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PHOTOACOUSTIC SPECTROSCOPY OF PURPLE MEMBRANE FRAGMENTS

EVIDENCE FOR DISORDER DURING PHOTOCYCLE

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A strong band at 412 nm has been observed in the photoacoustic spectrum of partially dried purple membrane, peaking sharply at a modulation frequency of about 70 Hz. This may be explained in terms of a disorder-order transition.

1. Introduction

There has been evidence for an initial molecular disordering, followed by a disorder-order transition, in the photocycle of bacteriorhodopsin (bR) [1–5]. Here we present additional evidence for this transition obtained by photoacoustic spectroscopy (PAS), but based on observations totally different from those presented previously [1,4,5]. PAS measurements on bR fragments at modulation frequencies between 60 and 80 Hz show an unusual phenomenon, in that a strong absorption band is observed at 412 nm, corresponding to the M_{412} intermediate in the photocycle, which peaks sharply at 70 Hz. It is suggested that 'short-circuiting' of the photocyle either by photoreaction [6,7] or by thermal relaxation [8–10], resulting in a sudden re-ordering or recrystallisation of the bR matrix, may be responsible for this effect.

Abbreviations: bR, bacteriorhodopsin; bR_{570}, M_{412}, M'_{390}, N_{530}, intermediates in the bacteriorhodopsin photocycle; PA, photoacoustic; PAS, photoacoustic spectroscopy.

2. Experimental

The PA spectrometer was similar to the instrument described by Garty et al. [4] and the same measuring protocol was followed. Purple membrane, prepared by the method of Oesterhelt and Stoeckenius [11] was used as a powder at room humidity (40–50%). PA spectra were obtained in the region 300–700 nm at modulation frequencies from 7 to 112 Hz.

3. Results

With the exception of the range of modulation frequencies between about 40 and 80 Hz the PA wavelength spectra obtained are similar to that found in aqueous suspension by Garty et al. [4]. However, in the range 60–80 Hz a strong peak appears (fig. 1) at 412 nm, which reaches its maximum height (relative to the peak at 570 nm) at about 70 Hz (fig. 2).

4. Discussion

Clearly the new peak is to be identified with the major long-lived photocyle intermediate M_{412}.

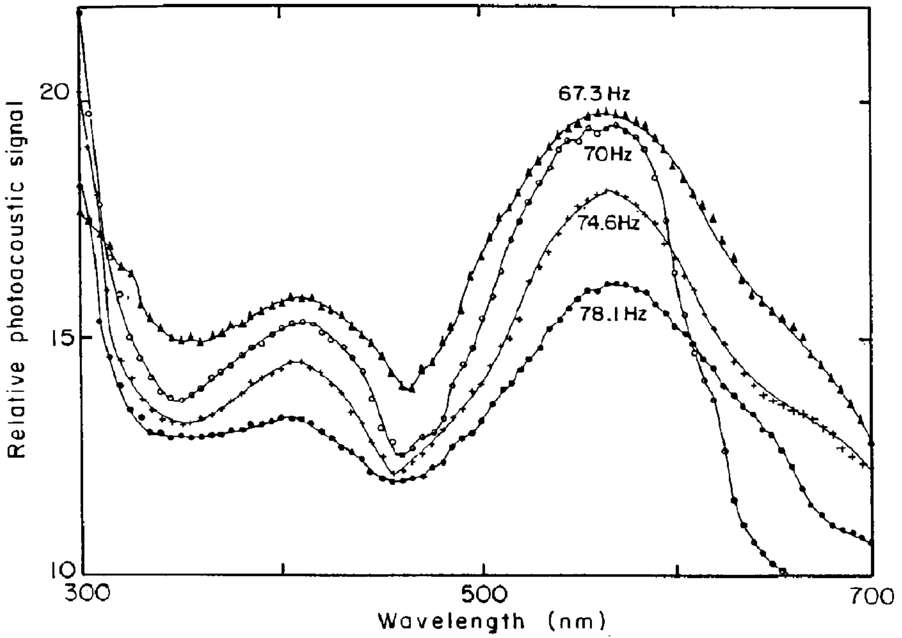

Fig. 1. Photoacoustic wavelength spectra of purple membrane fragments in powder form at 20°C and 40–50% relative humidity. Modulation frequencies as shown.

