EXISTENCE OF TWO FORMS OF BATHORHODOPSINS

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1. Introduction

A visual pigment, rhodopsin, decomposes via a series of intermediates to the final photo-products, all-*trans* retinal and opsin. Cattle bathorhodopsin (λ_{max}: 543 nm) [1] which has been thought to be the first photo-product of rhodopsin, was first observed by Yoshizawa and Kitô [2] who irradiated rhodopsin in liquid nitrogen. Afterwards, Grellmann et al. [3] carried out flash photolysis of cattle rhodopsin and found that the decay kinetics during the thermal conversion of bathorhodopsin to lumirhodopsin was fitted with three exponentials. However, three components were thought to be the same in absorption spectrum. The present paper shows that bathorhodopsin is composed of two molecular species of bathochromic photo-products, which are different in absorption spectrum at liquid nitrogen temperature.

2. Materials and methods

All the procedures were performed under dim red light. Cattle rod outer segments were isolated from retinas by method of Papermaster and Dreyer [4] except for use of 10 mM HEPES buffer (pH 6.8). From the purified rod outer segments, rhodopsin was extracted with 2% digitonin in 10 mM HEPES buffer (pH 6.8). After concentrating the rhodopsin, glycerol and neutralized hydroxylamine were added to it at the final concentrations of 67% and 0.1 M, respectively. The mixture thus obtained was used as a sample for low temperature spectrophotometry.

The absorption spectrum of the sample was measured at 77°K by use of a specially constructed glass-cryostat [5] attached to a recording spectrophotometer (323 Hitachi Co., Japan). The sample was irradiated with light from a xenon lamp (500 W, Ushio Co., Japan). The wavelengths for irradiation were selected by inserting a cut-off or a pair of cut-off and interference filters (Toshiba Co., Japan) between the sample and the xenon lamp.

3. Results and discussion

On cooling rhodopsin from room temperature to liquid nitrogen temperature (77°K), its λ_{max} moved from 498 nm to 504 nm. Irradiation with blue light (437 nm) shifted the spectrum to longer wavelengths with an isosbestic point at 513 nm (fig.1a). The kinetics of this conversion was plotted at various wavelengths in the range from 420 nm to 620 nm. All the kinetics showed one exponential (fig.1b), indicating that some formation of isorhodopsin is not effective on the kinetics and also rhodopsin is spectrally one form at 77°K. We also examined the kinetics of the conversion from isorhodopsin to bathorhodopsin, indicating that isorhodopsin is one form.

Alternatively the reversion of bathorhodopsin was kinetically examined. The photosteady state mixture was prepared by irradiating rhodopsin at 77°K with blue light (437 nm). This mixture consists of bathorhodopsin (51%), rhodopsin (33%) and isorhodopsin (16%). These percentages were calculated according to Yoshizawa and Wald [9]. On irradiating this mixture at 77°K with red light (>650 nm), the bathorhodopsin changed to a mixture of rhodopsin and isorhodopsin (fig.2a).

In this conversion an isosbestic point of the spectral changes shifted from 517 nm (fig.2a, above) to

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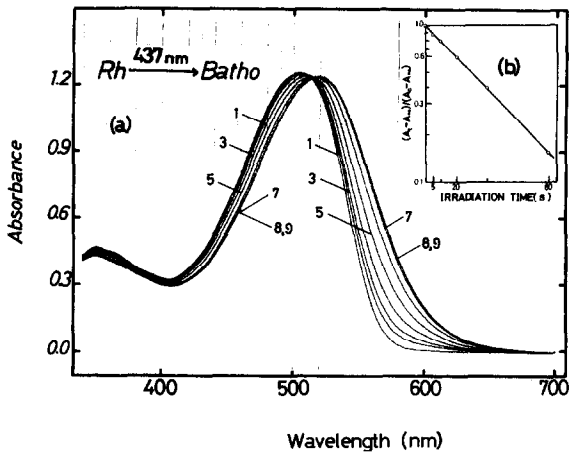

Fig.1. Course of photoconversion from rhodopsin to bathorhodopsin at 77°K. (a) Spectral changes. Curve 1; cattle rhodopsin (extracted with 2% digitonin) at 77°K. Curve 2–9, products of irradiation at 437 nm for successive periods of 5,5,10,20,40,80,160 and 320 s. The final spectra (curve 8 and 9) represent a photosteady-state mixture composed of rhodopsin, isorhodopsin and bathorhodopsin. (b) Kinetics. Absorbance changes were monitored at 480 nm.

