

Deposition Behavior and Photoelectrochemical Characteristics of Chlorophyll *a* Langmuir-Blodgett Films

Hyun-Goo Choi, Byung-Keun Oh, Won Hong Lee, and Jeong-Woo Choi*

Department of Chemical Engineering, Sogang University, C.P.O. Box 1142, Seoul 100-611, Korea

Abstract The deposition behavior and photoelectric response characteristics of chlorophyll *a* (Chl *a*) monolayers and multilayers were investigated under various film fabrication conditions. Chl *a* LB films were deposited onto quartz and pretreated ITO glass substrates under several fabrication conditions, including surface pressure and number of layers. The absorption spectra of Chl *a* in a solution state and solid-like state (LB films) were fairly consistent with each other, and two absorption peaks were found at 678 and 438 nm, respectively. The prepared Chl *a* LB films were set into an electrochemistry cell equipped with a Pt plate as the counter electrode, and the photoelectric response characteristics were obtained and analyzed relative to the light illumination. By considering the resulting photocurrents, the optimal fabrication conditions for Chl *a* LB films were determined as 20 mN/m of surface pressure and 20 layers. The action spectrum of the Chl *a* LB films was obtained in the visible region, and was found to be in good agreement with the absorption spectrum. The possible application of the proposed system as a constituent of an artificial color recognition device was suggested based on combining with the photoelectric conversion property of another light-sensitive biological pigment.

Keywords: chlorophyll *a* (Chl *a*), Langmuir-Blodgett (LB) films, photocurrent, artificial photoreceptor

INTRODUCTION

The photochemical and photophysical behavior of biological pigments have been important subjects in the fields of photobiology and photochemistry [1-5]. In connection with the photosynthetic primary process as well as light energy conversion, chlorophyll *a* (Chl *a*) has been one of the most widely studied pigments. From the absorption spectra of green plants, it has been revealed that multiple forms of chlorophyll (Chl) are present in green plants. It is also well known that Chl molecules on grana thylakoid membranes are arranged as a highly ordered state and the local concentration of porphyrin rings is relatively high [1]. Furthermore, reaction center Chl molecules, especially P700 in photosynthetic system I (PS I), would seem to have a specific structure due to the hydrophobic interaction between phytol chains and lipids/proteins, and an additional participation of hydrogen bonding.

In order to perform an *in vitro* investigation on the photoactivity of Chl *a*, several methods have been proposed to prepare the ordered structure of Chl *a*. There are two typical modes for fabricating a highly-ordered structure of Chl *a* molecules; one is the incorporation of Chl *a* molecules into a small lipid bilayer, and the other

is the deposition of ordered Chl *a* molecules onto solid substrates using the Langmuir-Blodgett (LB) technique [6,7]. The *in vitro* photoelectrochemical behaviour of the ordered structure of Chl *a* molecules has already been observed, and its characteristics investigated for developing a model system for the primary photosynthetic process. However, these works mainly focused on the primary process of photosynthesis, whereas further applications to other fields, such as bioelectronics, have rarely been reported.

In the present work, the deposition behavior of Chl *a* monolayers and multilayers was investigated. For application to artificial photoreceptors, the photoelectric response characteristics of prepared Chl *a* LB films were analyzed based on considering the fabrication conditions. The resulting photocurrent characteristics suggest the possible application of Chl *a* LB films as a constituent of an artificial color recognition device.

MATERIALS AND METHODS

Materials

Chlorophyll *a* (Chl *a*, extracted from spinach) and benzene were purchased from Sigma Chemical Co. (St. Louis, USA). The indium tin oxide (ITO) glass was kindly provided from Samsung Digital Interface Co. (Suwon, Korea). Other miscellaneous reagents were also

* Corresponding author

Tel: +82-2-705-8480 Fax: +82-2-711-0439

e-mail: jwchoi@ccs.sogang.ac.kr

purchased from Sigma Chemical Co. (St. Louis, USA) and used without further purification.

