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Proton-Pumping by Purple Membrane in Combined Multilayers with a Phthalocyanine Derivative

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Combined multilayers of purple membrane and octa-n-butoxyphthalocyanine as a pH sensitive dye were prepared by horizontal lifting method. The photo-induced proton-pumping activity of bacteriorhodopsin was observed by change of absorption spectra of the multilayers in water, and the one-way transport of proton was confirmed.

Bacteriorhodopsin (bR) is the only membrane protein which exists in purple membrane (PM) of *Halobacterium halobium*, and is well known as a 'light-driven proton pump'.1) The two-dimensionally ordered array of the bR molecules in the plasma membrane gives rise to one-way active proton transport across the membrane from cytoplasmic side to extracellular side.2,3) So far, several efforts have been made to obtain reorganized thin films of PM by Langmuir-Blodgett (LB) technique, retaining its proton-pumping function.4-8) In these studies, the activity of bR in the films has been discussed exclusively in terms of a generation of photovoltage and its polarity, aiming at some practical application. In the present work, we intended to develop an alternative way to demonstrate the proton-pumping by LB film of PM, that is, to prepare combined multilayers of PM and a dye whose visible absorption spectra vary depending on the ambient pH, and observe the changes of the spectra under photoirradiation in water. The direction of proton-pumping can be investigated by changing the sequence or orientation of the layers.

The freeze-dried sample of PM was provided by Dainippon Ink & Chemicals, Inc. (Biochem. Div.). 1,4,8,11,15,18,22,25-Octabutoxyphthalocyanine (Pc), as a pH-sensitive dye, was synthesized through the reaction described in the literature,9) and was purified by column chromatography and recrystallization.10) The PM fragments were dispersed in aqueous dimethylformamide of 25% and spread onto water (containing 5 mM $MgSO_4$) with the technique similar to that used by Furuno *et al.*8) to form the monolayer, which was compressed to the surface pressure of 18.8 mN/m immediately. The monolayer of Pc was spread from benzene solution onto aqueous solution of HCl ($pH \simeq 1$), since the monolayer of Pc on pure water was not sufficiently stable. Four kinds of combined multilayers (Fig. 1, A-D) were prepared at 18.8 mN/m (for both components) by horizontal lifting method,11) except that the bR layer in C was deposited by vertical dipping method (upward process). CaF_2 plates which were made hydrophobic with ferric stearate were used as substrates. The films were rinsed with water after each deposition of the bR monolayer. For the combined multilayers B, C and D, the films of Pc were kept in pure water for about 5 hours before subsequent deposition of bR layers, in order to make the Pc layers neutral.

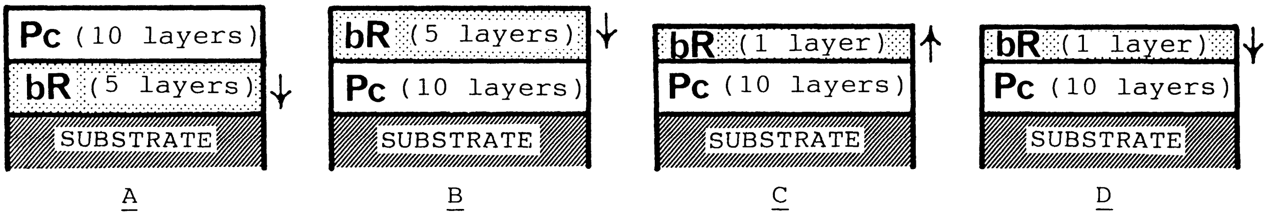

Fig. 1. Combined multilayers prepared. The arrows indicate the direction from the water side to the air side for the bR monolayers at the air-water interface.

The visible absorption spectra were measured with a setup shown in Fig. 2, using a multichannel photodetector MCPD-100 (Otsuka Electronics). The light of a halogen lamp was transmitted twice through the multilayer by reflection from a mirror placed at the bottom of the trough and was led to the multichannel photodiode array through optical fibers. A xenon lamp (500 W) was used for photoirradiation through a filter (max. transmittance at 500-600 nm, Toshiba G-55S). Before irradiation, the samples A and B were stored in pure water in a dark box for about 10 hours and dried gently also in the dark to make initial conditions as even as possible.

The spectrum of Pc 10 layers (in water : without bR layers) gradually responded to the ambient pH. As shown in Fig. 3, in a neutral or basic medium the spectrum showed an intense absorption at around 770 nm with a shoulder at 700 nm and a minimum absorption at 570 nm, whereas in an acidic medium the 770 nm peak diminished and the spectrum became rather flat with an increased absorption in the region of 500-600 nm. Since this response was reversible, the Pc film can be used as the pH-sensitive dye layer.