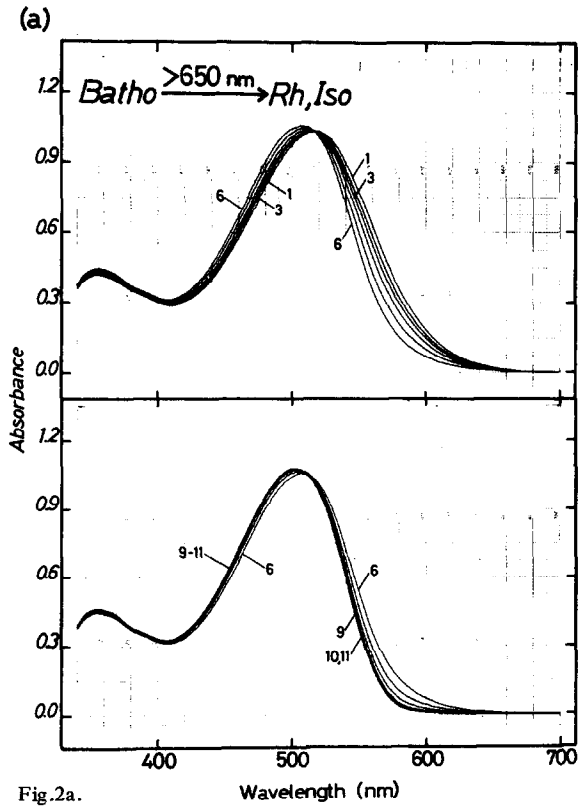

Fig.2a.

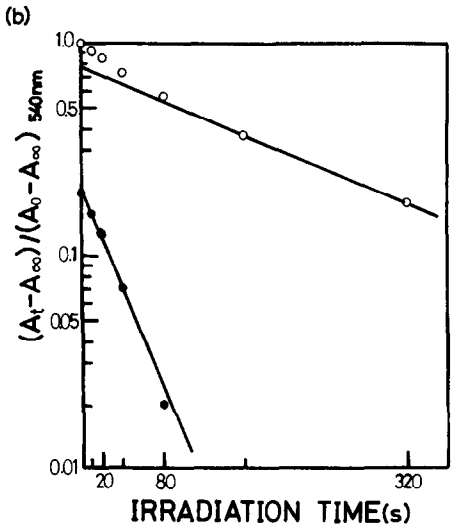

Fig.2. Course of photoconversion from bathorhodopsin to a mixture of rhodopsin and isorhodopsin at 77°K. (a) Spectral changes. Above; curve 1, a mixture of rhodopsin, isorhodopsin and bathorhodopsin produced by irradiation of rhodopsin with 437 nm light at 77°K. Curve 2–6, products of irradiation with red light (>650 nm) for 10,10,20,40 and 80 s. The spectral change shows conversion of bathorhodopsin$_1$ to mainly rhodopsin. The isosbestic point formed by curve 1–6 lies at 517 nm. Below; curve 6–11, products of irradiation with red light (>650 nm) for 80,160,320,640,1280 and 2560 s. The spectral change shows conversion of bathorhodopsin$_2$ to mainly rhodopsin. The isosbestic point formed by curve 6–11 lies at 513 nm. (b) Kinetics. Absorbance changes were monitored at 540 nm.

513 nm (fig.2a, below). The kinetics of the photoconversion was plotted at various wavelengths from 420 nm to 620 nm. All the kinetic curves were expressed by two exponentials (fig.2b), indicating existence of two forms of bathorhodopsins. We define that fast and slow processes are due to conversions of bathorhodopsin$_1$ and bathorhodopsin$_2$, respectively.

