PRESSURE INDUCED INTERMEDIATES IN THE PHOTOCHEMICAL REACTION OF SQUID RHODOPSIN

M. Tsuda, I. Shirotani*, S. Minomura*, and Y. Terayama

Department of Physics, Sapporo Medical College,
Sapporo 060, Japan

and

* Institute for Solid State Physics, University of Tokyo,
Roppongi, Tokyo 106, Japan

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SUMMARY Studies of the pressure effect on the photochemical reaction of squid rhodopsin have been initiated. On irradiation of rhodopsin with blue light at 6 kb, an intermediate having absorption maximum at 502 nm appeared, which we call p-lumirhodopsin. Upon release of pressure, a new intermediate having absorption maximum at 472 nm, which we call p-LM-rhodopsin. Molar free volume change takes place in the transformations of p-lumirhodopsin ⟶ p-LM-rhodopsin and p-LM-rhodopsin ⟶ Metarhodopsin.

INTRODUCTION

With caption of a photon, rhodopsin decomposes over a series of intermediates to the final products. To describe the molecular mechanisms of signal transduction in photoreception, it is important to elucidate the intermediate steps in the photobleaching sequence where change in conformation of rhodopsin and in the interaction of rhodopsin and its surrounding field takes place. Protein conformational change involves internal reorganization of the protein, so that one would expect activated entropy change and activated volume change accompanying the reaction of the protein conformational change (1,2). The low temperature spectroscopical method makes it possible to observe some intermediates in the thermal interconversion of rhodopsin accompanying the activated entropy change (3). If the interconversion involves a change in activated molar volume, a pressure induced intermediate may be expected to exist under a high pressure.

The investigation described here shows that the irradiation of squid rhodopsin under a high pressure at physiological temperature yields some intermediates, which are similar to those observed at cryogenic temperature (4-6).

MATERIALS AND METHODS

Squid (Surumeika, Todarodes pacificus) were used as experimental materials. Extraction and purification of rhodopsin were carried out by the method of Suzuki et al. (7). The outer segments of the photoreceptor cells were isolated

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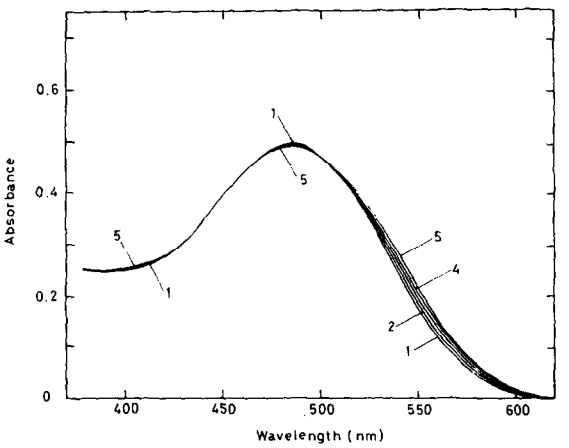

Fig. 1. Photoconversion of rhodopsin into p-lumirhodopsin at a pressure of 6 kb at 5°C by irradiation at 436 nm. Curve 1 ; squid rhodopsin in 2% digitonin at 5°C, pH 9.8 (0.1 M imidazole buffer) at 6 kb. Curve 2-5 ; products of irradiation at 436 nm for successive periods of 20, 60, 240, 360 sec.

by flotation with sucrose, washed and extracted with 2% digitonin in 0.1 M imidazole buffer at pH 10.2. The pH value of imidazole buffer used in this work increased by more than 0.1 on increasing the pressure from 1 b to 6 kb (8).

Absorption spectra were measured with a Shimadzu SV-50 spectrophotometer with a high pressure optical bomb. The optical bomb, with a maximum pressure rating of 10 kb, contained an internal optical cell of quartz (0.3 cm path length) and was thermostated by its jacket. The details of the apparatus used for pressure generation and measurement and optical bomb were described elsewhere (8). The sample was irradiated by blue light (λ = 435 nm) from a 150-W mercury lamp using glass filters (Toshiba VB-40, VY-42 and Hoya thermal filter). This blue light was focussed by an optical system and led to the sample cell through an optical glass fibre.

