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산성 pH가 박테리오로돕신의 분광학적 성질에 미치는 효과

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The Effect of Acidic pH on the Spectral Properties of Bacteriorhodopsin

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요 약. *Halobacterium halobium* 으로부터 분리 정제된 퍼플멤브레인을 7.5% 폴리아크릴아미드겔에 혼합시켰다.

이 겔을 사용하여 pH 변화에 따른 흡수 스펙트라와 원편광 이색성스펙트라를 얻었다. pH 7.0에서 보여준 이들 스펙트라의 성질들은 수용액내에 부유하고 있는 퍼플멤브레인으로부터 얻은 것들과 동일하였다. pH 2.7에서는 최대흡광도를 605 nm에서 나타내었으며 pH 0.8에서는 565 nm에서 보여주었다.

광에 노출되지 않았던 겔의 경우는 광에 노출되었던 겔과는 달리 등흡광점을 보여주었다. pH 2.7과 pH 0.8에서 측정한 원편광 이색성스펙트라는 pH 7.0에서 보여준 bilobed형을 유지하였으며 UV 영역에서 분자편광도나 스펙트라의 모양도 pH에 따라 큰 영향을 받지 않았다. pH 2.7에서 생성된 bR^{acid}_{605}가 퍼플멤브레인의 정상 광화학 순환기 중간 생성물인 O^{640}와 유사한 성질을 가진종이 아닐까 추측된다.

ABSTRACT. Purple membrane from *Halobacterium halobium* was incorporated into 7.5 % polyacrylamide gels. Absorption and circular dichroic spectra of puple membrane incorporated with gels were obtained at various pH. The spectra of these gels measured at pH 7.0 were essentially identical with those obtained in the aqueous suspension of purple membrane.

Acid titration of the gels showed the transition to a form absorbing at 605 nm (bR^{acid}_{605}) at pH 2.6, and to a second form at 565nm (bR^{acid}_{565}) at pH 0.8. Dark-adapted gels showed an isosbestic point for each transition whereas light-adapted gels did not. Visible CD spectra of bR^{LA}_{570}, bR^{acid}_{605} and bR^{acid}_{565} all showed the typical bilobed pattern. CD spectra measured at UV wavelength region were also independent of the variation of pH in terms of molar ellipticity and spectral shape. The protonated species bR^{acid}_{605} may be one of the intermediates formed during the normal photochemical cycle of purple membrane. Most probably, the species bR^{acid}_{605} is considered to be O^{640} in the cycle.

INTRODUCTION

The general features of the light-driven proton pump of bacteriorhodopsin (bR) in the purple membrane from *Halobacterium halobium* are well established. Purple membrane contains a single protein, bacteriorhodopsin which constitutes 75% of the dry weight of the membrane. Bacteriorhodopsin consists of the chromophore retinal bound *via* a Schiff base linkage to a lysine residue[1]. Electron diffraction study concerning the structure of this membrane shows that bR molecules are clustered in set of three about three fold axes[2]. Circular dichroism data of bR in purple membrane give an appreciable excitonic interaction between the retinal chromophores[3~4].

Bacteriorhodopsin can reside in two states: a dark adapted form, characterized by a visible spectral transition with $\lambda_{max}=558$ nm bR_{568}^{DA} and a light adapted form with $\lambda_{max}=568$ nm bR_{570}^{LA}. The light adapted form undergoes a photochemical cycle containing at least five intermediates which are designated by their visible spectral transitions as K^{635}, L^{550}, M^{412}, N^{540} and O^{640}.[5,7] Proton translocation across the membrane is coupled to this reaction cycle[8]. In order to fully understand the photochemical cycle, it is necessary to know the physicochemical properties of each intermediate.

Considering the rapid decay[9] (msec-μsec) of these intermediates, it is very difficult to trace the properties of each components produced during the cycle at its real aspects. As an alternative way to trap the phototransient M, ether saturation method[10] and guanidine hydrochloride method[11] have been reported.

In this paper, we present that the formation of stable protonated species of bacteriorhodopsin such as bR_{606}^{acid} may bear some relationship to the normal reaction cycle intermediates.

EXPERIMENTAL

Purple Membrane-Containing Gels. The purple membrane was isolated as described by Oesterhelt and Stoeckenius[12]. Purple membrane-containing gels were prepared from clamped microscope slides, and sealed with a 1% solution of warm agar. An acrylamide solution of 25 m*l* total volume was mixed, which contained 7.5% acrylamide, 0.2% *bis*, 0.03% tetramethylethylenediamine, 0.04 *M Tris*, 0.02 *M* sodium acetate, 0.02 *M* EDTA. Absorbance units of purple membrane were 70 at pH 7.4. The solution was degassed in vacuo, poured into the frame of slide glasses, and allowed to polymerize for one hour. The gels were then cut to a size (30×10×2 mm) and stored in water containing 0.01% sodium azide. Blank gels were prepared the same way, omitting purple membrane.

