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ON THE ORIGIN OF THE RED EMISSION OF LIGHT ADAPTED PURPLE MEMBRANE OF *HALOBACTERIUM HALOBIUM*

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1. Introduction

The light induced reaction cycle of bacteriorhodopsin (bR) in the light adapted (l.a.) purple membrane of *Halobacterium halobium* is described in the literature as consisting of a primary photochemical event followed by several thermal steps [1,2]. The initial chromophore, all-*trans*-retinal, is converted within 6 ps [3] after excitation by light into a product with an absorption maximum at about 630 nm [4,5]. This primary photoproduct is referred to as batho-form or K-form. A red luminescence centered around 700 nm has been observed from l.a. purple membrane [6,7].

Studying this luminescence from bR in aqueous suspensions of purple membrane at 77°K and room temperature we have obtained results which show that there exists a second primary photoproduct of bR. This is stable at 77°K and has an excitation spectrum with a maximum at 597 ± 3 nm. In view of this finding, we discuss some results taken from the literature for which no satisfactory explanation has yet been given.

2. Experimental

The luminescence data were obtained using a spectrometer consisting of two double-grating monochromators (Spex 1402), a 2.5 kW Xe-lamp and an ITT photomultiplier (F4013). Measurements were made using photon counting techniques. For illumination experiments at 77°K a 12 W argon ion laser (CR 12, Coherent Radiation) in combination with a rhodamine 6G dye laser was available. Furthermore the dye laser served as excitation source for room temperature emission spectra.

The suspensions of purple membrane (from strain R_1M_1) for luminescence experiments were 33 μM with respect to the protein (mol. wt 26000). These samples were contained in quartz tubes (diam. 4 mm, Suprasil) and degassed by several freeze-pump-thaw cycles at $p < 10^{-3}$ torr. Absorption spectra at 77°K were measured with 66 μM purple membrane suspensions (glycerol–water, 1:1, v/v) contained in a 1 mm absorption cell. The cell was mounted in a quartz dewar and could be inserted in a Perkin Elmer (Coleman 575) spectrometer. Light adaption was done by illuminating the samples at room temperature for about 10 min with 550 nm light (interference filter with a band-width of 30 nm) from a 1000 W tungsten lamp. The samples studied at 77°K were cooled in the dark in order to avoid trapping of thermal intermediates. All the spectra were corrected for the spectrometer response.

3. Results

In the figure three emission spectra as well as an absorption spectrum and an excitation spectrum are presented. The emission spectra from a l.a. sample after cooling down to 77°K in the dark (broken line) exhibits two maxima at 678 nm and 725 nm respectively. 570 nm light from the Xe lamp was used as

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excitation wavelength. The emission spectrum has been drawn magnified by a factor of 3. The corresponding excitation spectrum for the emission wavelength 670 nm is shown as a solid line in the left part of the figure. Its shape proved to be independent of the emission wavelength at least within a range from 670–740 nm. This spectrum exhibits a maximum at 597 nm and a shoulder at around 540 nm. Repetition of the emission spectrum after additional illumination of the frozen sample with 514.5 nm light from the argon ion laser ($I \approx 1$ W/cm^2) indicated an enhanced emission intensity. It reached a stationary level after about 5 min. This level was measured at 670 nm to be 3.5 times higher than before this treatment with green light. Parallel to this 3.5-fold increase, a change of the intensity ratio of the two emission peaks by about 20% in favour of the 670 nm peak was observed. Furthermore the two peaks shifted to 670 nm and 720 nm, respectively. Additional illumination with 623 nm light ($I \approx 100$ mW/cm^2) lead to another increase of the emission intensity by about 10%. These results are depicted by the solid line in the emission part of the figure. The 10% increase, however, was reversible. Subsequent illumination with 514.5 nm light restored the emission intensity to the level existing before the red illumination. The excitation spectrum did not exhibit measurable changes in the course of this procedure.

When the sample was then warmed up and cooled down to 77°K again in the dark, the first emission spectrum (broken line) was obtained. At room temperature excitation with 573 nm (dye laser) gave a broad emission band with one maximum at about 730 nm (dotted line in the figure). Its peak intensity was at least 10-times weaker than that of the emission at 77°K without preillumination (broken line).

