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Effect of high pressure on the light-induced structural change of bacteriorhodopsin reconstituted in liposome

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Bacteriorhodopsin (BR) is reconstituted into multilamellar phosphatidylcholine vesicles. When this reconstituted bacteriorhodopsin is under high pressure without illumination, a structural transition, in which the absorption maximum of the retinal changes from 560 nm to 500 nm, occurs reversibly. Upon illumination, bacteriorhodopsin reconstituted into vesicles is denatured irreversibly and, under high pressure, a 460-nm absorption peak of the chromophore appears transiently.

Introduction

Bacteriorhodopsin, a photoactive protein m purple membrane (PM) of *Halobacterium halobium*, has a unique absorption peak at 560 nm [1]. In the purple membrane, bacteriorhodopsin forms trimers which are arranged in a hexagonal lattice structure [2]. A BR monomer consists of seven membrane-spanning α-helices connected by short chains exposed to the aqueous environments [3]. Bacteriorhodopsin has 248 amino acid residues and its sequence [4,5] shows seven hydrophobic stretches which are thought to traverse the membrane. The seven α-helices are closely packed together to form an 'inside-out' structure [6]. Bacteriorhodopsin has one retinal which is attached to the polypeptide through a protonated Schiff base [7]. As a result of interactions between the retinal and vicinal amino acid residues of bacteriorhodopsin, the 380-nm absorption peak of the retinal is shifted to 560 nm [8]. Bacteriorhodopsin exists in two structural forms, the light-adapted and the dark-adapted forms; these are reversibly interconvertible with light [9]. When illuminated, the light-adapted bacteriorhodopsin goes through a photocycle with a number of intermediates with unique absorption peaks. This cycle brings about proton pumping [10]. This photocycle is accompanied by a structural change with a concomitant shift in absorption peak [11]. The intermediates generally recognized to date are LA-I-J-K-L-M-O-LA [12]. Under high pressure, the reaction time of the photocycle of the light adapted bacteriorhodopsin is retarded [13]. The existence of I_{460} intermediate preceding the J intermediate was reported [14]. More recent studies by Mathies et al. [15] and Zinth [16] showed that the singlet intermediate I_{460} is formed within 200 fs.

The present investigation extends the effect of high pressure on the photocycle of bacteriorhodopsin in purple membrane to the bacteriorhodopsin-reconstituted vesicles. It is hoped that further insight into the mechanism of the bacteriorhodopsin photocycle may be obtained from this study.

Materials and Methods

H. halobium R_1 was obtained from American Type Collection, Rockford, MA, U.S.A. Phosphatidylcholine was purchased from Sigma, St. Louis, MO, U.S.A.

The bacteria was grown and purple membrane was isolated using the procedures described in the literature [17]. Phosphatidylcholine (PC) vesicles were prepared by sonicating the PC suspension in 0.01 M Tris buffer (pH 7.5) with a 20 kHz sonicator. The vesicles were passed through a Sepharose 4B column and the fractions containing the vesicles of around 50 nm in diameter were collected. The incorporation of bacteriorhodopsin into the vesicles was achieved by using the sonication method of Racker [18]. The bacteriorhodopsin-reconstituted vesicles were passed through a Sep-

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harose 4B column to obtain an homogeneous size distribution.

High pressures were obtained with an oil pressure generator (Harwood Engineering Co., MA, U.S.A.). The high-pressure optical cell was a Daniel type [10]. The spectroscopic change was observed with a Cary-17D spectrophotometer. Pressure up to 4000 atm was exerted on the bacteriorhodopsin-reconstituted vesicles in 0.01 M Tris buffer (pH 7.5) in the dark at 20 °C. For a typical experiment, a spectrum was obtained after each 500 atm increment. When 4000 atm was reached, the pressure was lowered slowly to 1 atm and kept at 4 °C in the dark. These experiments were repeated under illumination with a lamp at 500 nm wavelength.

