diyne reacted with anthraquinone to give the same type of 1:1 adduct (**8**). The UV spectra and fluorescence spectra of the photoproducts (**3**, **4**, and **8** are shown in Figure 2 and Figure 3, respectively.

The IR spectrum of (**8**) shows the two C = O stretching bands at 1672 cm^{-1} and 1670 cm^{-1}, and C ≡ C stretching band at 2180 cm^{-1}. The mass spectrum of (**8**) showed the molecular ion peak at *m/e* 410. The peaks at *m/e* 105 and *m/e* 77 prove the existence of benzoyl group.

From the above results, conjugated diyne, such as diphenylbutadiyne, reacts with *p*-quinones to give 1:1 photoadducts. No 1:2 adduct, such as (**11**) and (**12**), was found in these reactions. The fluorescence of *p*-benzoquinone was quenched by diphenylbutadiyne. A plot of ϕ_F^0/ϕ_F versus [diphenylbutadiyne] is linear with intercept equal to 1.0 and slope ($k_q\tau$) equal to 265 M^{-1}. The values of the slope were 107, 20 and 0.85 M^{-1} for chloranil, anthraquinone and thioxanthone, respectively. In fact, the reactivity of thioxanthone toward diphenylbutadiyne was very low.

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Effects of SDS and Temperature on the Structural Changes of Bacteriorhodopsin

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Effects of SDS and temperature on the conformational changes of bacteriorhodopsin were studied using a, b, c bands of bacteriorhodopsin. **In the SDS denaturation, bacteriorhodopsin in purple membrane was more labile than bacteriorhodopsin reconstituted into PC vesicles. These rather interesting results may be understood by effective SDS concentration in lipid layer.**

Introduction

Bacteriorhodopsin (BR), a photoactive protein in purple membrane of Halobacterium halobium, has three absorption bands in the visible and near -UV: a band (e = 63000 M cm) at 570 nm, b band which lacks a clear maximum between 450 nm and intense band at 280 nm[1]. Both a and b bands are due to the retinyl chromophore and band to the aromatic protein residue, tyrosine and tryptophan[2].

BR has "inside -out" structure of which the hydrophibic amino acid residues are located in the outside of protein[3]. The hydrophobic interaction of those nonpolar residue and ionic interactions among inside polar amino acid residue contribute to the stability of BR[4]. As the environment around BR is perturbed, the stability of BR is altered via the change in above interaction. In extreme condition the denaturation of BR occured. Sodium dodecyl sulfate (SDS) is often used as a means of denaturation of protein[5,6].

In present investigation, BR was incorporated into the phosphatidyl choline vesicle and change in stability of BR was observed using SDS and temperature.

Materials and Methods

Halobacterium halobium R1 was obtained from American type Collection, Rockford, MD, U.S.A.. L-α-phosphatidyl

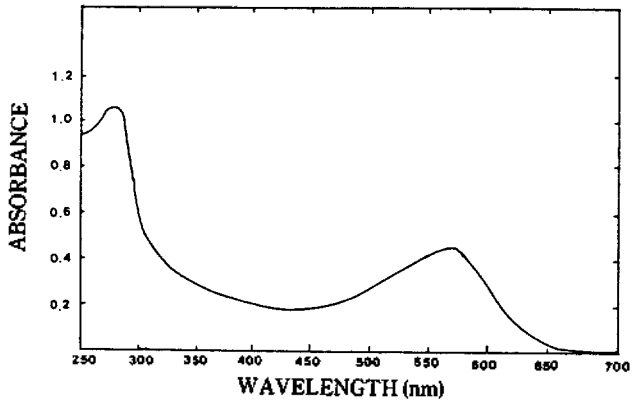

Figure 1. The absorption spectrum of purple membrane in distilled water at room temperature.

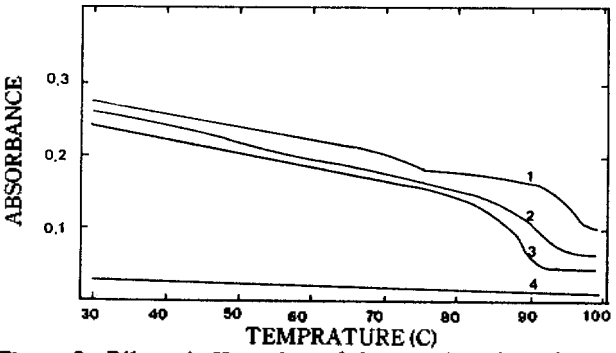

Figure 2. Effect of pH on thermal denaturation of purple membrane. Absorbance at 568 nm were taken from Gilford spectrophotometer; (1) pH 7.0 (2) pH 7.0 (2) pH 9.0 (3) pH 11.0 (4) pH 12.8.

choline and sodium dodecyl sulfate (SDS) were purchased from Sigma. Sepharose 4B was purchased from Pharmacia Fine Chemical Co.