4. Discussion

The emission with maxima at 678 nm and 725 nm has been previously reported by two groups [6,7]. However, different values for the emission maxima have been given in these two references (678 nm, 733 nm and 790 nm in ref. [6] and 680 nm, 710 nm and 740 nm in ref. [7]). As can be seen, agreement is perfect with respect to the short wavelength peak. Further the peaks at 733 nm [6] and 740 nm [7] might be compared with our peak at 725 nm obtained before illumination of the sample with 514.5 nm light at 77°K. In ref. [6] the emission was attributed to the parent bacteriorhodopsin, bR, in which the chromophore is all-*trans*-retinal. The observed structure was interpreted as a vibrational progression of $\Delta\nu \simeq 1000$ cm^{-1}.

Our experimental results, however, lead us to conclude that the emission originates from a primary photoproduct of bR. The spectroscopic data visualized in the figure provide two points of evidence for this conclusion. First, the increase in emission intensity by almost a factor of 4 in a sample which has been illuminated at 77°K with light of 514.5 nm and 623 nm clearly shows that the emission mainly comes from a species produced during the illumination. Second, the excitation spectrum with a maximum at 597 nm differs from the absorption spectrum of bR, which has a maximum at 574 nm [2,8]. The emitting species is thus expected to absorb slightly to the red of bR. The photoproduct cannot be the batho-form (K-form) since illumination of the sample with 623 nm light further increases the emission intensity. This behaviour is contrary to what one would expect since the batho-form is known to be reconvertable into bR by red light [2,8]. Furthermore the batho-form absorbs strongly around 630–650 nm [2,3,8] and therefore cannot be the cause for the observed excitation spectrum. We shall call the emitting species pseudo- or P-bacteriorhodopsin (P-bR) because its absorption lies close to that of bR.

The observed increase of the emission intensity upon preillumination at 77°K suggests the existence of a photochemical pathway of the following scheme:

$$\mathrm{bR} + h\nu \rightarrow \mathrm{bR}^* \rightarrow \mathrm{P\text{-}bR}.$$

The decrease of the emission intensity to the initial level after warming up the preilluminated sample and cooling it down back to 77°K in the dark indicates that P-bR can recycle back into bR thermally. But, of course, our present experiments do not allow us to decide whether this recycling occurs directly or via intermediates. The data presented so far cannot elucidate details of the postulated step bR* → P-bR. The following discussion and reconsideration of other results taken from the literature will give some more specific information. Kaufmann et al. [3] reported a

transient decrease of the absorbance of l.a. purple membrane samples at room temperature in the vicinity of 580 nm induced by picosecond laser pulses. Decay and recovery of the absorbance occurred with a half-time of about 15 ps (ref. [3], fig. 4). On the other hand, Hirsch et al. [9] measured a life-time of 15 ± 3 ps for the emission at 780 nm of such a sample. Together with our results these data can be interpreted as follows. Picosecond pulses excite bR into bR* which relaxes into (P-bR)*. (P-bR)* decays within about 15 ps into P-bR. Thus the recovery of the absorbance to almost the initial value within about 15 ps would be caused by the build up of the ground state of P-bR. This species absorbs strongly in the vicinity of 580 nm (see our excitation spectrum in fig.1). Since the ground state of the batho-form is produced within 6 ps or less, this sets an upper limit to the time interval during which (P-bR)* can be populated. Further it follows that P-bR is created parallel to the batho-form.

These conclusions have two consequences. First, they imply that Hirsch et al. have not necessarily measured the luminescence life-time of bR but rather of P-bR. Second, the early stage of the reaction cycle as understood at present needs a revision introducing a branched photochemical step, at least at 77°K. At room temperature our experiments show that P-bR reconverts thermally into bR. The existence of the photoproduct P-bR might be helpful to explain discrepancies in the quantum yields for the formation of the intermediate M [2] of the reaction cycle. In the literature one finds two very different values, 0.79 [10] and 0.28 [11]. The same can be expected for the reported quantum yields of the red luminescence $2 \cdot 10^{-4}$ [6] and $2 \cdot 10^{-5}$ [7]. Further the spectral shift of the bR absorption band after illumination with light of 500 nm at 77°K from 575 nm to about 600 nm as observed by Stoeckenius and Lozier [1] can be interpreted as the formation of P-bR. The different positions of the absorption maximum of the batho-form one finds in the literature, 590 nm [2], 610 nm [1] and 630 nm [5] constitute another discrepancy that can be resolved by the assumption of different concentrations of P-bR. Thus in fig.1 of ref. [1] the maximum of the batho-form is presumably at too short wavelength because P-bR which is also formed by irradiation at 77°K contributes to the calculated spectrum.

