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HYDRATION DEPENDENCE OF DRIED ORIENTED PURPLE MEMBRANE FILMS ACTIVITY

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Abstract — Dry orderly oriented purple membrane from Halobacterium halobium was obtained by a new technique of preparation. This oriented purple membrane film was very stable, nearly permanently, and showed long term reproducibility with respect to its photochemical behavior. In addition, we have investigated the photooptical properties in terms of the M_{412} intermediate of the bacteriorhodopsin photocycle with respect to the humidity of the film. The relative optical density, i.e. its apparent concentration of the M_{412} intermediate was decreased with the humidity increase as a function of the intensity of the exciting flash within our experimental range. It is suggested that the bound water molecules play an important role in the structure of the bacteriorhodopsin.

INTRODUCTION

Bacteriorhodopsin(bR) molecules embedded in the plasma membrane of Halobacterium halobium has a light-driven proton pump activity[1,2]. Light induces bacteriorhodopsin to move protons from the inside to the outside of the cell. The resulting electrochemical proton gradient across the membrane is finally used to synthesize ATP[3]. The kinetics of this light-driven proton pump has been studied so far mainly with purple-membrane sheets in aqueous suspension[4,5]. From these studies the spectral characteristics as well as the lifetimes of a number of intermediates in the photocycle could be obtained. The key to the remarkable photochemical behavior are the state of protonation of the chromophore around the bound protein structure, particularly near the protonated Schiff base linkage and electrostatic in nature, the separation of the positively charged protonated Schiff base from its negative counter-ion or solvating groups[6,7].

In the present paper, oriented purple membrane films were obtained by electrophoretic deposition of purple membrane on the surface of indium tin oxide(ITO) or tin dioxde. Such oriented purple membrane films permit not only to perform measurements of the spectroscopic method but also to perform measurements of the photopotential[8]. Our purpose was to obtain samples stable for several years with a large response from oriented purple membrane fragments and to measure their optical properties and related electrical properties. Here, we report on the optimum humidity conditions which maximize sample stability and response amplitude.

MATERIALS AND METHODS

Samples used in the experimental were prepared from an aqueous suspension of purple membrane isolated from Halobacterium halobium strain S9[9] and then the purple membranes were resuspended successively three times in bidistilled water after spinning at 17,000 g for 90 min.

In order to orient purple membrane sheets, anodic electrodeposition was performed on glass plates, 75 × 25 × 1.0 mm which were coated with layers of ITO or tin dioxide. A stainless plate served as a second electrode. The gap between the two plates could be adjusted as a teflon spacer(see Figure 1). A dark-adapted purple membrane suspension with protein concentration of O.D. 5.0 was injected with a syringe between the electrodes and 10 - 12 V electric potential was applied across the electrodes for 10 sec. The membranes being deposited on the substrate plate were dried in air environment for 24 h at ambient temperature. Samples were thereafter adjusted to the desired humidity by incubation in a sealed box whose ambient water vapor presence was controlled by saturating its atmosphere with appropriate NH_4Cl solutions. The area of the obtained purple membrane film was about 1.2 × 1.5 cm^2. This procedure of orientation did not markedly change

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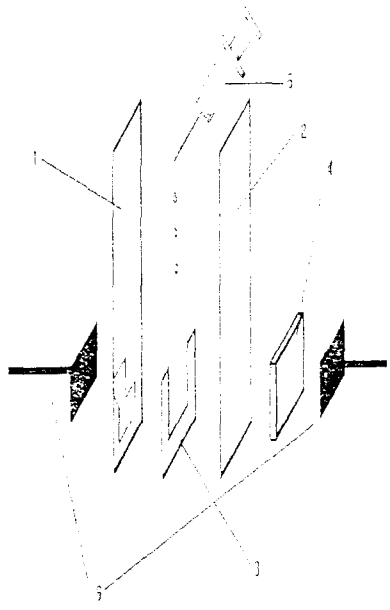

1. Glass plate('substrate' plate) ITO coating(+)
2. Stainless steel plate applied voltage(-)
3. Teflon spacer
4. Teflon supporter
5. Syringe
6. Clamp

Figure 1. Experimental set up for preparation of dry uniformly oriented purple membrane film.

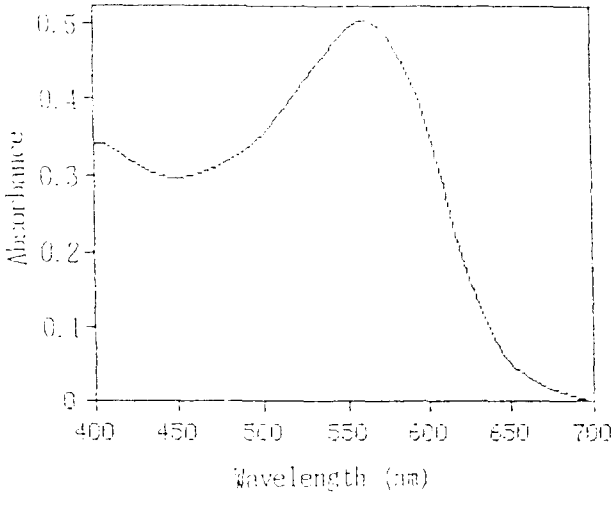

Figure 2. The absorption spectrum of purple membrane film.

the spectral characteristics of purple membrane films in the 400-700 nm region (see Figure 2). The optical densities of the films at the absorption maximum of bR were in the 0.3-1.5 range.