Garty et al. [4] observed a peak at 412 nm in the PA spectrum of purple membrane fragments incubated at 63% relative humidity using a modulation frequency of 40 Hz, and commented that even in the absence of actinic light some population of M_{412} is generated by the measuring beam (which is strong compared with that used in conventional transmission spectroscopy). In this regard it should be noted the three relaxation times describing M_{412} decay are drastically slowed down by lowering the hydration state of the preparation, for example, the first two relaxation times increase from 2 m and 5 ms in water to 244 ms and more than 1 s at 43% relative humidity [12,13]. Furthermore, it has been shown that the short-circuiting back-photoreaction, from M_{412} is initiated by the generation of a photoproduct, M'_{390}, which undergoes thermal decay to bR_{570} with a half-life of 150 ns; both its yield and decay rates are insensitive to the extent of hydration [6,13].

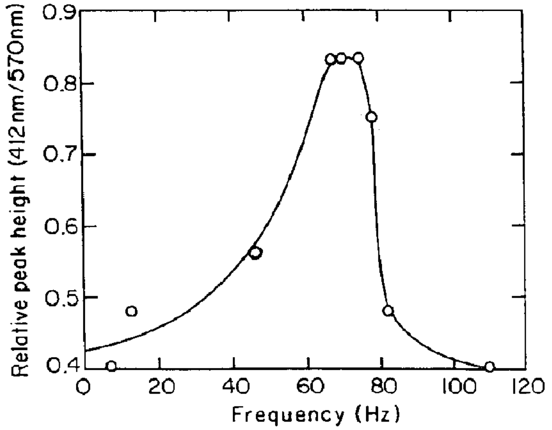

Fig. 2. Photoacoustic frequency spectrum of purple membrane fragments in powder form (from same data as fig. 1). Relative peak height (412 nm/570 nm) vs. modulation frequency.

Ort and Parson [1] have shown using calorimetric methods that during the photocycle of bR, there are considerable changes in the enthalpy and entropy. Over a time range of a few milliseconds, there is an enthalpy change of greater than 50 kcal/mol. There is accompanying decrease in entropy believed to be caused by a major increase in molecular order in the purple membrane.

It should be emphasised that the PAS signal is proportional to the heat flowing to the surface of the sample. Under normal circumstances the exciting light is simply degraded to heat and hence the PAS signal is directly related to the concentration of the absorbing species. If, however, the absorbed light promotes some photophysical or photochemical reaction giving rise to the output of heat, then the observed PAS signal will be larger than expected. Thus, short-circuiting of the photocyle by the irradiation of M_{412} will give rise to a signal which is much greater than expected from the concentration of this species.

The fact that the peak we have observed extends only over a very limited frequency range suggests some kind of resonance mechanism. Early workers found evidence of intramembrane rotational motion during the bR photocycle from measurements of linear dichroic relaxation [4] and this has recently been substantiated by the much more detailed studies of Ahl and Cone [2]. The work of Frankel and Forsyth [3] using pulsed X-ray techniques is consistent with this picture. Thus, resonance may well be occurring between the modulated input and the rotational or oscillational motion of monomer units of bR within the trimer suggested by Ahl and Cone [2]. At the same time, either photoreactive or thermal short-circuiting of the photocycle may take place, resulting in a direct transition to bR_{570} from either M_{412} (via M'_{390}) or N_{530}, respectively, which in turn gives rise to re-ordering of the disordered crystalline matrix. This would manifest itself as an exothermic effect and hence a large PAS signal. The above-reported observation strengthens the view that the photocycle of bR involves a disorder-order transition [1,5].

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