation in basal salt/ether [6] and this value was confirmed by Kouyama et al. [15] using the same system. Using resonance Raman scattering experiments as an alternative method to measure the quantum yield a value of 0.67 [16] was determined. The molar extinction coefficients of BR and K and the shape of their spectra are only compatible with a quantum yield of >0.6, as requested by semi-empirical quantum mechanical calculations of spectral properties of retinals [17]. Semiempirical molecular dynamic calculation yielded a value of 0.27 for the quantum yield of the BR ⟶ K reaction [18] if the counterion of the Schiff base was fairly close, whereas this value increased to 0.74 if the counterion was further away. This was interpreted to indicate the occurrence of two different states of BR. In a very detailed analysis the value of 0.32 for the quantum yield at room temperature was confirmed [19].

In this report we have determined the quantum yield measured at 602 nm about one ms after the excitation

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*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/90/$3.50 

flash from the depletion of BR from its initial state. All measurements were done at room temperature and the photoreaction was quantitated as BR molecules in the M state. Systematic variation of experimental conditions verified the determined value of 0.64 ± 0.04 as a minimal value of the quantum yield.

## 2. MATERIALS AND METHODS

PMs were isolated by standard methods [20] and stored at −20°C. Freshly thawed samples were used after short sonication (Bransonic type 12, ~1 min) in order to disrupt aggregates of PM patches. Light adaptation of BR was ensured by illumination of the sample for 10 min with white light at an intensity of 10 $mW \cdot cm^{-2}$ before the measurements. All measurements were carried out at room temperature (~19°C).

### 2.1. *Optical setup*

The optical setup used is shown in Fig. 1. Measuring light from a 50 W halogen lamp was filtered through a glass filter (OG515 Schott, Mainz, FRG) and reached the sample through a variable slit of 3 × 8 mm. Irradiance at the place of the sample was measured with an optometer (40 × United Detector Technology Inc., Madison). The value of 150 $\mu W/cm^{-2}$ was low enough so that photostationary accumulation of intermediates M or N could be neglected. The sample cuvette had the inner dimensions of 10 × 10 × 30 mm and was filled with 3 ml PM suspension. Transmitted light was measured with a pbJ 22 photomultiplier (Maurer, Nürtingen, FRG) which was protected with an interference filter (maximal transmittance at 602 nm, halfwidth 8 nm, Schott Mainz, FRG). The increase of transmitted light about 1 ms after a flash was due to the molecules in intermediate states of the photocycle which absorb differently from the initial state. None of the early intermediates J, K or L existed 1 ms after a flash. The remaining intermediates were M, N and O. Fig. 2 shows by the time-dependent changes in absorption at selected wavelengths 570, 660 and 410 nm. The N intermediate does not accumulate to a measurable extent because the recovery of the absorbance at 570 nm coincides with the decay of 660 nm absorbance. Furthermore, the half time of absorbance increase at 660 nm is the same as that of the decrease at 410 nm. The experiment of Fig. 2 was carried out in a grating spectrophotometer dispersing the light which has passed the sample. A detailed description of the apparatus is given in [21].

The selected measuring wavelength of 602 nm is at the isosbestic point of BR and O spectra. Thus the O intermediate would not contribute to the determination of the number of BR molecules which have left the initial state of BR after the flash. These are found, however, at the selected time windows exclusively in the M state because in Fig. 2 the ratio of absorbance at 570 and 660 nm of 13.5 is indicative of the absence of the O intermediate. In addition, any residual amount of intermediates other than M would result in an underestimation of the quantum yield.

The beam of a dye laser (Lambda Physics, FL 3001, pulse duration 16 ns) was focused 5 mm before the entrance of a light pipe (8 mm diameter) to achieve a homogeneous output light at the exit of the light pipe. Homogeneity was checked after appropriate attenuation by eye and on polaroid film (data not shown). The end of the light pipe was rectangular (25 × 2 mm) so that all laser light reached the sample. Because of the use of a multimode light-guide no special care was necessary for polarized actinic light.

The possible influence of a gradient of the actinic light in the suspension was tested by opening the slit at a given PM concentration.

Only at higher concentrations (>1 OD) this gradient caused measurable decrease of transmission changes when the slit was fully opened (8 mm).