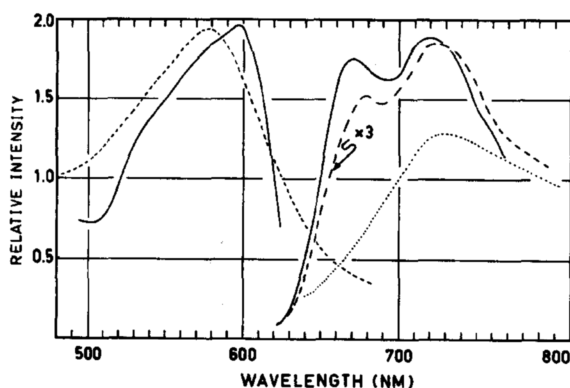

Fig.1. This figure summarizes our spectral data obtained from samples of light adapted purple membrane at 77°K. In the short wavelength region an absorption spectrum in water–glycerol (1:1 v/v, dashed line) and an excitation spectrum in H_2O (emission wavelength 670 nm, solid line) are represented for comparison. In the long wavelength region emission spectra for a sample with and without preillumination (solid and dashed line respectively) have been drawn. The emission spectrum obtained without preillumination has been magnified by a factor of 3 for better representation. A room temperature emission spectrum (dotted line) is also shown. The scaling of the emission spectra does not reflect the experimentally established weakness of the emission at 298°K as compared to 77°K (see text).

Finally we have to take into account the structural difference in the emission spectra shown in the figure. The most straightforward interpretation is that there are two emitting species at 77°K. The dashed line then represents a combination of two emissions, namely from the P-form and from a so-far unidentified species, which gives rise to our room temperature emission (dotted line). This interpretation offers a simple explanation for the larger increase in the emission intensity at 670 nm as compared to 720 nm as the P-form is produced. We then assume that the emission with a maximum at 730 nm remains constant or even decreases in intensity as the sample is illuminated with green light. It is thus not necessary to assume that the blue shift in the emission upon cooling is caused by unrelaxed dipolar groups etc. [9].

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References

[1] Stoeckenius, W. and Lozier, R. H. (1974) J. Supramol. Struc. 2, 769–774.
[2] Lozier, R. H., Bogomolni, R. A. and Stoeckenius, W. (1975) Biophys. J. 15, 955–962.
[3] Kaufmann, K. J., Rentzepis, P. M., Stoeckenius, W. and Lewis, A. (1976) Biochem. Biophys. Res. Commun. 68, 1109–1115.
[4] Chu Kung, M., Devault, D., Hess, B. and Oesterhelt, D. (1975) Biophys. J. 15, 907–911.
[5] Dencher, N. and Wilms, M. (1975) Biophys. Struct. Mechanism 1, 259–271.
[6] Lewis, A., Spoonhower, J. P. and Perreault, G. J. (1976) Nature 260, 675–678.
[7] Alfano, R. R., Yu, W., Govindjee, R., Becher, B. and Ebrey, T. G. (1976), Biophys. J. 16, 541–545.
[8] Tokunaga, F., Iwasa, T. and Yoshizawa, T. (1976) FEBS Lett. 72, 33–38.
[9] Hirsch, M. D., Marcus, M. A., Lewis, A., Mahr, H. and Frigo, N. (1976) Biophys. J. 16, 1399–1409.
[10] Oesterhelt, D. and Hess, B. (1973) Eur. J. Biochem. 37, 316–326.
[11] Goldschmidt, C. R., Ottolenghi, M. and Korenstein, R. (1976) Biophys. J. 16, 839–843.