RESULTS AND DISCUSSION

Under high pressure an absorption band of rhodopsin increased in intensity and shifted slightly to a longer wavelength. The absorption maximum moved from 480 nm at 1 b to 487 nm at 6 kb. Its intensity increased 1.11 times due to the contraction of the solvent under pressure. These changes were completely reversible upon release of the pressure even when the samples were kept under the pressure for more than two hours.

The rhodopsin was irradiated with blue light (the mercury blue line at 436 nm) at 6 kb at 5°C. As the irradiation went on, the spectrum shifted to longer wavelengths, with slight decrease in absorbance at the maximum. The result was shown in Fig. 1 and the behavior was similar to that of the production of lumirhodopsin on irradiation of rhodopsin at a cryogenic temperature

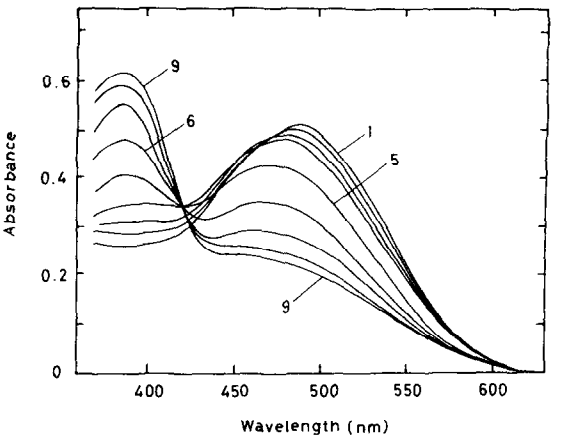

Fig. 2. Interconversion of p-lumirhodopsin to p-LM-rhodopsin and alkaline metarhodopsin upon release of pressure in the dark at 5°C. Rhodopsin at 6 kb was irradiated at 436 nm for 360 sec (curve 1 which is the same as curve 5 in Fig. 1). Curve 2-9, the pressure upon the preparation was released down to 5.7, 5.3, 5.0, 4.5, 3.5, 2.5, 1.5 kb, and 1 b, respectively. The spectra were measured at 6 kb.

(4,5). In order to evaluate the pressure-induced intermediates, the pressure upon the preparation was released to an appropriate value and re-pressed to 6 kb in the dark to measure the spectra. At a released pressure, observation of the spectra was repeated at an interval of 10 min until the last two spectra were considered to be indentical. The spectra were shown to equilbriated at each pressure. Figure 2 shows the spectra of the preparation (due to release of the pressure) observed at 6 kb. In the early stage of release of the pressure in the dark, the spectrum shifted to a shorter wavelength, with slight decrease in absorbance at the maximum. The isosbestic point of the spectra shifted to a shorter wavelength down to a pressure of 4.5 kb (curve 5 in Fig. 2). Upon successive release of the pressure, the absorption band around 470 nm fell and the absorption band around 390 nm rose, which was ascribed to the production alkaline metarhodopsin. A sharp isosbestic point was observed at about 420 nm. The above results showed that an intermediate produced by light under a high pressure transformed via another intermediate to alkaline metarhodopsin upon release of the pressure in the dark. The existence of the intermediate at a lower pressure was confirmed by the fact that the irradiation of rhodopsin with blue light at 3 kb shifted the spectrum slightly to shorter wavelengths with the maximal absorbance falling. We named the intermediate at a higher pressure p-lumirhodopsin and that at a lower pressure p-LM-rhodopsin