Now the absorption spectra of two forms of bathorhodopsins were determined by following procedures. First the photosteady-state mixture including two forms of bathorhodopsins produced by irradiating rhodopsin at 77°K with blue light (437 nm) and the spectrum of the mixture of two forms of bathorhodopsins was measured (fig.3, curve 1) by the method described by Tsukamoto et al. [6]. The mixture has its λ_{max} at 543 nm which is consistent with the bathorhodopsin reported by Yoshizawa and Wald [1]. We tentatively call this mixture Batho$_{437}$. Next Batho$_{437}$ was separated into two forms of bathorhodopsins by

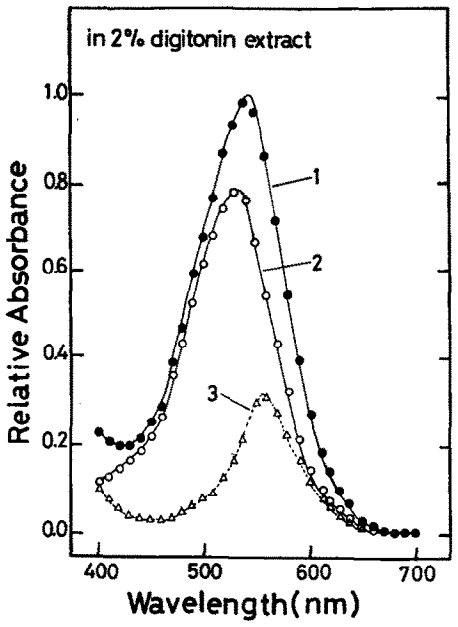

Fig.3. Curve 1, absorption spectrum of bathorhodopsin ($Batho_{437}$) at 77°K. The maximum absorbance of bathorhodopsin (λ_{max}: 543 nm) is normalized at its λ_{max}. The bathorhodopsin is composed of $bathorhodopsin_1$ (25%) and $bathorhodopsin_2$ (75%). Curve 2, absorption spectrum of $bathorhodopsin_2$ (λ_{max}: 538 nm). Curve 3, absorption spectrum of $bathorhodopsin_1$ (λ_{max}: 555 nm) which was calculated from the difference between curve 1 and curve 2.

irradiation with the red light at 77°K. Thus we obtained a mixture composed of rhodopsin, isorhodopsin and $bathorhodopsin_2$ without $bathorhodopsin_1$. Using this mixture the absorption spectrum of $bathorhodopsin_2$ was measured by Tsukamoto's method [6]. The amount of $bathorhodopsin_2$ in the $Batho_{437}$ was estimated to be 75% (±5%; average of five experiments) from the kinetics of photoconversion of $Batho_{437}$ with red light (>650 nm). In fig.3, the subtraction of curve 2 from curve 1 gave curve 3, the spectrum of $bathorhodopsin_1$ (25%). The maximum absorbance of $bathorhodopsin_1$ and $bathorhodopsin_2$ lies at 555 nm (±2 nm) and at 538 nm (±2 nm), respectively.

Our results showed that rhodopsin is a homogeneous molecular species spectroscopically, while bathorhodopsin is composed of two molecular species which are different in absorption spectrum. Recently, Stewart et al. [7,8] showed that the kinetics of conversion of metarhodopsin-I to metarhodopsin-II at room temperature could be expressed by two exponentials and suggested that all the intermediates of rhodopsin should be in multiforms. Thus, they infered two forms of rhodopsins which are in equilibrium with each other.

However, we failed to observe the multiforms of rhodopsin on the kinetics of photoconversion of rhodopsin. Therefore, the existence of two forms of bathorhodopsins which we have found, should be not due to the heterogenity of rhodopsin itself, but probably to some difference in twisted all-*trans* form of chromophoric retinal. Recently, we found two forms of bathorhodopsins in frog retina and its rod outer segment suspension. The detail results including cattle and frog rhodopsins will be published elsewhere.

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