THE PHOTOCHEMICAL QUANTUM YIELD OF BACTERIORHODOPSIN IS pH INDEPENDENT

A photoacoustic study

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1. Introduction

Bacteriorhodopsin, the pigment of the purple membrane of *Halobacterium halobium*, undergoes upon illumination a photocycle consisting of several intermediates [1,2].

$$bR_{570} \rightleftarrows K_{590} \rightarrow L_{550} \rightarrow M_{412} \rightarrow N_{530} \rightarrow O_{660}$$
$$\uparrow \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \downarrow$$

This photochemical process is the driving force for the translocation of protons from the cell interior into the extracellular fluid [3–5]. The determination of the number of protons extruded per photocycle implies the measurement of the quantum efficiency of the photochemical cycle (QY_{cy}) as well as that of the proton pumping mechanism (QY_{H^+}).

Recent determinations of QY_{cy} yielded values close to 0.3 [6–9]. Since QY_{H^+} is $\simeq$0.6 at pH<7.0 [10–14] but decreases to ~0.3 at pH $\geqslant$8.0 [14], it was worthwhile to investigate the pH dependence of QY_{cy}.

We have used photoacoustic spectroscopy to measure the pH influence on the conversion of light energy into chemical energy. Results demonstrate that QY_{cy} is pH-independent over pH 5–9. This observation implies that ~2 H^+/photocycle are pumped in the low pH range whereas only 1 H^+ is extruded at alkaline values.

2. Material and methods

2.1. *Strain, culture conditions and sample preparation*

Halobacterium halobium strain R_1M_1 was grown in shaking cultures as in [15]. Cells were harvested by centrifugation (10 min, 12 000 × *g*, 4°C) ~90 h after the end of the exponential growth phase. The purple membrane sheets were obtained as in [16]. Samples were prepared from a stock solution of membranes in distilled water. Bacteriorhodopsin was 8 mg/ml as determined from absorbance at 570 nm using ϵ = 63 000 $M^{-1} \cdot cm^{-1}$ [16]. Samples of 50 µl were spread on 80 mm^2 filter papers. They were dried overnight in a dessicator then impregnated with 10 µl buffer. A 66 mM phosphate buffer was used at pH 5.1 and 6.9 and a 66 mM Tris–HCl buffer at pH 8.9.

2.2. *Reflectance measurements*

The absorption spectra of purple membrane sheets spread on filter papers were measured with a Cary 14 spectrophotometer equipped with an integrating sphere accessory (Varian accessory 1411750). A light-detecting box coated with MgO was placed in the reference compartment in order to ensure a double-beam recording [14].

2.3. *Photoacoustic measurements*

These were done with a single beam spectrometer built in this laboratory. The light source was a Varian 300 W Xenon lamp (VIX 300 UV). A variable speed chopper (Princeton Applied Research, model 192) was used for the modulation. The monochromator (*f*/3.5 Farrand Co.) had a resolution of 5 nm. The microphone was from Brüel and Kjaer Ltd. (type 4166). The sample cell was designed and built in this laboratory from aluminium and quartz. The lock-in amplifier was from Princeton Applied Research (model HR 8). The spectra were recorded between 350

and 700 nm at 90 Hz with a time constant of 1 s. They were normalized using carbon black as reference. The division was performed on a IBM 370 computer.

3. Results and discussion

Typical photoacoustic spectra of purple membrane sheets in buffer are shown in fig.1 and compared to the corresponding reflectance spectra obtained with the same samples. The principle underlying the photoacoustic spectroscopy (PAS) measurement is the following [17,18]: the sample is placed in an airtight cell and illuminated with a modulated light beam. The fraction of the absorbed energy which is dissipated thermally leads to a modulated heating of the surrounding layer of air. This layer can be regarded as a vibratory piston creating the acoustic signal which is detected by the microphone [17]. The PAS signal is thus proportional to the heat released following illumination.

