PHOTOREACTION OF *TRANS*-BACTERIORHODOPSIN AT LIQUID HELIUM TEMPERATURE

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Received 26 February 1979

1. Introduction

In bacteriorhodopsin (bR) in purple membrane of *Halobacterium halobium*, there are two different forms: One is a light-adapted bacteriorhodopsin (λ_{max} 570 nm) which has all-*trans* retinal as its chromophore (we call this *trans*-bR) and another is a dark-adapted bacteriorhodopsin (λ_{max} 560 nm), the chromophore of which is a mixture of all-*trans* and 13-*cis* retinals in an equal amount [1–3]. This paper deals with only *trans*-bR. On absorption of light, *trans*-bR converts to several intermediates sequentially, e.g., batho-*trans*-bR, lumi-*trans*-bR and meta-*trans*-bR, which finally revert to the original *trans*-bR. These intermediates have been identified by flash photolysis [4–6] and low temperature spectrophotometry [7,8]. They are quite similar in spectral properties to the intermediates appearing in the process of photo-bleaching of visual pigment.

Cattle rhodopsin was irradiated [9,10] at liquid helium temperature (4–9 K) and hypsorhodopsin found. On warming, it converted to bathorhodopsin. It was confirmed [11] by a picosecond laser photolysis that squid hypsorhodopsin was an earlier intermediate than bathorhodopsin.

Now a question arises whether or not there is an intermediate earlier than batho-*trans*-bR. In order to settle this question, we investigated the photoreaction of *trans*-bR at liquid helium temperature.

2. Materials and methods

Purple membrane was prepared by the method similar to that in [12]. Purified purple membrane was suspended in 10 mM phosphate buffer (pH 6.8), to which glycerol was added at 75% final conc.

For the spectral measurements at liquid helium temperature, MPS 5000 recording spectrophotometer (Shimazu) equipped with a double vacuum glass cryostat described [9] was used. The sample was filled in an optical cell composed of a rubber ring, a quartz plate and an opal glass. The cell was set in the copper block screwed on a cold finger of the cryostat. The temperature of the sample was continuously measured with an Au–Co versus chromel thermocouple attached to the copper block.

A 500 W Xe lamp (Ushio) was used as a light source for irradiation of the sample. The wavelengths of the irradiation light were selected by interference filters and/or glass cut-off filters (Toshiba).

3. Results

In order to prepare *trans*-bR, the preparation was irradiated at 510 nm at 0°C until the further irradiation caused no additional spectral change. At 0°C, *trans*-bR showed its λ_{max} at 570 nm and on cooling to 77 K, it changed to 580 nm. Further cooling it to liquid helium temperature did not cause any additional shift of λ_{max} (curve 1, fig.1). On cooling from liquid nitrogen temperature to liquid helium temperature, the absorbance λ_{max} increased ~7% and the absorption band was sharpened.

To examine the presence of earlier intermediates than batho-*trans*-bR, *trans*-bR was irradiated at liquid helium temperature with light at wavelengths

