SPECTROSCOPY AND ENERGETICS OF THE PURPLE MEMBRANE OF *HALOBACTERIUM HALOBIUM*

A photoacoustic study

David CAHEN, Haim GARTY and S. Roy CAPLAN
The Weizmann Institute of Science, Rehovot, Israel

Received 3 May 1978

1. Introduction

Photoacoustic spectroscopy (PAS) on solids, semi-solids, and liquids has proven to be a valuable method for the optical investigation of samples that are difficult or impossible to study by other means [1–3]. In PAS, a sample situated in a closed chamber filled with gas (generally air) is illuminated by light modulated at an acoustic frequency. Part of the absorbed radiation is converted into heat which is transferred to the gas medium. Because of the modulation this induces pressure waves in the sample chamber, which are detected by a microphone. The measured signal is proportional to the amount of heat released by the absorbing species, i.e., its concentration and the fraction of absorbed energy converted into heat. The technique has been used mostly as an alternative to transmission or diffuse reflectance methods. Because of its insensitivity to scattered radiation it is of particular utility in the study of biological suspensions. Also, since only the fraction of the absorbed radiation that is converted into heat is sensed by PAS, it is an attractive method for the study of energy conversion processes. Here we report on the spectroscopy and energetics of several preparations of the purple membrane from *Halobacterium halobium*. It is well established that this membrane contains a single protein, bacteriorhodopsin, covalently bound to a retinal molecule [4,5]. Absorption of light by the retinal brings about a cyclic photochemical process [6–8], which drives the translocation of protons from one side of the membrane to the other [9]. Presumably this occurs by way of conformational changes in the protein [10].

Our results show PAS to have a surprising sensitivity as compared to conventional spectroscopic methods. Furthermore, the data indicate that PAS can provide information on the energetics, by clearly distinguishing between samples in which part of the absorbed energy is stored or used to do work, and those in which all the absorbed energy is dissipated in the photocylce as heat. The results also provide experimental evidence for a theoretical treatment of the influence of photochemical processes on PAS signals [11].

2. Experimental

Purple membrane suspensions were prepared from *H. halobium* R_1 as in [12]. Lyophilized samples were ground finely and measured as a flaky powder. Dried samples were obtained by sorption of a concentrated suspension of purple membrane fragments on cotton wool or millipore filter paper, and letting it dry in air. Samples of whole cells were prepared by sorption of a concentrated suspension on millipore filter paper; measurements were then carried out on the wet, impregnated paper. In this way acceptable signal to noise and signal to background ratios were obtained, so that there was no need to resort to the less reproducible method of sorption on cotton wool previously used for chloroplast membranes [13]. Measurements were carried out in the home-made photoacoustic apparatus [14] using both single-ended and differential cells (for background correction). A Cary 15 spectrom-

eter was used for diffuse reflectance measurements. Side-illumination was obtained from a quartz halogen projector lamp (24 V, 150 W), filtered through a 3484 Schott filter.

The dependence of the photoacoustic signal on the light intensity of the modulated beam was checked and found to be linear in the range used (10–50 mW/cm^2). All PAS data were corrected for background (empty cell, empty cell + wt millipore paper or dry cotton wool) and for the variation of lamp intensity with wavelength. The last correction was done by using a totally absorbing carbon black sample in the photoacoustic cell.

3. Results and discussion

Figure 1 shows the absorption (from diffuse reflectance) and photoacoustic spectra of lyophilized purple membrane fragments. The two spectra show essentially the same features except that in the photoacoustic