The bateria was grown and purple membrane was isolated using the procedures described in the literature[7]. Phosphatidyl choline (PC) vesicles were prepared by sonicating the phosphatidyl choline 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 cotaining the vesicles around 50 nm dia were collected. The incorporation of BR into the vesicles was achieved by using the sonication methods of Racker[8]. These samples were used uncolumned -incorporated BR vesicle. Uncolumned-incorporated BR vesicles were passed through Sepharose 4B column. After column treatment, elimination of lipid aggregation around vesicles was checked with electromicroscope JEOL 100-C(80 KV). The change of absorbance with temperature was observed with ultraviolet and visible spectrophotometer Gilford Model 260 attached with temperature programmer. The spectral change with SDS was observed with Cary-17D spectrophotometer. The condition was fixed into pH 7.5, 20 °C. Ionic strength was held constant at 0.03 with NaCl at 0.01 M Tris buffer solution.

Result and Discussion

The ultraviolet and visible spectrum of purple membrane was shown at Figure 1. The peak at 568 nm and peak at 280

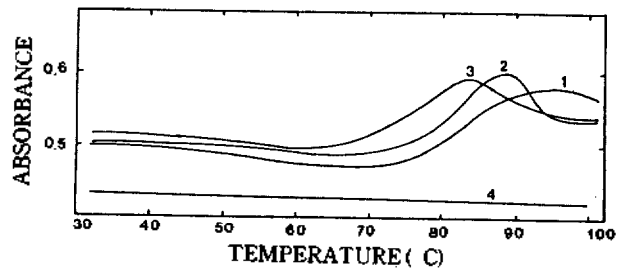

Figure 3. Effect of pH on thermal denaturation of BR in purple membrane. Absorbance at 280 nm were recored from Gilford spectrophotometer; (1) pH 7.0 (2) 9.0 (3) pH 11.0 (4) pH 12.8.

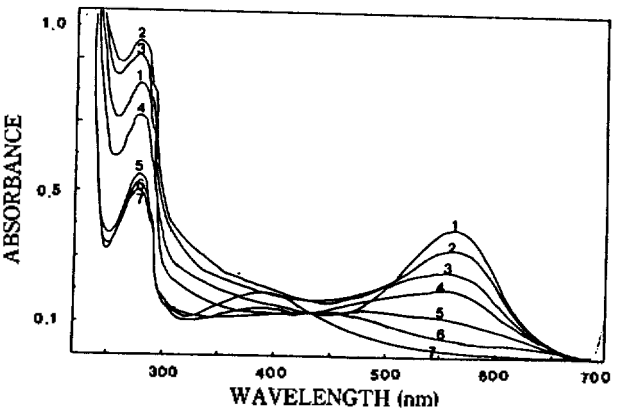

Figure 4. Effect of SDS on absorption spectra of purple membrane. The pH was 7.5 at 20 °C. The solutions contained (1) 0% SDS (2) 0.01% SDS (3) 0.02% SDS (4) 0.03% SDS (5) 0.04% SDS (6) 0.05% SDS (7) 0.05% SDS (4 hours later).

nm can be seen. The change of absorbance at 568 nm and 280 nm was observed at several pH up to 100 °C (Figure 2 and Figure 3). As the pH of purple membrane dispersed solution was increased, the abrupt change of absorbance at 568 nm occured at lower temperature. This means that the purple membrane became unstable at higher pH[9]. The absorbance at 280 nm decreased as the temperature was increased, but at certain temperature the large increase in absorbance, followed by abrupt decrease, can be seen. The increase of 280 nm absorbance at high temperature region seems to be due to the increase of scattering by the lipid molecules which were extruded from the purple membrane layers. As the tyrosines and tryptophans which contribute to the 280 nm peak were exposed to the polar solvent from hydrophobic environment of BR, the decrease of absorbance at 280 nm occured[6]. The absorbance decrease at 280 nm by the exposure of tyrosines outweighed the turbidity increase by lipid molecules after some threshold temperature. The exposure of tyrosines and tryptophanes means that the tertiary structure and a part of secondary structure of BR were destroyed[6], so to speak, the denaturation of BR occured. Therefore, by observing the absorbance change at 280 nm, we can discern the temperature at which the denaturation of BR occured. At Figure 3, as the pH of solution increased, the temperature at which the 280 nm absorbance decrease occured was lowered. This trend is in accord with the result at Figure 2.