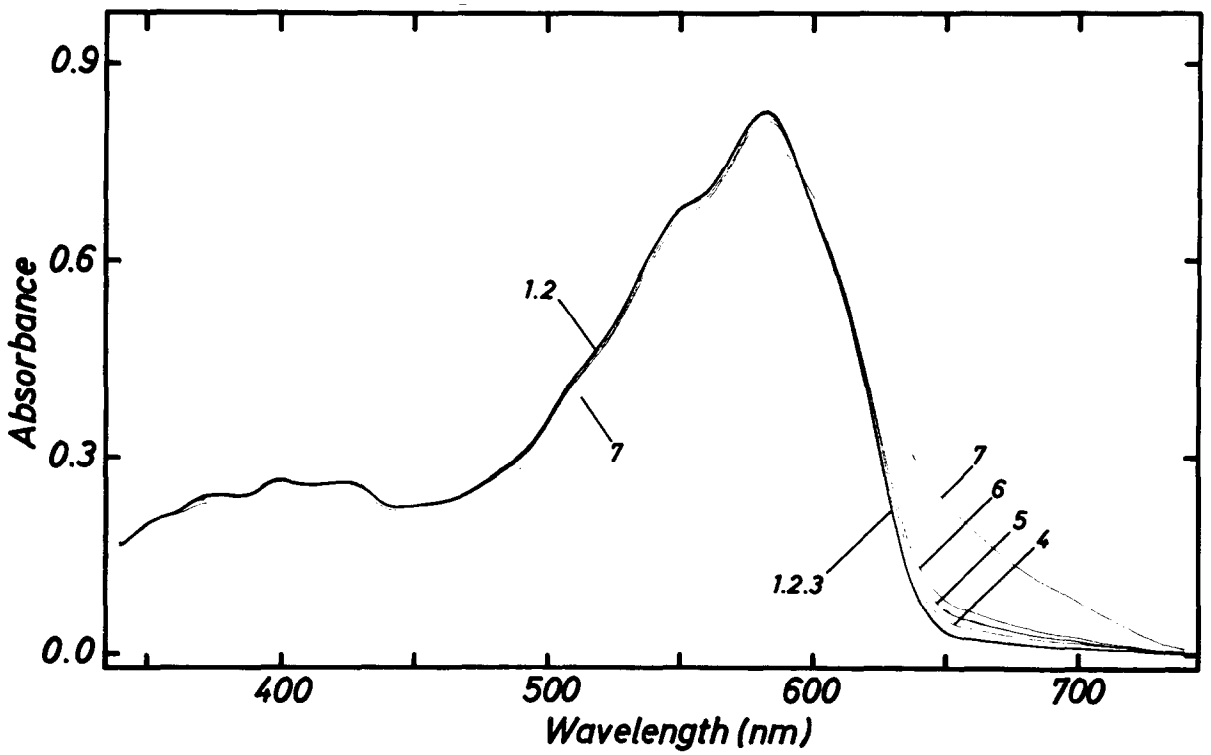

Fig.1. Photoconversion of *trans*-bR at liquid helium temperature. All the spectra were measured at 9 K. Curve 1: *trans*-bR. Curve 2–7: photoproducts formed by the successive irradiation of *trans*-bR with light at wavelengths >660, 640, 610, 580 and 560 nm, and finally with light at 510 nm. Each irradiation time was 10 min and it was confirmed that the further irradiation caused no additional spectral change.

>660 nm. We observed no spectral shift (curve 2, fig.1). Then we irradiated *trans*-bR with light at wavelengths >640 nm. We observed no hypsochromic shift but a very small bathochromic shift (curve 3, fig.1). The irradiation with light at wavelengths >610 nm caused a further bathochromic shift (curve 4, fig.1).

Further irradiations with light at wavelengths >580 nm then 560 nm caused similar additional spectral changes and the absorbance increased in a longer wavelength region (curves 5, 6, fig.1). These spectral changes formed an isosbestic point at 595 nm, which lies at the same wavelength as that between *trans*-bR and batho-*trans*-bR at liquid nitrogen temperature [7].

The irradiation at 510 nm caused a big absorbance change in a longer wavelength region (curve 7, fig.1). The spectrum passed through the isosbestic point at 595 nm. From these results, we concluded that the irradiation of *trans*-bR at liquid helium temperature does not produce such an intermediate which has λ_{max} at a shorter wavelength than *trans*-bR, as hypsorhodopsin in a rhodopsin system.

Next, we examined whether or not the batho-product formed at 9 K (contained in samples of curves 3–7, fig.1) is the same molecular species as batho-*trans*-bR. The isosbestic point of the spectral change in fig.1 suggests that it may be the same species as batho-*trans*-bR. The difference spectrum between curve 1 and 6 (○) and that between curve 1 and 7 (△) in fig.1 were plotted in fig.2, and compared with the difference spectrum between *trans*-bR and batho-*trans*-bR at 77 K (solid line). All three difference spectra were almost identical with each other. This fact indicates that the irradiation of *trans*-bR at 9 K produced only one molecular species, which is

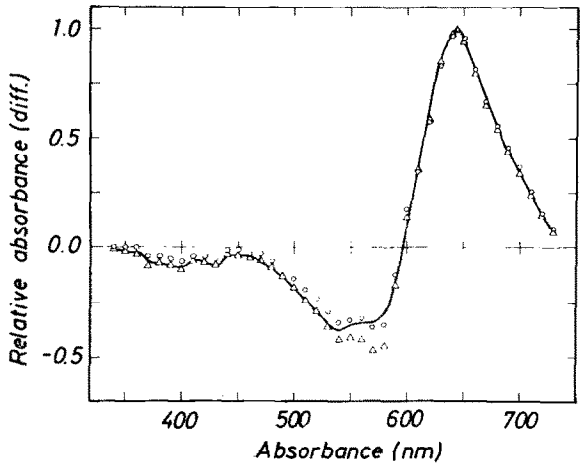

Fig.2. Difference spectra between *trans*-bR and its batho-product by irradiation at 9 K. Circles (○) and triangles (△) represent difference spectra between curve 1 and 6 in fig.1, and between curve 1 and 7 in fig.1, respectively. Solid line is a difference spectrum between batho-*trans*-bR and *trans*-bR at 77 K, which was calculated from curve 4 and 5 in fig.3b. These spectra are normalized with their difference maximum at 645 nm as 1.0.