Results and Discussion

Purple membrane. Within the range of pH 7–10, 20–40 °C and 1–4500 atm, no change in the spectra of purple membrane was observed. It appears that the structure of bacteriorhodopsin in the purple membrane is stable and no structural change occurs at high pressure under the experimental conditions described above.

Bacteriorhodopsin-reconstituted vesicles. Fig. 1 shows the spectral change of bacteriorhodopsin-reconstituted vesicles under pressure without illumination. It can be seen that the 560-nm absorption maximum of dark-adapted bacteriorhodopsin at 1 atm shifted to 500 nm at a pressure above 2000 atm. Beyond 3000 atm, there are small increase in absorbance without further change in the maximum absorption wavelength. This increase in absorbance may be due to a decrease in the specific volume of water at high pressure [20]. The 500-nm peak

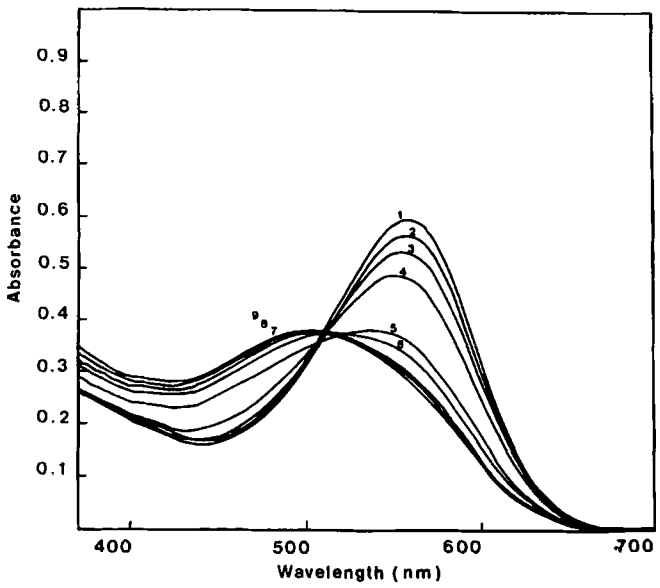

Fig. 1. Effect of pressure on absorption spectra of bacteriorhodopsin-reconstituted vesicles in 0.01 M Tris buffer solution (pH 7.5, 20 °C) with no illumination. Each hour the pressure was increased with 500 atm: from 1 atm in (1) until 4000 atm in (9).

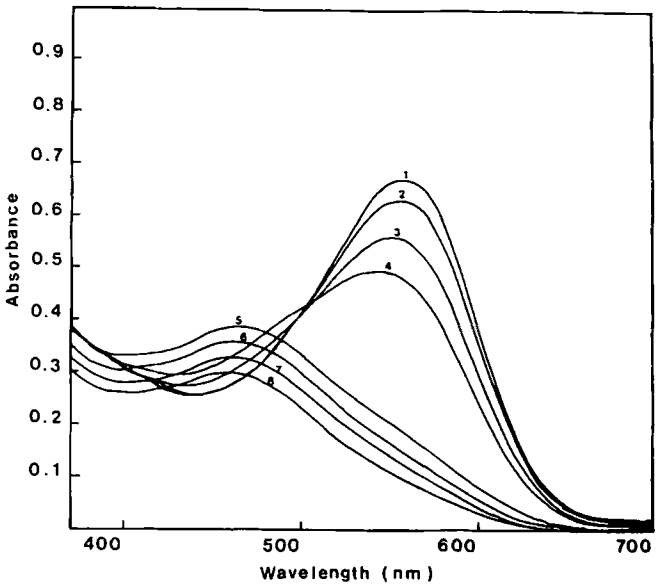

Fig. 2. Effect of pressure on the absorption spectra of bacteriorhodopsin-reconstituted vesicles in 0.01 M Tris buffer solution (pH 7.5, 20 °C) with illumination of 500 nm. Each 1–2 hour the pressure was increased with 500 atm, except at (5) where te pressure was kept during 6 hours. The pressure increases with 500 atm continued until 3500 atm in (8).

seems to be stable above 3000 atm. When the pressure was brought down to 1 atm after reaching 4000 atm and kept in the dark at 4 °C for 36 h, the 560-nm absorption peak was regained.