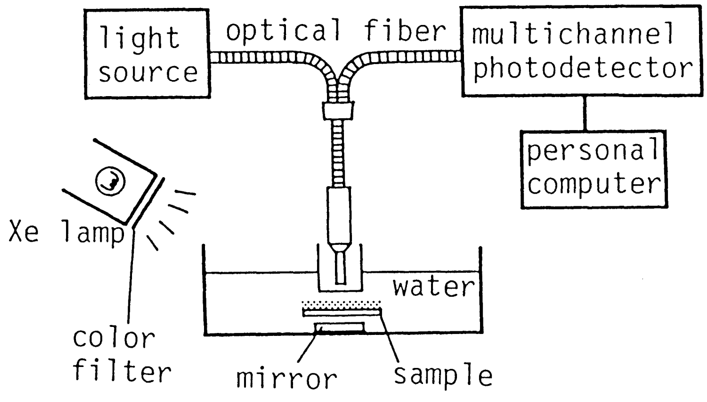

Fig. 2. Setup for measurement of visible absorption spectra.

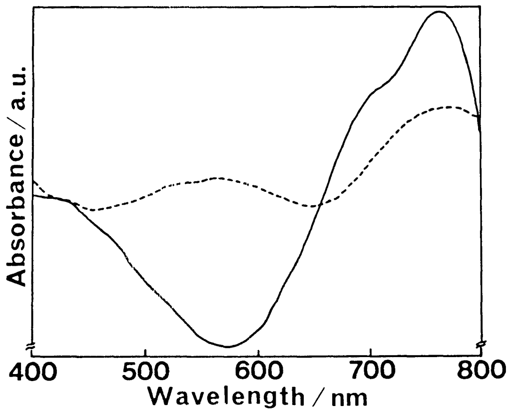

Fig. 3. Typical absorption spectra of Pc multilayers in neutral-basic(——) and acidic (- - -) water.

Figure 4 shows the spectra of Pc layers in the combined multilayers A and B after irradiation (*ca.* 8 hours) in water. (The absorption of bR layers were subtracted from the total spectra of the combined layers.) Spectral changes of A and B on the photoirradiation were contrastive with each other. The relatively weakened absorption at around 770 nm in B is indicative of acidic environment for the Pc layers, whereas the enhanced absorption in A suggests rather basic environment. This implies that the photo-induced proton transport really occurs across the bR multilayers in direction from the outer surface toward the substrate.

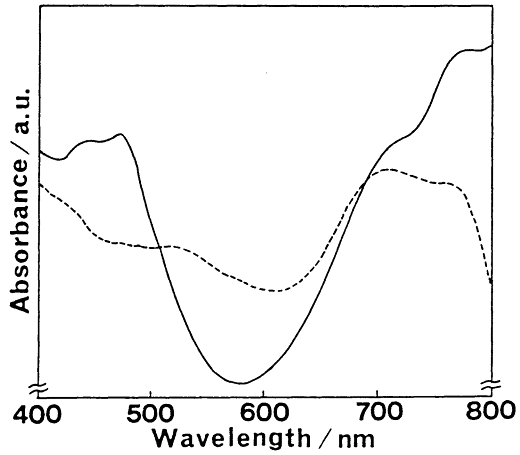

Fig. 4. Absorption spectra of Pc layers in A(——) and B(- - -) after photoirradiation.

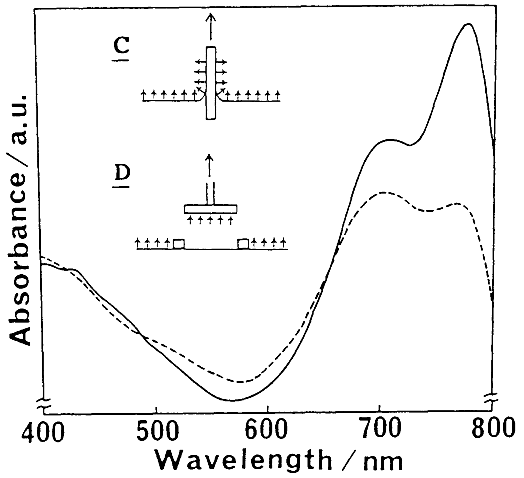

Fig. 5. Absorption spectra and deposition models of multilayers C(——) and D(- - -) after irradiation. Small arrows represent the direction of proton transport in the monolayer.

Since the monolayers of bR at the air-water interface were transferred onto the solid substrate by the horizontal lifting method in the cases of A, B,and D, the water side and the air side of the monolayer must be directed toward the outer surface and the substrate, respectively, in the transferred multilayers. It is acceptable that the 'overturn' of molecules during deposition[12] hardly takes place for polymeric monolayers such as bR. It is reasonable to consider that, in the monolayer at the air-water interface, hydrophilic intracellular side of the bR molecule[3] faces toward the aqueous subphase, and consequently in the transferred multilayer, hydrophobic extracellular side faces toward the solid substrate. Therefore, the direction of proton transport observed in our experiments is from intracellular to extracellular in accordance with that in the bacteria cell. This result is consistent with the surface potential study of mixed monolayers of PM and a lipid by Hwang *et al.*[5]

Further evidence for the one-way active proton transport was obtained by the spectra of the multilayers C and D after 1 hour irradiation, which are shown in Fig. 5, together with deposition models and the expected direction of proton transport in the monolayer. The distinction between C and D is that the bR monolayer is turned inside out. A clear difference in the absorption at around 770 nm implies that protons were accumulated into the Pc layers in D to some extent, but were not in C, as expected.

The phenomena described above assure us that the bR molecules mostly retain the photo-induced proton-pumping function in the reconstructed multilayers with the specific order of molecular orientation, and that they are active even in a monolayer.

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