The flash-induced BR-depletion was calculated according to

$$\Delta A = \epsilon_{602} \cdot \Delta C \cdot d$$

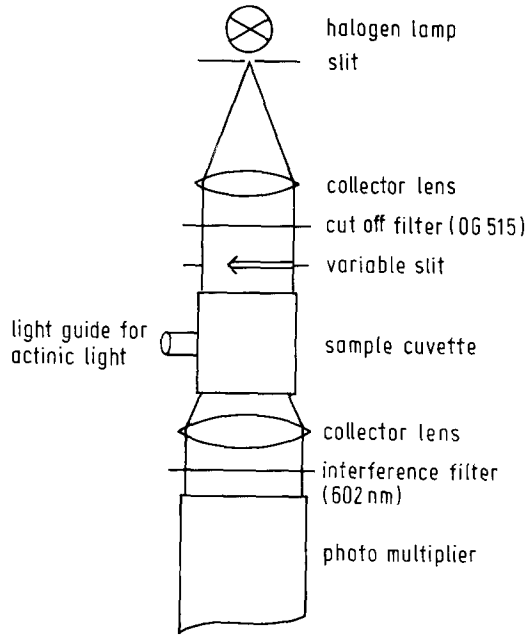


Fig. 1. Optical setup for the quantum yield measurements.

with $\epsilon_{602}$ of the initial state = 43000 $M^{-1} \cdot cm^{-1}$ and a pathlength $d$ = 1 cm. Absorbance change $\Delta A$ was calculated from the measured transmission change according to

$$\Delta A = {}_{10}\log(1 + \Delta T/T)$$

and for small $\Delta T/T$ this converts to

$$= 0.43 \cdot \Delta T/T$$

using the serial development of the above equation. $T$ is the transmission of the sample measured in a conventional spectrophotometer (Aminco DW2). The conversion $\Delta T/T$ was maximally 8% so that the latter formula could be used.

To investigate the effect of light scattering, experiments at different sample concentrations were performed and will be shown and discussed in the next section.

Incident laser light energy was measured with two independent methods. First, a calibrated thermophile detector was used to measure the energy at the end of the light guide and due to the highly

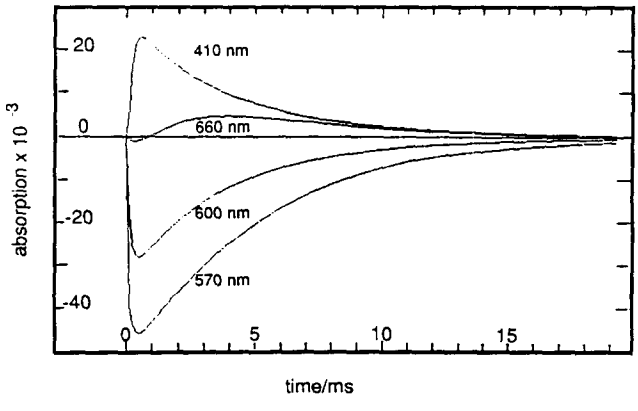


Fig. 2. Time dependent absorbance changes of BR after flash excitation. 27 $\mu$M of BR in 10 mM Tris pH 7.0 with a transmission of 0.23 at an optical pathlength of 4 mm were excited at 580 nm with a power of 48 $\mu$J. Absorbance changes at indicated wavelengths are shown.

monochromatic light ($\Delta\lambda$ = 0.08 nm) the number of photons could be calculated according to Planck's formula. The other method was based on an irreversible photoreaction with an actinometer solution, Actinochrome N (PTI, Tornesch, FRG), absorbing in the appropriate region (475–610 nm). Differences of not more than 10% were found between the two methods and, thus, the thermophile detector was used throughout the measurements. To account for instabilities in the laser output, averages of 32 or 64 flashes were taken. The number of absorbed quanta was calculated from the number of incident quanta and the transmission of the sample at the given wavelength. Three laser dyes were used to cover the wavelength range of 500–600 nm of the actinic light (Coumarine 334, Coumarine 153 and Rhodamine 6G, Lambda Physics, Göttingen, FRG).