The decay kinetics of M_{412} intermediate were measured using a single beam spectrophotometer. The actinic pulse was provided by the frequency doubled pulse (532 nm, 10 ns duration, 30 mW/pulse) of a Q-swithed Nd-YAG laser (Laser system 2000, JK Lasers, Rugby, England). The flash intensity was adjusted by moving two diffusing silica

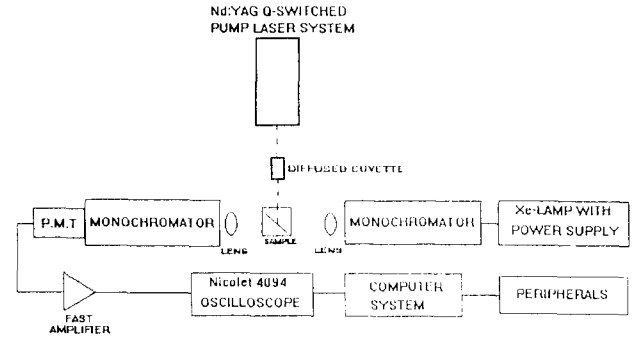

Figure 3. The schematic diagram of the measuring for photoresponse signal.

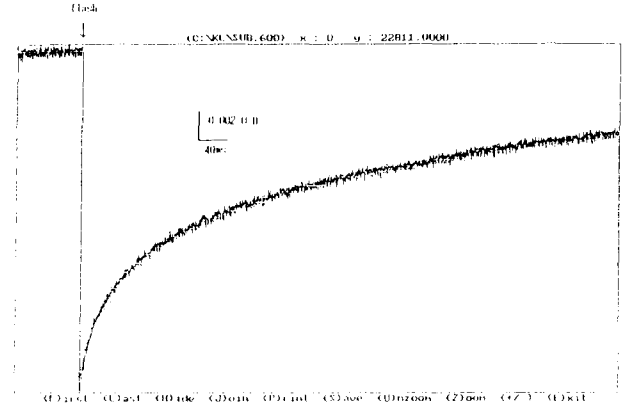

Figure 4. One of the typical light-induced decay curves of the M_{412} intermediate.

plates in the light path. The energy of the actinic light incident on the sample site was measured using a joule meter.

Measuring light from a grating monochromator was focused on the purple membrane sample. After the measuring light passed through the sample, followed by the second monochromator. A photodiode (50 ns rise time) or PMT was attached at the exit slit of the second monochromator. The signal was amplified by a current amplifier and then fed into digital oscilloscope(Figure 3). The acquisistion of kinetic data get the average of 32 or 64 shots (see Figure 4). Figure 4 shows a typical light-induced decay curve of the M_{412} intermediate. These dried samples were very useful for studying optical responses of the purple membrane as these films are very stable for several years and show high reproducibility.

RESULTS AND DISCUSSION

The photocycle of bR in dry films of oriented purple membranes obtained by electrophoretic sedimentation was investigated as a function of the humidity. The large change in time and intensity of the signal was influenced the hydration level of the

Table 1. Humidity effect on the M_{412} formation time constant and ΔA of the dried oriented purple membrane film on the SnO_2(a) and InSnO(b) substrate, respectively.

a)

Humidity(%)	τ(μs)	ΔA
38	14	0.032
50	17	0.030
54	25	0.027
60	26	0.026

b)

Humidity(%)	τ(μs)	ΔA
31.8	10	0.040
50.0	13	0.035
52.0	23	0.0317
62.0	27	0.030
72.0	35	0.029

dry film. Table 1 shows the humidity dependence of M_{412} intermediate on the tin dioxide substrate and the ITO substrate, respectively. M_{412} formation time constant of dry film is longer with the increase of humidity content and the relative absorbance of M_{412} intermediate is lower with the increase of humidity. It means that flash induced light can affect only the proton binding groups in the closest vincinity to the Schiff base because the proton translocation activity is to restricted to local scales in dried oriented purple membrane. Therefore, the different proton pump activity might be caused by the change due to the hydration state of the dry film. Hildebrandt and Stockburger[8] have found that water molecules exert a strong influence on the structure and function of bR chromophores in the resonance Raman (RR) spectroscopy. They explained as follows. There is thus convincing evidence that water molecules are located in the vicinity of the retinal chromophore. But evidently the chromophore cannot be immersed completely in a pool of water since otherwise the Schiff base bond would not be stable. The fact that the unprotonated intermediate, M_{412} and M_{412} (dehydrated), in the hydrated and dehydrated membrane have a similar conformational structure[9] implies that in the absence of ionic interactions with the Schiff base group water molecules do not determine the chromophore's structure but rather the nonpolar interactions with the protein environment[10] are largely responsible. They concluded that water molecules are confined to the vicinity of the terminal Schiff base group, where they stabilize ionic