Preparation of Chl *a* LB Films

For the deposition of Chl *a*, quartz and ITO glass were used for the spectroscopic analysis and photocurrent measurements, respectively. Prior to use, all the substrates were cleaned according to the appropriate procedure [8]. The LB film deposition of Chl *a* was carried out with a circular-type Langmuir trough (Model 2011, Nima Tech., UK). Chl *a* molecules were dissolved into thiophene-free benzene at a concentration of 1 mM, and stocked in a frozen state at -20°C without light illumination. The Chl *a* solution was then carefully applied onto the aqueous subphase (1 mM phosphate buffer, pH 7) at room temperature, and the resulting monolayer was compressed until a collapse occurred thereby producing the surface pressure-area (π - A) isotherm of Chl *a*. The deposition of the Chl *a* LB films was started with an upward stroke. This means that the substrate was immersed into the subphase first, the Chl *a* solution was applied onto the aqueous subphase, and the monolayer was compressed to the target pressure and subsequently transferred onto the substrate by moving the substrate upward using a computer-controlled stepping motor. The dipping speeds of the upward and downward strokes for the Chl *a* LB film deposition were 6 mm/min.

Spectroscopic Analysis and Photocurrent Measurement

For the absorption spectroscopy, the Chl *a* LB films were transferred onto quartz substrates, and their spectra were obtained using a UV/VIS spectrophotometer (V-550, JASCO, Japan) with a resolution of 1 nm. A bare-quartz was used as the reference substrate.

For the analysis of the photoelectric response, the fabricated Chl *a* monolayers and multilayers were set into an electrochemistry cell equipped with a Pt plate as the counter electrode. KCl solution (0.1 M) was used as the supporting electrolyte, and the pH of the electrolyte was adjusted to 7.0 with the KOH solution. The electrochemistry cell was serially connected to low-noise current preamplifier (SR570, Stanford Research System, USA), amplifier (DC10000H2O, B&H Electronics, USA), digital oscilloscope (HP54610B, Hewlett Packard, USA), and personal computer. The effective area of the LB films for light sensing was 1 cm^2 ($1\text{ cm} \times 1\text{ cm}$). The experimental setup for the photocurrent measurement is schematically illustrated in Fig. 1.

A 300 W Xenon lamp system (Model-66984, Oriol Co., USA) was used as the light source. Monochromatic light was illuminated onto the fabricated LB films using several optical filters, and the resulting photoelectric responses were analyzed through the photocurrent measurement system. To obtain the correlation between the incident light intensity and the photocurrent generation, the incident light intensity was measured

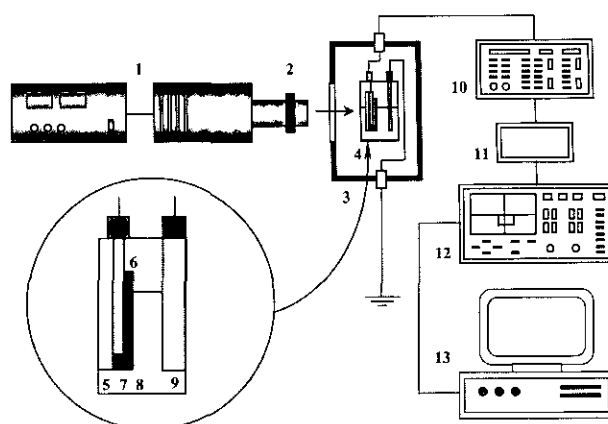


Fig. 1. Schematic illustration of experimental setup for photocurrent measurements: 1. 300 W Xenon Lamp; 2. Optical Filter; 3. Shield Box; 4. Electrochemistry Cell; 5. Glass; 6. ITO; 7. Chl *a* LB Films; 8. Electrolyte; 9. Pt Electrode; 10. Low-Noise Current Preamplifier; 11. Amplifier; 12. Oscilloscope; 13. Personal Computer.