The change of absorption spectra of purple membrane, dispersed into 0.01 M pH 7.5 Tris buffer solution, with SDS

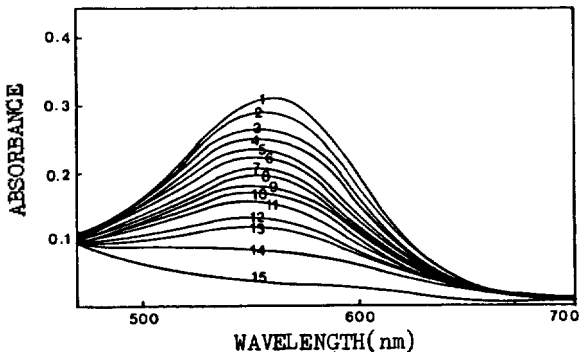

Figure 5. Effect of SDS on absorption spectra of Uncolumned-Incorporated BR solutions with 0.01 M Tris buffer. The pH was 7.5 at 20 °C. The solutions contained (1) 0% SDS (2) 0.01% SDS (3) 0.02% SDS (4) 0.03% SDS (5) 0.04% SDS (6) 0.05% SDS (7) 0.06% SDS (8) 0.07% SDS (9) 0.08% SDS (10) 0.09% SDS (11) 0.10% SDS (12) 0.11% SDS (13) 0.12% SDS (14) 0.13% SDS (15) 0.14% SDS.

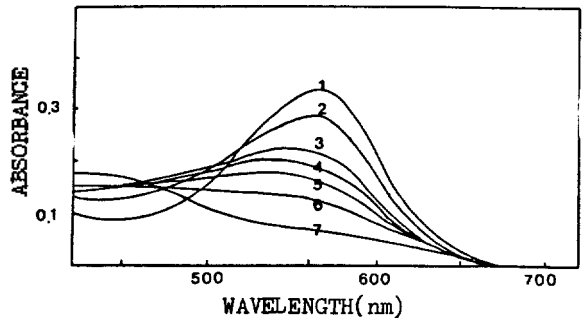

Figure 6. Effect of SDS on absorption spectra of incoporated BR in solutions with 0.01 M Tris buffer. The pH was 7.5 at 20 °C. The solutions contained (1) 0% SDS (2) 0.01% SDS (3) 0.02% SDS (4) 0.03% SDS (5) 0.04% (6) 0.05% SDS (7) 0.06% SDS.

addition was shown in Figure 4. The complete denaturation of BR occured at 0.05% SDS addition. The absorption peak at 370 nm which appeared after 0.05% SDS addition shows the presence of free retinals detached from BR as the tertiary structure of BR was destroyed by SDS. The change of absorption spectra of uncolumned-incorporated BR was observed at Figure 5 with SDS addition. Because of intense scattering in the ultraviolet region due to the vesicles and lipid residues, the 280 nm peak could not be observed. So, complete disappearence of 568 nm absorption peak was chosen as the denaturation of BR. With the creteria, the denaturation of uncolumned-Incorporated BR occured at 0.13% SDS addition. The denaturation of incorporated BR occured at 0.06% SDS addition as can be seen from Figure 6.

The thermal denaturation of incorporated BR, the denaturation criteria is same as above, was observed at various SDS concentration (Table 1). On addition of 0.03% SDS, thermal denaturation occured at about 53 °C in Tris buffer solution and at 56-66 °C in phosphate buffer solution, which are lower than the thermal denaturation temperature of incorporated BR without SDS addition. In the presence of 0.07% SDS, that temperature was lowered to 47-52 °C in Tris buffer solution.

Table 1. Denaturation Temperature of Uncolumned-Incorporated BR in 0.01 M Buffer Solution. The pH was 7.5

SDS	Buffer	
	Tris	Phosphate
0.03%	53 °C	56-66 °C
0.07%	47-52 °C	53-56 °C

On the thermal denaturation, incorporated BR is less stable than BR in the purple membrane. And the effects of SDS and temperature are additive. But the sole effects of SDS to the BR denaturation, shows different phenomena; BR in purple membrane is less stable than incorporated BR and uncolumned-incorporated BR(Figure 4,5,6). Because SDS is quite well mixed with lipid, SDS molecules are diffused into bilayer, and the effective concentration of SDS around BR is lowered in incorporated BR, and especially in uncolumned-incorporated BR. In the purple membrane, the lipids are only 25% of total mass. Therefore, even small amounts of SDS are enough to change the conformation of BR in purple membrane. Uncolumned-incorporated BR whose BR to lipid ratio is 1/100, needs more SDS. Effects of SDS on the structural changes of BR can be understood by effective SDS concentration in lipid layer.

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