## 3. RESULTS AND DISCUSSION

### 3.1. *Intensity of the actinic light*

The linearity range of the photoreaction was checked by variation of the laser flash intensity at 520 nm. The maximal output energy was 4 mJ and neutral glass filters were used to dim the actinic light flashes. As can be seen from Fig. 3, product formation upon flashing was linear with flash intensity for all conditions and maximally 8% of the BR molecules were excited. These conditions ruled out the possibility of appreciable photon absorption by a photocycle intermediate existing in the time range of the laser pulse duration because maximally 8% conversion of that intermediate would occur again assuming the same quantum yield and extinction at 520 nm. This would lead to a contribution of maximally 0.64% to the overall process and therefore would not contribute significantly to the transmission change measurements.

### 3.2. *Concentration dependence*

Determination of the quantum yield at different concentrations of purple membranes in the sample could yield information about the effect of light scattering by the purple membranes and its contribution to transmission changes. In Fig. 4 the results of measurements in the concentration range of OD 0.1–2 at 568 nm are shown. In this experiment the pathlength was set to 4 mm and a number of 0.64 ± 0.04 from 13 independent measurements is obtained. Taking a pathlength of 8 mm, decreasing values above the concentration of OD 1 were observed, indicating the effect of light scattering of the actinic light.

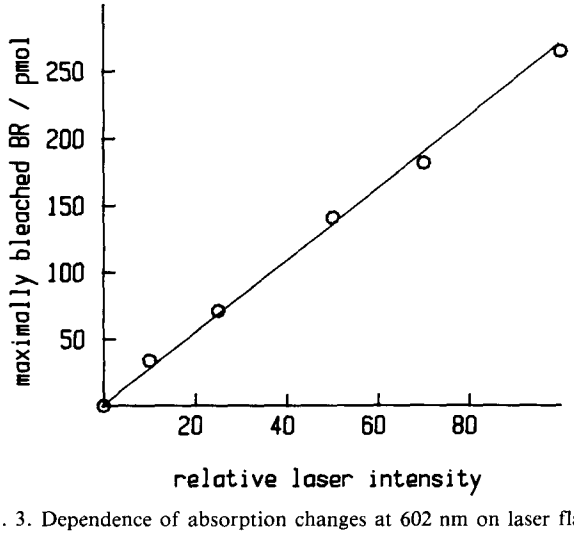


Fig. 3. Dependence of absorption changes at 602 nm on laser flash intensity. 8 $\mu$M of PM suspended in 10 mM sodium phosphate (NaPi) pH 7.0 were illuminated at 520 nm. 100% laser intensity corresponds to 4 mJ.

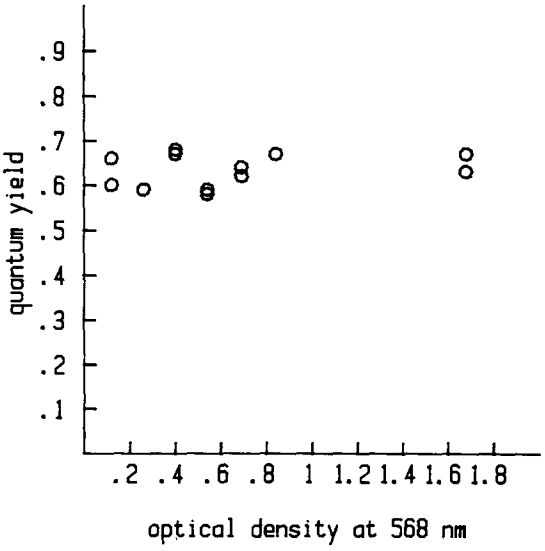


Fig. 4. Dependence of the measured quantum yield on BR concentration. Purple membranes were suspended in 10 mM NaPi pH 7.0 and excited at 520 nm.

### 3.3. *Wavelength dependence*

The quantum yield of a single photon reaction of a given chromophore is expected to be independent of the wavelength of the actinic light. If deviations from this independence are observed, a two photon event involving excited states or intermediates photochemically active during the flash or photoselectivity of the chromophore have to be invoked. To exclude these

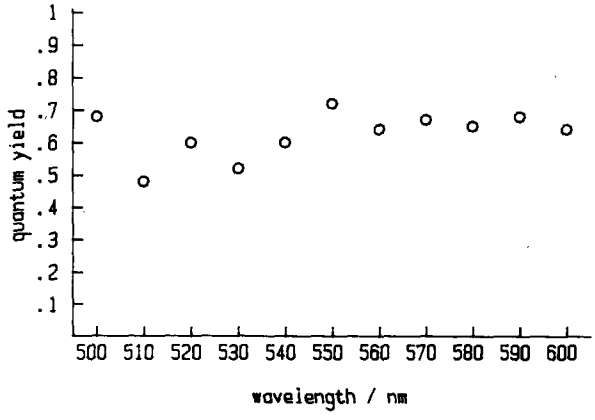


Fig. 5. Wavelength dependence of the quantum yield. PMs at 6.4 $\mu$M concentration of BR in 10 mM Tris pH 7.0 were used.

