SQUID HYPSORHODOPSIN AND BATHORHODOPSIN BY A PICOSECOND LASER PHOTOLYSIS

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1. Introduction

The formation of bathorhodopsin (formerly prelumirhodopsin) was first observed by Yoshizawa and Kitô [1] who irradiated cattle rhodopsin at liquid nitrogen temperature (77 K). Afterwards, the spectra of batho-intermediates in various animals were measured by low temperature spectrophotometry [2]. Therefore it is generally believed that bathorhodopsin is the earliest photoproduct in the photobleaching process of rhodopsin. Recently it was found that irradiation of cattle [2] or squid rhodopsin [3] at liquid helium temperature yielded two thermolabile photoproducts – hypsorhodopsin (λ_{max}: cattle 430 nm, squid 446 nm) and bathorhodopsin (λ_{max}: cattle 543 nm, squid 534 nm). Now the question arises: which is an earlier photoproduct of rhodopsin, hypsorhodopsin or bathorhodopsin? In order to elucidate this problem, we tried a picosecond laser flash photolysis of squid rhodopsin using a 347 nm light pulse from a mode locked ruby laser.

2. Materials and methods

Squid (*Todarodes pacificus*) rhodopsin was extracted by a method described in a previous paper [4], i.e., the rhabdomeres were isolated from the retinas by the sucrose (40%) flotation method, and then rhodopsin in the rhabdomeres was extracted with 2% digitonin in 0.1 M sodium carbonate buffer (pH 10.5). If necessary, the extract was concentrated by ultracentrifugation at 105 000 × g for more than 12 h. The absorbance of preparation used for the experiment is 4.0–15.0 in 1 cm light path at the absorption maximum (480 nm).

An apparatus for picosecond laser spectroscopy described by Kobayashi and Nagakura [5] was used with slight modifications. A selected single pulse at 694.3 nm from a mode rocked ruby laser system (JEOL) was frequency-doubled with a phase matched ADP crystal. The second harmonic at 347.2 nm (20 ps pulse width, ca. 5 mJ) thus obtained was used as an exciting pulse. For monitoring pulses, the single pulse (694.3 nm) was focussed at a self phase modulation cell containing phosphoric acid (H_3PO_4) in order to generate a broad continum pulse, and then it was converted into 14 pulse train at intervals of 19 ps by an echelon. Both the exciting pulse and the third pulse in the monitoring pulse train were adjusted to arrive simultaneously at a sample cell (light path: 0.2 cm) containing the rhodopsin (0.3 ml). Two sets of monochromator (Jarrell Ash : JE-25) and Vidicon with multichannel analyzer (PAR, OMA 1205A/1205D) were used for measurements of light intensities of the monitoring pulses and the analysis of the data was made by a computer (YHP 9825A). Maximal absorbance change was 0.2–0.3 in the optical path where the exciting laser pulse traveled in our experimental condition.

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3. Results

On the excitation of rhodopsin at 21°C, the absorbance at 430 nm (near the maximum of difference spectrum between rhodopsin and hypsorhodopsin [3]) increased within 19 ps and then gradually decreased (fig.1). These processes represent a rapid formation and a relatively slow decay of hypsorhodopsin. The decay time constant $\tau_{1/e}$) was estimated at about 50 ps from a logarithmic plot. We also measured the absorbance at 550 nm (near the maximum of difference spectrum between rhodopsin and bathorhodopsin [3]) after the excitation of rhodopsin at 21°C. The increase of the absorbance indicates the formation of bathorhodopsin (fig.2). The formation time constant of bathorhodopsin ($\tau_{1/e}$) was calculated at about 50 ps, which is consistent with the decay time constant of hypsorhodopsin. This is the first evidence of the formation of hypsorhodopsin at room temperature, which is an earlier intermediate than bathorhodopsin.

In the course of these experiments, particular care was taken against the mixing of recovering process of alkaline metarhodopsin to rhodopsin, because a small amount of alkaline metarhodopsin (< 5%), which is rather stable at 21°C, existed in our preparation after the first excitation. As a consequence, the sample was never used after the second excitation. Our preliminary experiment showed that the excitation of alkaline metarhodopsin increased the absorbance at 430 nm with the time constant of about 60 ps. Therefore the increase of the absorbance at 430 nm (fig.1) can not be explained as the recovering process of alkaline metarhodopsin to rhodopsin.

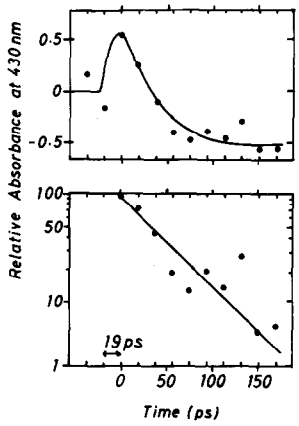

Fig.1. Formation and decay of hypsorhodopsin after the excitation of rhodopsin with a laser pulse (wavelength: 347 nm, pulse width: 20 ps) at 21°C. The points in the figure represent averages of three measurements. Initial increase in absorbance at 430 nm is due to the formation of hypsorhodopsin and successive decrease indicates the decay of hypsorhodopsin to bathorhodopsin.

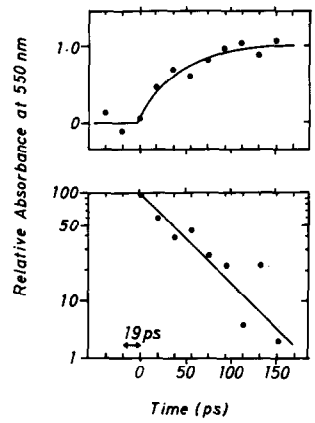

Fig.2. Formation of bathorhodopsin after the excitation of rhodopsin at 21°C. The points in the figure represent averages of three measurements. The formation time constant is comparable to the decay time constant of hypsorhodopsin.

4. Discussion

From these results, it may be clear that hypsorhodopsin is a physiological intermediate in the photobleaching process of rhodopsin and a precursor of bathorhodopsin. It is interesting that the formation of cattle bathorhodopsin at room temperature is one order faster (within 6 ps [6]) than that of squid bathorhodopsin. As to the difference in the formation time constant between cattle and squid bathorhodopsins, three explanations may be possible. The first is the difference in wavelength of the excitation pulse between squid (347 nm) and cattle (530 nm) experiments. The second is the difference in detergent for extraction of rhodopsin between digitonin (squid) and LDAO (cattle). The third difference is due to the intrinsic difference of opsin structure. In fact, it was found in 2% digitonin that squid hypsorhodopsin

converts to bathorhodopsin above 35 K [3], while cattle hypsorhodopsin above 24 K [2].

In preliminary experiments of picosecond laser photolysis at liquid nitrogen temperature, the formation of squid hypsorhodopsin was observed without any formation of bathorhodopsin within 260 ps. The formation time constant of hypsorhodopsin at liquid nitrogen temperature was estimated at about 70 ps, which was much slower than that at room temperature. These results suggest that a conformational change of the retinal chromophore may occur in the conversion of rhodopsin to hypsorhodopsin, because the rate of the conversion was affected by the cooling which may induce the increase of viscosity of the environment around the retinal chromophore. Thus an isomerization of twisted 11-*cis* retinal to twisted 11-*trans* form may occur in the conversion of rhodopsin to hypsorhodpsin. Further experiments on this hypothesis are being carried out.

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