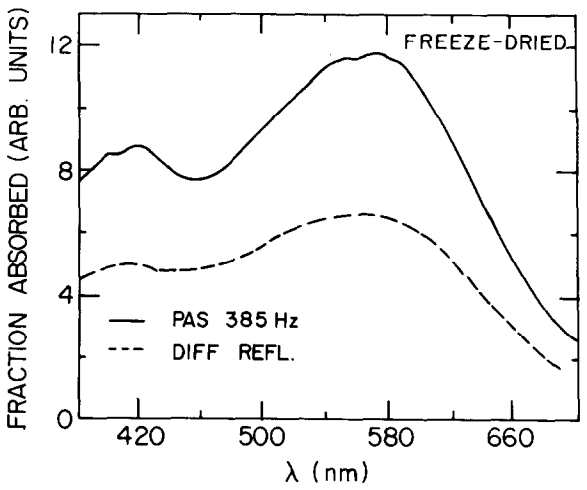

Fig.1. Photoacoustic and diffuse reflectance spectra of lyophilized purple membranes. A 450 W Xenon arc lamp, monochromator, and light guide were used for illuminating samples for PAS. Detection was by Knowles Electronics BT-1753 microphones and Princeton Applied Research JB-6 or Brookdeal 9502 lock-in amplifiers. Modulation frequency, 385 Hz. The two spectra (which are expressed as fraction of light absorbed by the samples) have been adjusted to the same concentration of chromophore. The proportionality between optical absorption and the photoacoustic signal has been derived [16].

spectrum a stronger signal around 415 nm was observed and a peak at about 550 nm. These differences can be explained by the scheme for the photocycle suggested in [15]. According to this scheme excitation of the photointermediates absorbing at 550 nm and 412 nm can induce their thermal decay to the ground state, thus giving rise to an increased (compared to optical absorption) PAS signal. At 72 Hz and 770 Hz similar results are obtained but the 565 nm/415 nm peak intensity ratio, which is 1.6 for the diffuse reflectance spectrum, increases from 1.2 (± 0.03) at 72 Hz to 1.3 at the higher frequencies. This frequency dependence is probably caused by slow decay (ms) of 415 nm species in lyophilized samples which results in a larger modulated accumulation of the 415 nm species at lower modulation frequencies. Dried samples (on cotton wool) give spectra similar to these and here, too, the 415 nm peak decreases at high modulation frequencies relative to the 565 nm peak. The fact that no dramatic differences are seen between the PAS and the absorption spectrum is to be anticipated in these preparations as all the absorbed energy is dissipated as heat and no net energy storage processes are expected to take place (except steady-state accumulation of phototransients). In whole cells such processes do take place, but the much reduced signal-to-noise ratio made it difficult to obtain results accurate enough for direct comparison with the absorption spectrum (see, however, fig.3).

In fig.2 the effect of continuous side illumination on the PAS spectrum of dried membrane fragments is shown. The relatively strong continuous illumination caused an increase in the signals measured around 415 nm and 660 nm and a decrease of the signal around 565 nm. This is due to light-induced accumulation of the photointermediates absorbing at 415 nm and 660 nm (which can be observed either by flash [6,7] or modulation [17] excitation) and thus a decrease in the population absorbing at 565 nm. In table 1 such light-induced changes are compared for different samples at two modulation frequencies.

The accumulation of the 415 nm intermediate is larger in dried membrane fragments than in wet intact cells and in both cases a smaller signal was obtained at higher chopping frequencies. These two observations can be accounted for by the build-up and decay times of this intermediate. At higher chopping frequency the technique senses earlier events and part of the

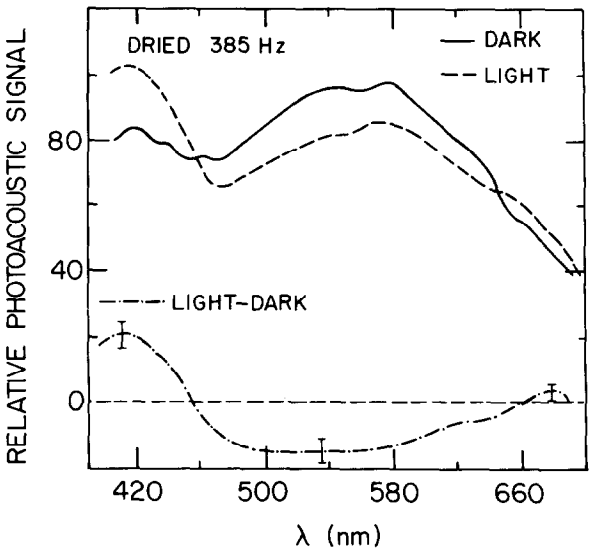

Fig.2. PAS of dried purple membrane fragments on cotton wool, with and without 200 mW/cm² continuous side illumination. Error bars indicate approximate uncertainties at different wavelengths in the difference spectrum.

