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FORMATION OF HYPSORHODOPSIN IN FROG RETINA

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Summary

Hypsorhodopsin was formed in frog retina by irradiation at liquid helium temperature and converted into bathorhodopsin above about 29 K.

Since 1958, bathorhodopsin had been regarded as the first photoproduct of rhodopsin, until Yoshizawa and Horiuchi (1972) [1] observed the formation of hypsorhodopsin by irradiating digitonin-extracted cattle rhodopsin at liquid helium temperature (4 K). On warming above 23 K, the hypsorhodopsin converts to bathorhodopsin. Therefore, it appears that hypsorhodopsin is an earlier intermediate in the photobleaching process of rhodopsin than bathorhodopsin. However, bathorhodopsin was also formed at the same temperature. In addition, in the early stage of the irradiation, isorhodopsin appears. Thus, Wald (1973) [2] insisted that hypsorhodopsin may be a photoproduct produced from isorhodopsin.

In order to obtain conclusive evidence that hypsorhodopsin is an earlier photoproduct of rhodopsin than bathorhodopsin under physiological conditions, one must confirm the formation of hypsorhodopsin in the retina at physiological temperature. Recently, picosecond laser spectroscopy confirmed that the excitation of squid rhodopsin in digitonin micelle yielded hypsorhodopsin within 19 ps at room temperature and then decayed with life time of 50 ps [3]. The present paper is a record of the first observation on the formation of hypsorhodopsin in retina by irradiation at liquid helium temperature.

A dark-adapted bull frog (*Rana catesbeiana*) retina was isolated in Ringer, held between two pieces of filter paper with a hole according to the method reported previously [4], and then set in an optical cell containing 66% glycerol-solution N [4] with 33 mM hydroxylamine in a final concentration. Finally the optical cell was fixed in a copper block in an optical Dewar described in Fig. 4b of Yoshizawa's review [1].

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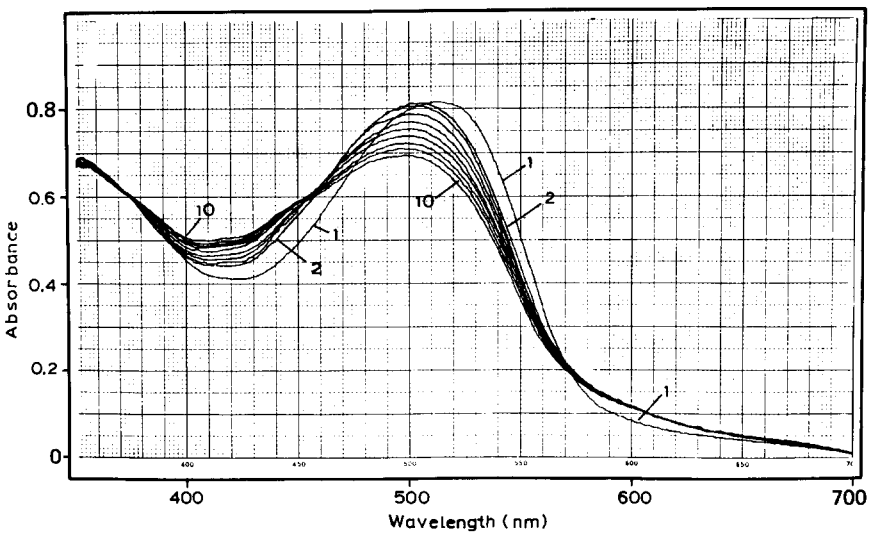

Fig. 1. The formation of hypsorhodopsin in a bull frog retina. The frog retina was cooled down to 4 K and irradiated with yellow light (>500 nm). Curve 1: rhodopsin, curves 2—10: products of irradiation for a total of 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 min. The final spectrum represents a mixture of rhodopsin, isorhodopsin and hypsorhodopsin with a small amount of bathorhodopsin.

On cooling the retina to liquid helium temperature (4 K), similar to a digitonin extract, the spectrum of rhodopsin in the retina shifted to the red (λ_{max}: 503 → 515 nm) and its absorbance increased.