The existence of 500 nm chromophore, which is stable in an alkaline solution saturated with diethyl ether, was reported by Oesterhelt [21]. It was also found that the pH-induced change between 560 nm chromophore and that of 500 nm is reversible and the 500 nm chromophore also has an M_{412} intermediate. These phenomenon are very similar to our observation above that the 500 nm chromophore exists as a stable intermediate at high pressure in the dark and the pressure-induced structural transition is also reversible.

Fig. 2 shows the effect of pressure on the absorption spectra of bacteriorhodopsin incorporated into vesicles when illuminated with 500 nm light beam. With the increase in pressure, there is a gradual decrease in absorbance accompanied by a blue shift. Beyond 200 atm, however, the maximum absorption at 460 nm is stabilized, although a further decrease in absorbance can be seen. When the pressure was brought back to 1 atm and kept in the dark at 1 atm and after the bacteriorhodopsin-reconstituted vesicles were pressurized at 4000 atm for 1 h, there was no reversal to 560 nm absorption maximum. This irreversible structural change occurred even at 1 atm when illuminated with 500 nm light. When the illumination was terminated after the 460 nm absorption peak was reached, the structural transition still continued as shown in Fig. 3. A similar phenomenon was observed when the 500 nm

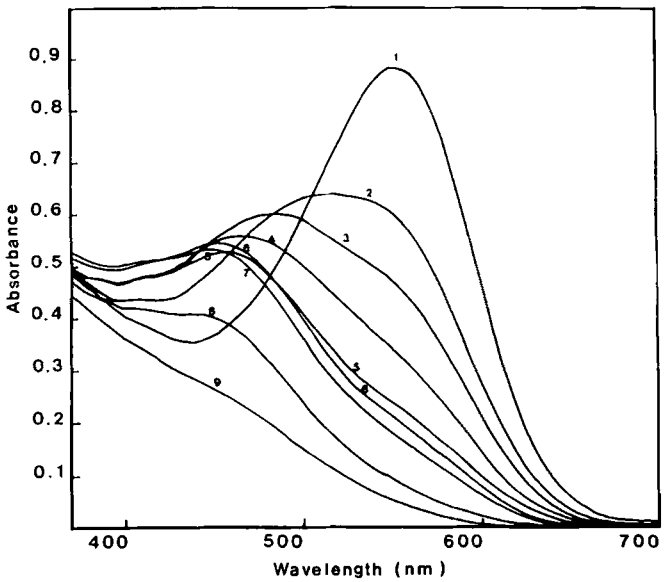

Fig. 3. Change of absorption spectra of bacteriorhodopsin-reconstituted vesicle in 0.01 M Tris buffer solution (pH 7.5, 20 °C) under the pressure (2000 atm). 500 nm illumination was applied from (1) to (5) and turned out after (5). Illumination was on during 5 h at (1); idem at (2), (3), (4) and (5); during 4 h at (6); during 8 h at (7), during 48 h at (8) and finally 80 h without illumination at (9).

absorption peak was obtained first under pressure and the vesicle-incorporated bacteriorhodopsin was subsequently illuminated (Fig. 4). Therefore, there seems to be no reversibility between 460 nm and 500 nm absorption maxima under high pressure. This may arise from the irreversible structural transition of the light-adapted bacteriorhodopsin.