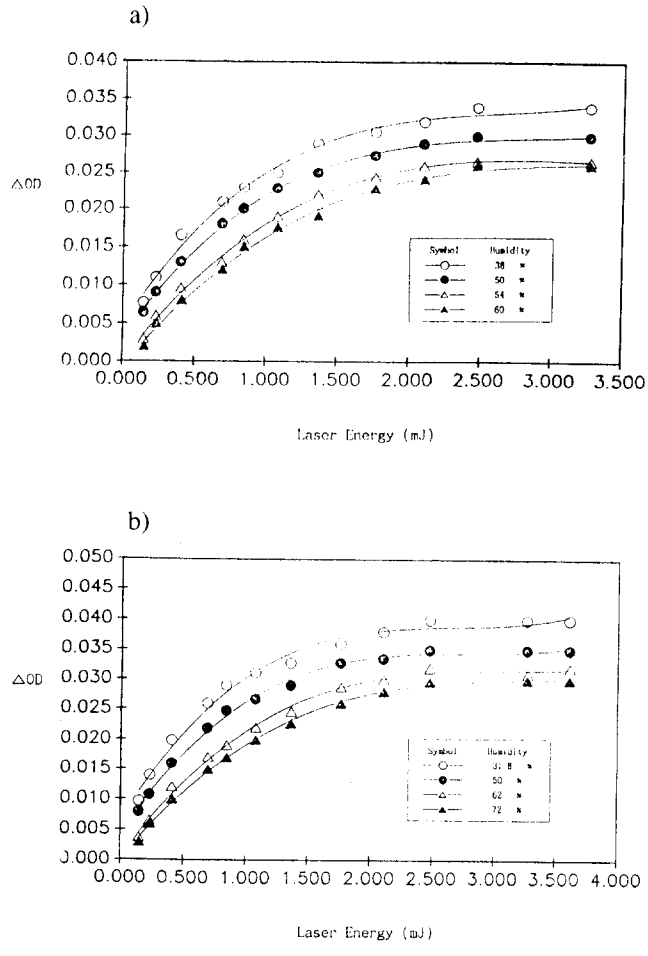

Figure 5(a :SnO_2 and b :ITO substrate). The relative optical density of the M_{412} intermediate as a function of the energy of the exciting flash at all fixed humidity.

structures and also contribute to keep the chromophore in a fixed position within the protein pocket. In the presence of water content the bound water molecules allow to bridged between the proton-binding group and the retinal Schiff base in the M_{412} intermediate. Also it could not exclude the hydrogen bonding between them.

The correlation between the intensity of the actinic light and humidty was also investigated. Figure 5 shows that the relative optical density of the M_{412} form increases in a nonlinear manner with an increase in the laser flash intensity at all fixed humidities. It is clear that the relative absorbances consist of at least two exponential components with laser energy. This is very similar to the results obtained under other experimental conditions[11]. Accorrding to their trimer model, the intermediate M_{412} produced by flash excitation will be distributed in the membrane as monomers, dimers and trimers because bacteriorhodopsin molecules are arranged in a two dimensional lattice as trimeric clusters[12]. In the case of weak

excitation, most of the intermediate M_{412} will be proportional to intensity of the exciting flash. However, when the intensity of the exciting flash is strong enough, the amount of intermediate M_{412} taking trimer structure will no longer be negligible. Also, at present conditions a nonlinear function of exciting intensity must be maintained in a trimer structure which is accompanied with hydration dependence in dried oriented film.

Nonetheless, there is enough water to allow normal hydration to persist in a dry film of bacteriorhodopsin under normal atmospheric conditions. Because the relative absorbance of M_{412} intermediate decreases with the increase of humidity at fixed laser intensity, we could not exclude the proton pumping activity depends mainly on the hydration level. One way of explaining such results is that a water molecule is hydrogen bonded between the Schiff base and it's counter-ion[13]. Because it is generally believed that the Schiff base binding site is quite accessible to aqueous solvent[13] and the variation in the amount of bound water induce the change in the charge distribution around the membrane[14]. Therefore, we suggest that the change of the relative optical density of the M_{412} intermediate with an increase of humidity content results from the loosening of the structure due to the bound water molecules between the proton-binding group and the retinal Schiff base in M_{412} intermediate.

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