On irradiation of rhodopsin in the retina at 4 K with yellow light (>500 nm), λ_{max} shifted to the blue with increase of absorbances in the ranges above 570 nm and between 376 nm and 508 nm and with decrease of absorbance between 508 nm and 570 nm (Fig. 1, curves 1 and 2), then the

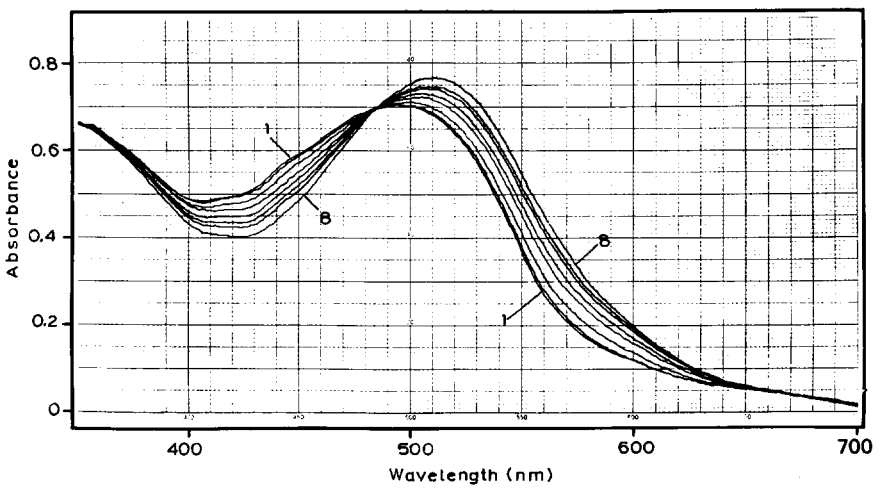

Fig. 2. The conversion of hypsorhodopsin to bathorhodopsin by warming. The mixture of rhodopsin, isorhodopsin and hypsorhodopsin was produced by irradiating rhodopsin with yellow light (>500 nm) for 3 h 54 min in liquid helium. After evaporation of the liquid helium, the sample was allowed to warm spontaneously and the spectra were measured at 26, 29, 32, 35, 38, 42, 53 and 77 K (curves 1—8). The final spectrum (curve 8) represents a mixture of rhodopsin, isorhodopsin and bathorhodopsin.

spectrum shifted gradually to the blue with the progressive decrease of maximal absorbance. The intersecting points among the spectra shifted from 508 to 455 nm. After the irradiation for 128 min, λ_{max} reached 498 nm and the maximum absorbance decreased remarkably. The spectral change of curve 1 to curve 2 is mainly due to the formation of isorhodopsin, because the intersecting point between curve 1 and curve 2 lies near the isosbestic point (510 nm at liquid nitrogen temperature [5]) between rhodopsin and isorhodopsin. While, the spectral change of curve 2 to curve 10 indicates the formation of hypsorhodopsin, because it is very similar in profile to the case of cattle [1] or chicken [6] rhodopsin-digitonin extract. On warming the irradiated sample above about 29 K in the dark, the frog hypsorhodopsin in the retina converted into bathorhodopsin with an isosbestic point at 485 nm, indicating that there is no stable intermediate in the process of the conversion from hypsorhodopsin to bathorhodopsin (Fig. 2). The hypsorhodopsin finally decayed to retinal-oxime by warming to room temperature.

The hypsorhodopsin in frog retina was estimated by the absorption maximum at about 430 nm and its extinction at about 0.9 times that of rhodopsin at liquid helium temperature. These spectral properties are similar to those of cattle and chicken rhodopsins [1,6]. While, bathorhodopsin are quite different in absorption maxima from one rhodopsin system to the other (cattle: 543 nm [7], chicken: 550 nm [6], frog retina: 565 nm, digitonin-extract (from frog): 555 nm [6]). Thus, the proximity of absorption maxima of hypsorhodopsin in cattle, chicken and frog systems (approx. 430 nm) may imply that the interaction between the chromophore and the opsin is a naked Schiff base. Squid hypsorhodopsin, however, shows its λ_{max} near 440 nm, so that the following explanations cannot be excluded: (1) Hypsorhodopsin may be a deprotonated Schiff base [1], (2) The conjugated double bond system of the retinal chromophore in hypsorhodopsin may be separated by some rotation at the 7—8 double bond, as suggested from an analogue rhodopsin having a retro-γ-retinal as the chromophore [8].

We confirmed hypsorhodopsin production in the retina by the irradiation, and showed that it is not an artifact in detergent-extracted sample. However, hypsorhodopsin cannot be detected at room temperature even using picosecond laser flash photolysis [9] except for the squid rhodopsin system [3]. The investigation whether or not hypsorhodopsin is produced in the retina at room temperature is our future subject.

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