Absorption and Circular Dichroic Spectra. Absorption spectra were taken at room temperature on a Cary 14 outfitted with a scattered transmission accessory and an EMI 9657 photomultiplier. CD spectra were obtained on a home built instrument at the Chemical Biodynamics Laboratory at the University of California, Berkley. A Dumont 2703 phototube with S-20 response was used as a detector, from which the sample cuvette was held 3.8 cm away by a cell holder constructed for that purpose. The purple membrane gels were placed at the phototube side of a 1 cm cuvette filled with buffer solution at the desired pH.

RESULTS

Electronic absorption spectra of light-adapted purple membrane in solution are shown as a function of pH in *Fig.* 1. The lack of isosbestics occurs because dark adaptation becomes very rapid at low pH. The red shift of the maxi-

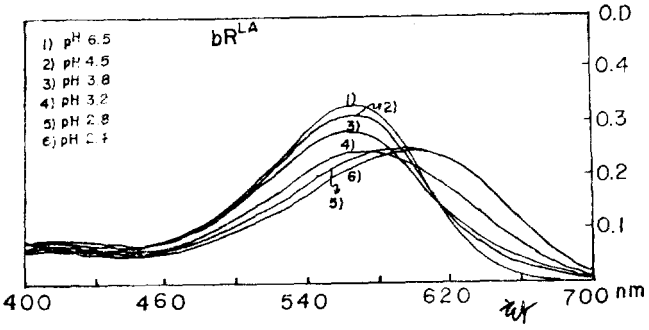

Fig. 1. Absorption spectra of purple membrane (light-adapted) suspensions at different acidic pH.

imate pK's for the formation and decay of bR^{acid}_{605} were measured by the absorbance changes at that wavelength. The pK for the formation of bR^{acid}_{605} is approximately 2.6, whereas that for the formation of bR^{acid}_{595} is around 0.7 (*Fig.* 4). The shoulder at pH 3.0 suggests that another site is being titrated.

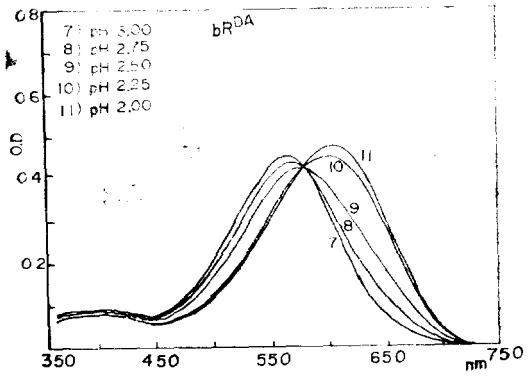

Fig. 2. Absorption spectra of the gels incorporated with purple membrane (dark-adapted) from pH 3.0 to pH 2.0.

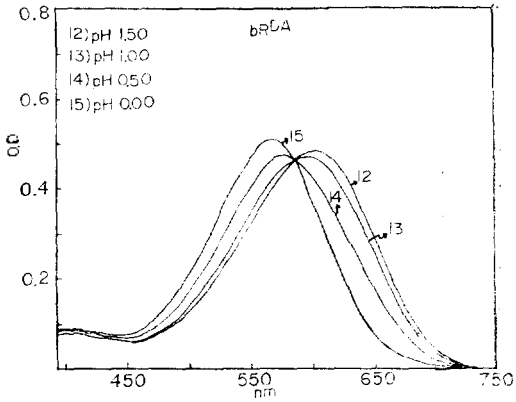

Fig. 3. Absorption spectra of the gels incorporated with purple membrane (dark-adapted) from pH 1.5 to pH 0.0.

um absorption band to 605 nm at pH 3.0 is consistent with the measured pK of 3.25 for the shift by Oesterhelt *et al.*[1] Quantitative studies at lower pH were impossible due to coacervation. However, we qualitatively noted that the membrane particles turn purple in 1 M HCl solution.

Absorption spectra of dark-adapted purple membrane gels as a function of pH are shown in *Fig.* 2 and 3. Two isosbestic points are seen-one for the bR^{DA}_{560} to bR^{acid}_{600} transition at 578 nm (*Fig.* 2), and one for the bR^{acid}_{605} to bR^{acid}_{595} at 585 nm. Since there is no spectral contribution from bR^{LA}_{568} or bR^{acid}_{605} at 690 nm, the approx-

The circular dichroism spectra of membrane-impregnated gels were taken as a function of pH. The spectra (*Fig.* 5) are qualitatively the same as those published by Becher and Cassim[12]. The bilobed pattern characteristic of exciton splitting is maintained even at pH 2.0. There is no essential change of molar ellipticity and spectral shape of CD at 220 nm (*Fig.* 6), indicating no gross change in the secondary structure of the protein.

DICUSSION

It is well known that the photochemical cycle

of light adapted bacteriorhodopsin is directly involved in the proton translocation across the purple membrane. Recently, resonance Raman spectroscopy[6,13,14] has provided evidences that proton uptake and release occur during this cycle. In order to understand the mechanism of this proton uptake and release behavior, it is necessary to study the properties of intermediate formed during the cycle.