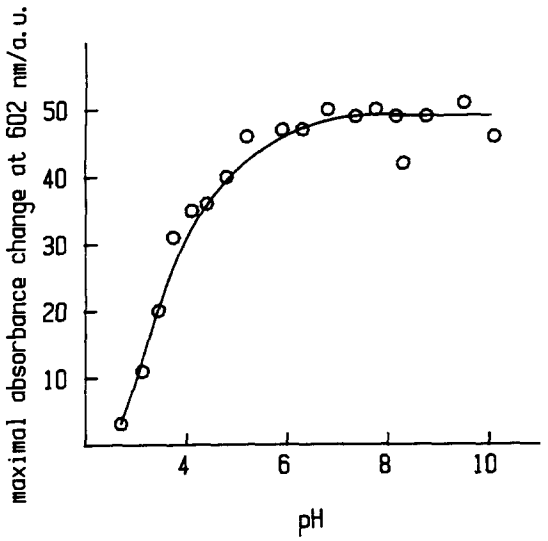


Fig. 6. pH dependence of the quantum yield. pH was adjusted by adding HCl or NaOH from 0.1 N stock solutions. The surface of the sample was continuously flushed with nitrogen to avoid pH changes due to dissolution of atmospheric $CO_2$.

possibilities the wavelength dependence of the quantum yield of BR was investigated. Fig. 5 shows that variation of the wavelength between 500 and 600 nm had no effect on the quantum yield indicating that the experimental conditions were suited for measuring correctly the quantum yield of the primary reaction of BR. This wavelength independence was also reported earlier [10,16].

### 3.4. *Effect of ionic strength*

Measurements of the quantum yield of proton release by BR revealed a dependence on ionic strength [12]. In order to discriminate between the possibilities of altered primary photochemistry of the chromophore or altered protein properties by changing membrane surface potential, we investigated the effect of increasing the concentration of salt from 0 to 4 M. Within the accuracy of the measurements, no effect of salt concentration changes on the quantum yield could be detected.

### 3.5. *pH dependence*

BR equilibrates with different isoforms at different pH values [22,23]. Below pH 2.7 an acidic form absorbing maximally at 605 nm becomes dominant. The photocycle of this form is characterized essentially by the lack of an M species [24]. On the other hand, at basic pH (>8.5) one intermediate of the photocycle, N, becomes predominant. This intermediate is also photochemically active and has a quantum yield similar to that of BR itself [15]. A pH dependence of the quantum yield measurement was carried out to address the question whether the different forms have different photochemical behaviours. As seen in Fig. 6, the absorption change at 602 nm is independent of pH in the range pH 5–11, whereas at pH values below 5 a decrease was observed. This is mainly due to the fact that no M intermediate exists in the photocycle at these pH values, so the amplitude of absorbance change at 602 nm decreases. Thus, we interpret the observed pH dependence to be mainly due to the different photocycles of these isoforms and not as an altered photochemical behaviour affecting the quantum yield of the primary step in BR.

## 4. CONCLUSIONS

In summary, we have shown that the quantum yield of BR in water at neutral pH and room temperature is 0.64 ± 0.04. This value is close to the one found in the basal salt/ether system and therefore rules out selection of a different photocycle with a higher quantum yield under the basal salt/ether condition. Determinations of significant lower values of the quantum yield rely on photostationary mixtures at low temperatures (−196°C for BR-K and −30°C for BR-M) and the question arises whether these experimental conditions affect the primary photochemistry of the molecule more than assumed.

*Acknowledgements:* We gratefully acknowledge the help of Dr Lilly Maglova in an early stage of the work. Very helpful and intense discussions with Drs H.W. Trissl, W. Zinth and P. Hegemann improved the work in progress of more than two years. The permanent help and patience of Dr Judy Shiozawa in improving the English style is also gratefully acknowledged.

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