heat released due to decay of the 415 nm species is not modulated and thus not detected. Reducing the water content of the membrane increases the decay-time of the 415 nm intermediate [18] resulting in an increase in its steady state concentration, and hence a larger PAS signal. For the 565 nm species in whole cells, changes in chromophore concentration and storage of energy other than as photointermediates

Table 1
Light-induced photoacoustic signal changes[a]

Sample	Wavelength 415 nm	565 nm	660 nm
Whole cells			
72 Hz[b]	+15	−10	+20
385 Hz	+ 5	− 5	+ 5
Dried membranes			
72 Hz	+45	− 5	+20
385 Hz	+25	−10	0

[a] Changes are expressed as % dark signal; accuracy ~ ± 5%
[b] Modulation frequency

should both play a role, but apparently the second effect which should be sensitive to modulation frequency too is over-shadowed by the first. However, preliminary results on the kinetics of the 565 nm and 415 nm signal changes upon side-illumination seem to discriminate between the two effects.

To investigate the effect of the photochemical process more directly, we calculated the dissipation spectrum of both lyophilized membranes in which the absorbed light drives the photocycle only, and whole cells in which part of the absorbed energy is eventually stored as ATP and ion gradients [19–21]. According to theory [11], the corrected photoacoustic signal, ρ, for a photochemically active system is given by:

$$\rho = I/I_{\text{inc.}} \cdot \left(1 - \frac{\phi \cdot \Delta E_p \cdot \lambda}{\text{const.}}\right) \quad (1)$$

where $I/I_{\text{inc.}} = \alpha$ is the fraction of light absorbed, ϕ and ΔE_p are the quantum yield and internal energy change in the photochemical reaction, respectively, and λ is the wavelength. From eq. (1):

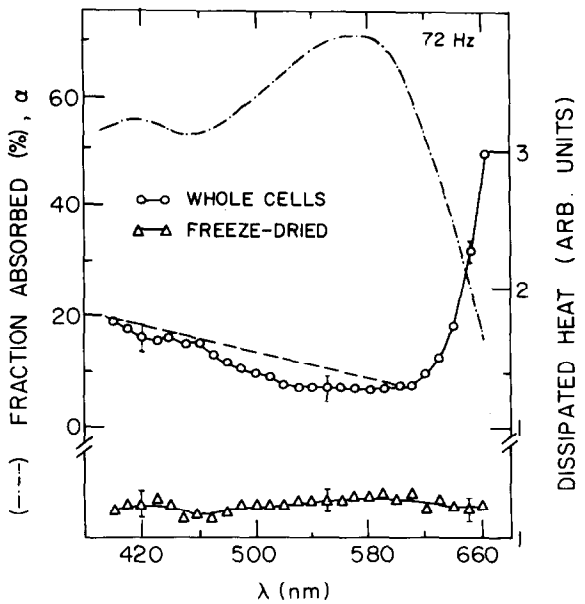

Fig.3. Absorption and dissipation spectra of whole cells, and dissipation spectrum of lyophilized purple membrane fragments. Error bars indicate approximate uncertainties at different wavelengths. Modulation frequency, 72 Hz. For details, see text.

$$\rho/\alpha = 1 - \frac{\phi \cdot \Delta E_p \cdot \lambda}{\text{const.}} \quad (2)$$

which represents the relative dissipation of the photochemical reaction, as sensed by PAS. If $\phi \cdot \Delta E_p = 0$ (no photochemical reaction or cyclic reaction with $\Delta E_p = 0$ at the modulation frequencies used), then ρ/α will be independent of λ. However, if some of the absorbed energy is stored in the products sensed by PAS a valley-shaped curve should be obtained, with the lowest values of dissipation in the region of highest energy storage and $\rho/\alpha = 1$ in regions of zero energy storage (e.g. at long wavelengths where $\phi = 0$).