Fig.1 shows that the reflectance and the PAS signals have similar features, although they are not identical because:

(i) The reflectance spectrum can be related to the number of photons absorbed by the sample at a given wavelength whereas the PAS signal is a measurement of the energy converted into heat following the monochromatic light absorption [18].

(ii) The PAS signal depends upon both the optical and the thermal properties of the sample [18]. The interference of the thermal diffusion processes into the PAS signal may cause a saturation

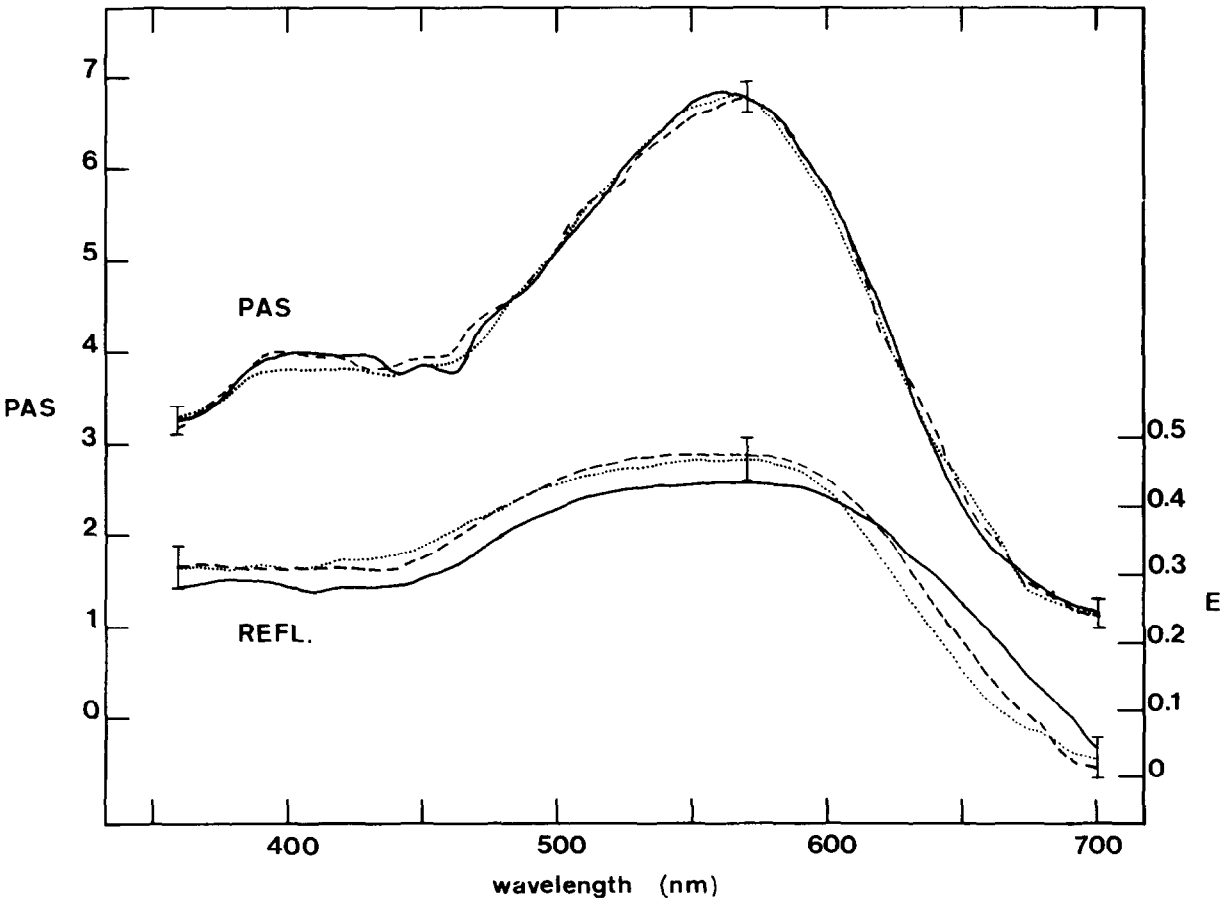

Fig.1. Photoacoustic (PAS) and reflectance (REFL.) spectra of purple membrane sheets of *Halobacterium halobium* spread on filter papers (40 mg bacteriorhodopsin/ml). The PAS spectra are normalized at 570 nm. The units are arbitrary. For attenuance units, see [14]: (——) pH 5.1; (– – –) pH 6.9; (· · ·) pH 8.9.

effect which in turn produces a perturbation of the spectral lineshapes [19].