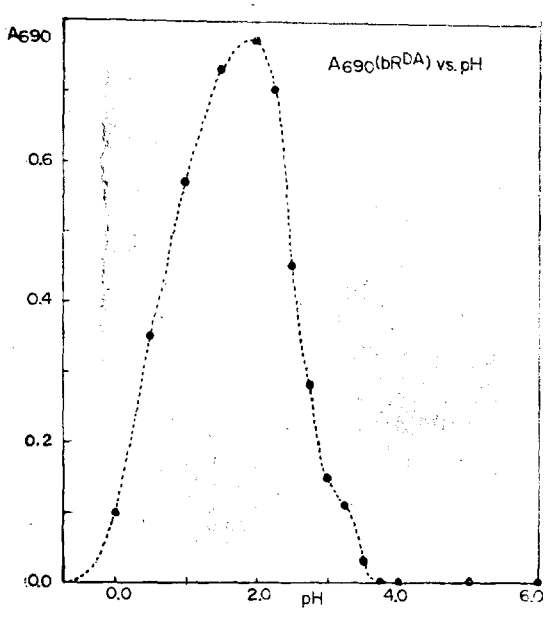

Fig. 4. Acid titration curve for the gels incorporated with purple membrane (dark-adapted). This is the plot of A_{690} as a function of pH.

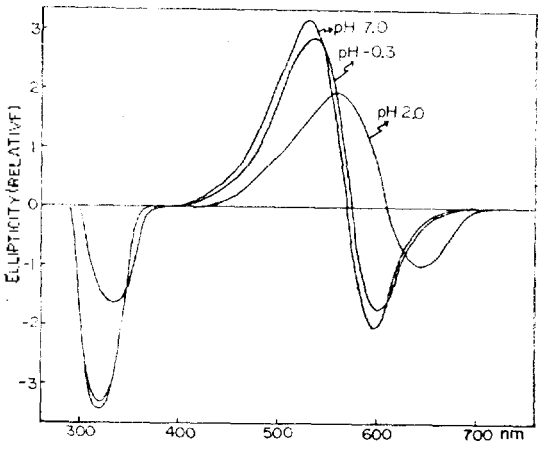

Fig. 5. Circular dichroic spectra of the gels incorporated with purple membrane (dark-adapted) at pH 7.0, pH 2.0 and pH0.3.

pH dependent absorption spectra (*Figs.* 1, 2 and 3) show three discrete entities as bR^{LA}_{568}, bR^{acid}_{605} and bR^{acid}_{565} which are physically isolable. The formation of stable protonated species of bR having different absorption spectra is of interest since the groups being titrated may be groups which are transiently protonated during the normal reaction cycle of bR^{LA}_{568}. At the moment, it is not possible to say that these entities are one of the intermediates formed in the cycle.

However, it is assumed that bR^{acid}_{605} may bear some relationship to the phototransient O which has absorption maximum at 640 nm. If so, a protonation process appears to be involved in the formation of intermediate O. The shoulder at pH 3.0 shown in the titration curve (*Fig.* 4) suggests that another site of the membrane is being titrated. In other words, a protonation of the Schiff-base is not the only way to form O. Further photochemical studies on bR^{acid}_{605} must be carried out in order to confirm that bR^{acid}_{605} is similar species with the phototransient O.

Circular dichroic spectra (*Fig.* 5 and 6) measured at both visible and UV wavelength region tell us that intact membrane structure is still maintained even at such an extreme low pH. This implies how bR molecules are rigidly clustered in the membrane bilayer. The bilobed pattern at visible wavelength region as reported previously[3,4] clearly indicates that the exciton geometry of bR molecules is not destructed by acidic pH. Molar ellipticity shown at 220 nm suggests us that secondary structure of bR is also not changed significantly by lowering the pH. These two CD results make us be easier to assume that the species formed by acid

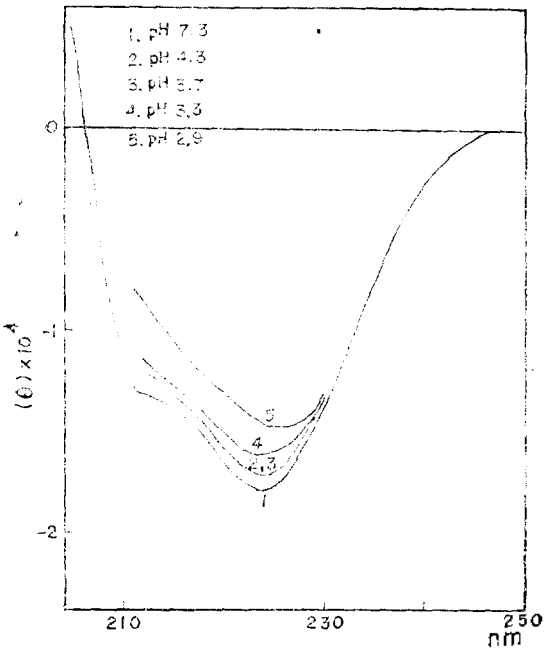

Fig. 6. Circular dichroic spectra of purple membrane suspensions at UV wavelength range as a function of pH(7.3, 4.3, 3.3, 2.9).

titration could be one of the phototransients produced during the normal reaction cycle.

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