In fig.3 our results with freeze-dried purple membrane fragments and intact whole cells are shown, and it can be seen that the above predictions are essentially borne out by experiment. In the freeze-dried purple membrane fragments, where presumably no energy is stored (on our time scale) in the steady state, ρ/α is constant within the experimental error. However, in intact cells ρ/α passes through a minimum in the 540–620 nm region, and increases both at shorter and longer wavelengths. The middle part of the curve is not linear as expected from eq. (2) for $\phi \cdot \Delta E_p$ independent of λ (dashed line in fig.3). Although it is possible that ϕ and/or ΔE_p are wavelength-dependent, a more probable reason for this behaviour can be found in the different light intensities used for measuring ρ (10 mW/cm^2) and α (0.1–0.3 mW/cm^2). At higher light intensities different absorption characteristics are obtained because of accumulation of photointermediates (see fig.2), thus leading to too large a value for α at 565 nm, resulting in too small a value for ρ/α at that wavelength.

Plots such as those shown in fig.3 can be used in principle to calculate $\phi \cdot \Delta E_p$ for the various intermediates sensed at different modulation frequencies and future experiments will concentrate, inter alia, on this use of the technique.

Acknowledgement

We thank Knowles Electronics, for microphones and advice on their use.

References

[1] Harshbarger, W. R. and Robin, M. B. (1973) Acc. Chem. Res. 10, 329–334.
[2] Rosencwaig, A. (1975) Anal. Chem. 47, 592A–604A.
[3] Adams, M. J., King, A. A. and Kirkbright, G. F. (1976) Analyst 101, 73–85; Adams, M. J., Beadle, B. C., King, A. A. and Kirkbright, G. F. (1976) Analyst 101, 553–561; Adams, M. J., Beadle, B. C. and Kirkbright, G. F. (1977) Analyst 102, 569–575.
[4] Oesterhelt, D. and Stoeckenius, W. (1971) Nature New Biol. 233, 149–152.
[5] Bridgen, J. and Walker, I. D. (1976) Biochemistry 15, 792–798.
[6] Lozier, R. H., Bogomolni, R. A. Stoeckenius, W. (1975) Biophys. J. 15, 955–962.
[7] Dencher, N. and Wilms, M. (1975) Biophys. Struct. Mech. 1, 259–271.
[8] Chu Kung, M., DeVault, D., Hess, B. and Oesterhelt, D. (1975) Biophys. J. 15, 907–911.
[9] Lozier, R. H., Niederberger, W., Bogomolni, R. A., Hwang, S.-B. and Stoeckenius, W. (1976) Biochim. Biophys. Acta 440, 545–556.
[10] Stoeckenius, W., Lozier, R. H. and Bogomolni, R. A. (1978) Biochim. Biophys. Acta. in press.
[11] Malkin, S. and Cahen, D. (1978) Photochem. Photobiol., submitted.
[12] Oesterhelt, D. and Stoeckenius, W. (1974) Math. Enzymol. 31, 667–678.
[13] Cahen, D., Malkin, S. and Lerner, I. (1978) FEBS Lett. in press.
[14] Cahen, D., Lerner, I. and Auerbach, A. (1978) Rev. Sci. Instr. in press.
[15] Hurley, J. B., Becher, B. and Ebrey, T. G. (1978) Nature 272, 87–88.
[16] Rosencwaig, A. and Gersho, A. (1976) J. Appl. Phys. 47, 64–69.
[17] Slifkin, M. A. and Caplan, S. R. (1975) Nature 253, 56–58.
[18] Korenstein, R. and Hess, B. (1977) Nature 270, 184–186.
[19] Danon, A. and Caplan, S. R. (1976) Biochim. Biophys. Acta 423, 133–140.
[20] Garty, H. and Caplan, S. R. (1977) Biochim. Biophys. Acta 459, 532–545.
[21] Eisenbach, M., Cooper, S., Garty, H., Johnstone, R. M., Rottenberg, H. and Caplan, S. R. (1977) Biochim. Biophys. Acta 465, 599–613.