(iii) Some spectral variations may also result from the different capabilities of resolution of the two spectrometers.

In fact, the two techniques are complementary. For example, at 570 nm, the reflectance signal is related to the number of photons absorbed by the bacteriorhodopsin molecules whereas the PAS signal measures the heat production consequent on the photon absorption. In our experiments, the PAS modulation frequency is 90 Hz so that the period of illumination is 11 ms. Since the bacteriorhodopsin photocycle lasts from 5–10 ms [2,5], it follows that in our experimental conditions, the 570 nm PAS signal represents an integration over the heat generated during the whole photocycle.

The balance between the energy absorbed by the bacteriorhodopsin molecules and that converted into heat is of course the energy stored within the biological system, i.e., the fraction of the incident light used to drive the photocycle. A theoretical outline has been proposed for the evaluation of the latter parameter [20–22]. Since severe difficulties were associated with its measurement in absolute terms, we felt that a comparison of the relative values was actually more feasible.

Fig.1 and table 1 show that the reflectance spectra of bacteriorhodopsin are unaffected by the pH over pH 5–9. E_{570} is the attenuance [14] at the maximum of the bacteriorhodopsin absorption band and E_{360} is used as a reference wavelength since at this wavelength the pigment absorbance is small. The results imply that a constant fraction of the incident flux is absorbed whatever the pH. If, on the other hand, the cycling quantum yield was pH-dependent, so would be the energy balance. In that hypothesis, the PAS spectra should be pH-sensitive. Fig.1 shows that the PAS spectra observed at pH 5.1, 6.9 and 8.9 are very similar. This is also revealed in table 1 which compares the spectral amplitudes measured at some specific wavelengths. The 700 nm PAS signal is essentially due to the white noise of the cell since, at this wavelength, the absorbance of the membranes has vanished. Hence, the ratio PAS_{570}/PAS_{700} represents a normalized measurement which allows a direct comparison of different samples. It can be seen that the variations are inferior to the experimental errors. The PAS_{570}/PAS_{360} ratio is also pH-independent. One can conclude that a constant fraction of the absorbed light is converted into chemical energy, whatever the pH.

Table 1
pH effects on the reflectance and PAS spectra of purple membrane sheets

pH	5.1	6.9	8.9
$\frac{E_{570}}{E_{360}}$ [a]	1.55 ± 0.2	1.53 ± 0.2	1.49 ± 0.2
$\frac{PAS_{570}}{PAS_{700}}$	6.17 ± 0.9	6.17 ± 0.9	6.24 ± 0.9
$\frac{PAS_{570}}{PAS_{360}}$	2.12 ± 0.3	2.16 ± 0.3	2.21 ± 0.3

[a] Light scattering prevents the measurement of the true absorbance [14]

The sole photochemical reaction of the bacteriorhodopsin photocycle is the one leading to the formation of the intermediate K. The following intermediates L, M, N and O result from dark reactions [1,2,8]. The quantum yield values reported concern either the primary photoprocesses [6,9] or the formation of the intermediate M [7]. The values are in good agreement and close to 0.3. Although this work does not lead to an absolute measurement of QY_{cy}, it demonstrates that the quantum yield of the whole photocycle is unaffected by pH over pH 5–9. Since, within this range, QY_{H^+} decreases from 0.68–0.28 [14], it is tempting to conclude that 2 H^+/cycle are pumped in the low pH range whereas only 1 H^+ is extruded at high pH values.

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