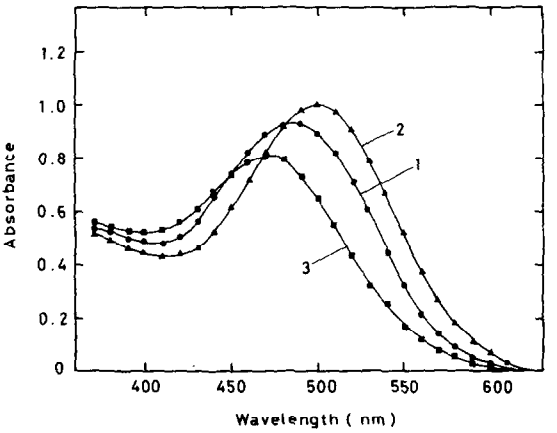

Fig. 3. Absorption spectra of squid rhodopsin (curve 1) p-lumirhodopsin (curve 2), and p-LM-rhodopsin (curve 3) at 5°C at 6 kb. The maximum absorbance of rhodopsin (λ_{max} : 487 nm) is plotted arbitrarily at 1.0. P-lumirhodopsin (λ_{max} : 502 nm) and p-LM-rhodopsin (λ_{max} : 475 nm) of of equivalent concentration possess maximum absorbance of about 1.07 and 0.65 times, respectively, as high as that of rhodopsin.

after the intermediates at cryogenic temperature (4-6), where p implies the pressure induced intermediate.

The scheme for the photoconversion of rhodopsin to the pressure-induced intermediate and its interconversion to alkaline metarhodopsin upon release of the pressure in the dark can be illustrated as,

$$\text{Rhodopsin} \xrightarrow{h\nu} \text{P-lumirhodopsin} \longrightarrow \text{P-LM-rhodopsin} \longrightarrow \text{Alkaline Metarhodopsin.}$$

Ebina et al. (9) showed by the method of flash photolysis that acid metarhodopsin was also formed transiently from mesorhodopsin, which corresponds to LM-rhodopsin observed at a cryogenic temperature, in alkaline wolution. We could not detected acid metarhodopsin under alkaline conditions, probably owing to its short life time compared with the time for measurement.

Spectra of the pressure-induced intermediates were evaluated as follows. The molar equivalent of the difference spectrum of p-lumirhodopsin and alkaline metarhodopsin was calculated from the difference spectrum of curve 1 and curve 9 in Fig. 2 and from the content of rhodopsin in the preparation in curve 9 in Fig. 2. Content of rhodopsin in curve 9 was estimated as 30% with complete bleaching of the preparation in alkaline solution on the irradiation of light (λ > 480 nm) at 1 b and 5°C. The addition of the difference spectrum to the spectrum of alkaline metarhodopsin of an equivalent concentration yields the spectrum of p-lumirhodopsin (curve 2 in Fig. 3).

The existence of an isosbestic point at about 465 nm exhibited in the interconversion between p-lumirhodopsin and p-LM-rhodopsin is of considerable help in obtaining the content of p-LM-rhodopsin and alkaline metarhodopsin in the preparation. From curve 6 in Fig. 2, content of p-LM-rhodopsin in the preparation was estimated as 62% at 3.5 kb. Using this and the difference spectrum of curve 6 and curve 9, the spectrum of p-LM-rhodopsin was evaluated with the same procedure as that of p-lumirhodopsin and shown in curve 3 in Fig. 3.

P-lumirhodopsin posseses an absorption maximum of about 502 nm and a maximal absorbance 1.07 times that of lumirhodopsin. This spectrum is similar to that of lumirhodopsin, though its absorbance is somewhat larger (5). The absorption maximum of p-LM-rhodopsin is at 472 nm and the maximal absorbance is 0.65 times, which is somewhat smaller than that of LM-rhodopsin (5). Due to a similarity of the spectral pattern, the pressure induced intermediates were considered to be the same intermediates which appeared in the photobleaching step of rhodopsin at cryogenic temperatures. Molar volume seemes to increase in the transformations of lumirhodopsin ⟶ LM-rhodopsin and LM-rhodopsin ⟶ metarhodopsin, since the intermediates, p-lumirhodopsin and p-LM-rhodopsin, only existed under high pressure at 5°C. Several authors considered the possible source of molar volume changes for the conformational change of proteins and suggested that the solvation of side chains exposed during a conformational change should lead to a large change in the molar volume (1,2). The above results suggest that the change in the interaction between rhodopsin and its surrounding field as well as the conformational change in the protein part of rhodopsin takes place in the transformations of lumirhodopsin ⟶ LM-rhodopsin and LM-rhodopsin ⟶ metarhodopsin.

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