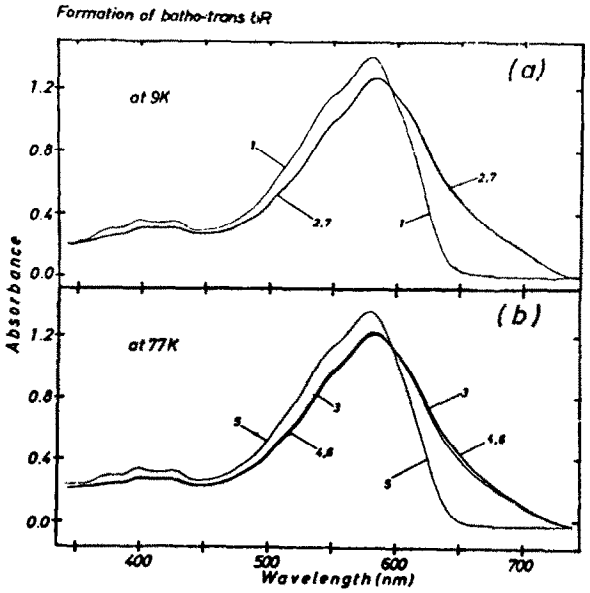

Fig.3. An experiment for confirmation of formation of batho-*trans*-bR by irradiation of *trans*-bR at liquid helium temperature. Curve 1: spectrum of *trans*-bR at 9 K. Curve 2: A photosteady state mixture (PSS 510) composed of *trans*-bR and batho-*trans*-bR which was formed by irradiation of *trans*-bR with green light (510 nm) at 9 K. Curve 3: spectrum of PSS 510 warmed to 77 K. Curve 4: a photosteady state mixture formed by irradiation of PSS 510 at 77 K (curve 3) with green light (510 nm). Curve 5: spectrum of *trans*-bR at 77 K which was formed by irradiation of PSS 510 (curve 4) at 77 K with deep-red light (>660 nm). Curve 6: PSS 510 at 77 K formed from *trans*-bR (curve 5) by irradiation with the green light (510 nm). Curve 7: spectrum obtained by re-cooling the PSS 510 at 77 K (curve 6) to 9 K. This spectrum is almost identical with curve 2.

the same species as the batho-*trans*-bR formed at 77 K. Very little inconsistency between plots and the solid curve may be due to the sharpening of the spectra by the cooling to 9 K.

For further confirmation of these findings, the photosteady state mixture formed by the irradiation of *trans*-bR (curve 1, fig.3a) at 510 nm (designated as PSS 510) (curve 2, fig.3a) was warmed from 9 K to 77 K (curve 3, fig.3b), and irradiated with light at 510 nm (curve 4, fig.3b). The spectrum scarcely changed. This indicates that PSS 510 formed at 9 K is the same as that at 77 K. We also compared PSS 510 formed at 77 K with that formed at 9 K by recooling it. PSS 510 (curve 6, fig.3b) formed from *trans*-bR at 77 K (curve 5, fig.3b) was recooled to 9 K (curve 7, fig.3a). The spectrum was identical with that at 9 K (curve 2, fig.3a). This result indicates that the batho-product formed at 9 K is batho-*trans*-bR as suggested above, and also that the amount of batho-*trans*-bR formed at 9 K is almost the same as that formed at 77 K. This fact may suggest that the ratio of photosensitivity between conversion of *trans*-bR to batho-*trans*-bR and its reversion does not change on warming from 9 K to 77 K.

4. Discussion

The irradiation of *trans*-bR at 9 K caused a bathochromic shift of its absorption spectrum and formed only batho-*trans*-bR. Any other molecular species was not observed.

According to the piscosecond laser flash photolysis [13], there is a precursor of batho-*trans*-bR, which has its λ_{max} in a longer wavelength region than batho-*trans*-bR. It was formed within 6 ps at 77 K and converted to batho-*trans*-bR with the half time of 11 ps. We did not observe such an intermediate at 9 K. Probably the photoproduct they observed may be the

singlet excited state and batho-*trans*-bR is the first photoproduct of *trans*-bR.

A study of *trans*-bR luminescence at 77 K [14] showed that the pre-illumination of *trans*-bR with light of the wavelength at 514.5 nm or 623 nm caused the increase of intensity in emission spectrum. It was concluded that the emitting molecular species was an intermediate (λ_{max} 597 nm), which was different from batho-*trans*-bR, and produced simultaneously with batho-*trans*-bR [14]. It was named P-bR. We failed to detect such a photoproduct.

The spectral change in the conversion of *trans*-bR to such intermediates as batho-, lumi- and meta-*trans*-bR resembles those of visual pigment. Our present results, however, show that *trans*-bR has no earlier intermediate than batho-*trans*-bR, which is unlike animal rhodopsin systems. In chicken iodopsin system, one of cone visual pigments, no hypso-product was observed by irradiation at liquid helium temperature [15]. Iodopsin has its λ_{max} at 560 nm which is close to those of bR. Bathoiodopsin and also batho-*trans*-bR convert to iodopsin and *trans*-bR on warming, respectively, without decomposing to protein and chromophoric retinal [16]. Those similarities may suggest that bacteriorhodopsin is similar to iodopsin in the interaction between the chromophoric retinal and the apoprotein rather than rhodopsins.

It should be noted that the amount of batho-*trans*-bR formed at 9 K by irradiation with light at 510 nm was the same as that formed at 77 K. This means that the ratio of photosensitivity of *trans*-bR to that of batho-*trans*-bR at liquid nitrogen temperature is the same as that at liquid helium temperature. Probably, this suggests that the photosensitivity of *trans*-bR is temperature independent, since it was observed [17] that the quantum efficiency of *trans*-bR at 77 K was the same as that at room temperature. The more detailed investigation is now in progress in cooperation with Dr Ebrey.

Acknowledgement

This work was supported in part by the grant from Japanese Ministry of Education to T. Y.

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