These studies show that reversible structural change occurred in bacteriorhodopsin reconstituted into the vesicles when subjected to high pressure without illumination. This reversible change from 560 nm chromophore and vice versa seems to be caused by water

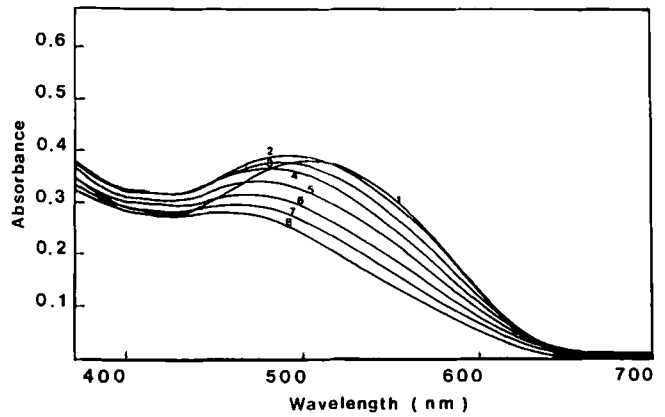

Fig. 4. Effect of 500 nm light on the absorption spectra of BR-reconstituted vesicle under the pressure of 400 atm. The 500 nm light was illuminated after 500 nm peak. (1) was gained under no light. (1) 4000 atm under no light. Illumination with 500 nm light and pressure of 4000 atm was given during 1 hour at each of the stages (2)–(8).

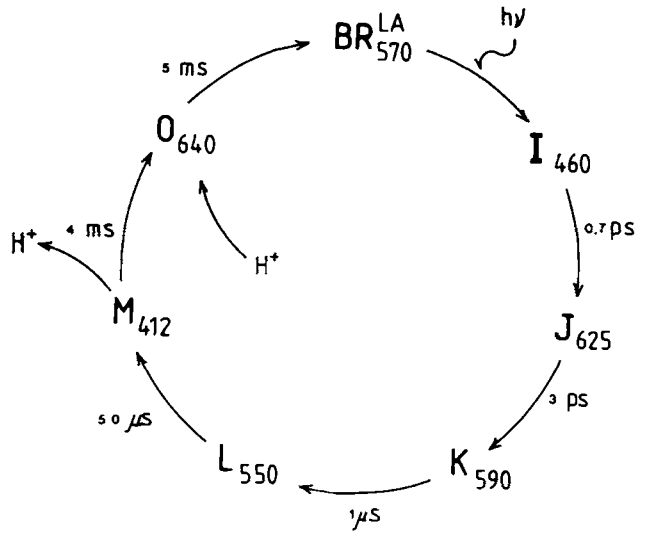

Fig. 5. Photoreaction cycle of light-adapted bacteriorhodopsin. I_{460} intermediate was inserted.

molecules which were extrude from bacteriorhodopsin under high pressure [20].

It has been suggested that high pressure retards the reaction time of the photocycle after the second step, but does not retard it during the first step which absorbs photons [13]. Fig. 5 represents the photocycle of light-adapted bacteriorhodopsin. It appears that irreversible denaturation of bacteriorhodopsin in vesicles occurs after a certain intermediate when the bacteriorhodopsin-incorporated vesicle conducts the photocycle upon illumination, because the bacteriorhodopsin in the vesicle is less stable than that that in purple membrane. This intermediate may be the M_{412}, as can be seen from the small shoulder at a wavelength of 412 nm in Fig. 3. When pressure is exerted on bacteriorhodopsin-reconstituted vesicles, with concomitant illumination with 500 nm light, there seems to be an accumulation of an intermediate. This is likely to I_{460}, because there are no other intermediates absorbing light at around 460 nm. This means that the lift-time of the intermediate is increased. It should be noted that the absorbance at 460 nm is comparably large and no other absorption peak appeared. This may mean that the value of the volume increment, ΔV, is large for step of I_{460} to J_{635} and there must be a large structural change with this transition. The detailed studies by Mathies et al. [15] and also by Zinth [16] clearly demonstrated that first a relaxed excited single state intermediate I_{460} is formed within 200 fs and this is converted on the 500-fs time scale into J_{625}. Our results, however, showed a long-lived intermediate suggested to be the I_{460}. Additional studies on the structures of I_{460} and J_{625} using the fs optical measurement technique are needed to clarify the reason for pressure prolonging the life-time of I_{460} and for bacteriorhodopsin in the purple membrane not showing this phenomenon.

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