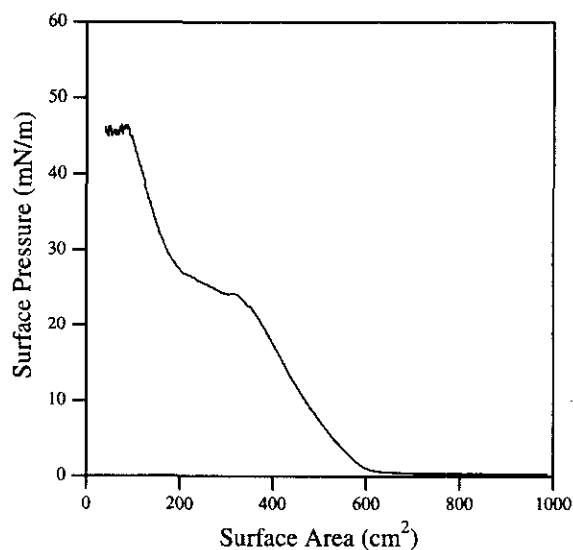


Fig. 2. π - A isotherm of Chl *a* monolayer at air-water interface; aqueous subphase, phosphate buffer (1 mM, pH 7, and at room temperature).

with a commercially available photodiode (SmartSensor LM-3 and LM-10, Coherent-Earling, USA) and a broadband power-meter (LaserMate, Coherent-Earling, USA).

RESULTS AND DISCUSSION

Deposition Behavior and Spectral Properties of Chl *a* LB Films

A surface pressure-area isotherm of the Chl *a* monolayer is shown in Fig. 2. The isotherm shows that the

surface pressure constantly increased until it reached about 20 mN/m as the monolayer was compressed. This first condensed monolayer region was followed by a transition region, with a surface area ranging from 400 to 200 cm² which exhibited a sudden increase in compressibility, and then became a second condensed monolayer region as the monolayer was further compressed. This transition can be considered as a characteristic of a mixed monolayer [9,10]. The average purity of the Chl *a* used in this work was around 90%, according to the data of Sigma Chemical Co. The principal impurity was most likely chlorophyll *a'*, and other possible impurities were carotene, zanthophylls, and carotenoid compounds. Since these impurities are not completely miscible with Chl *a*, this immiscibility could lead to the discontinuity of the compressibility. However, after the transition region, there was no readily observed discontinuity of compressibility as the monolayer state reached a fully condensed region. The collapse of the monolayer was found at around 45 mN/m. Based on the obtained π -*A* isotherm, in all experiments, the Chl *a* monolayers were deposited at 20 mN/m, the first condensed monolayer region, before any transition occurred.

The deposition type of the Chl *a* LB films was found to be Z-type; the transfer ratio were 100±10% in upward and 0±10% in downward strokes. In order to confirm the deposition of Chl *a* monolayers onto a solid substrate, the absorption spectrum of the Chl *a* LB films was measured. For the absorption spectroscopy, quartz was used as the substrate and a bare-quartz plate was used as the reference. As shown in Fig. 3, the absorption spectrum of the Chl *a* LB films was fairly consistent with that of the Chl *a* solution. It has a broad band ranging from 400-700 nm with two peaks at around 438 nm and 678 nm, respectively. There were also slight red-shifts by 10-15 nm in the LB films, which is well consistent with previous reports [1,2,11-13]. These results could be due to the close-packed structure of the Chl *a* LB films. Accordingly, it can be deduced that the Chl *a* monolayers were well transferred onto the solid substrate, with maintaining their spectroscopic properties.

Photoelectric Response Characteristics of Chl *a* LB Films

The typical time course behavior of the photocurrent generation from the Chl *a* LB films (5 layers) is shown in Fig. 4. According to the monochromatic light illumination (680 nm), the anodic current was generated and returned to its baseline, *i.e.* background dark current level, when the light was turned off. The magnitude of the photocurrent was about 22 nA/cm² and the rising time (response time) was slightly less than 300 ms. The photocurrent generation of the fabricated Chl *a* LB films was very stable, and the reproducible (almost the same) response profiles were obtained with repeated light illuminations.

The photocurrent generation of the Chl *a* LB films

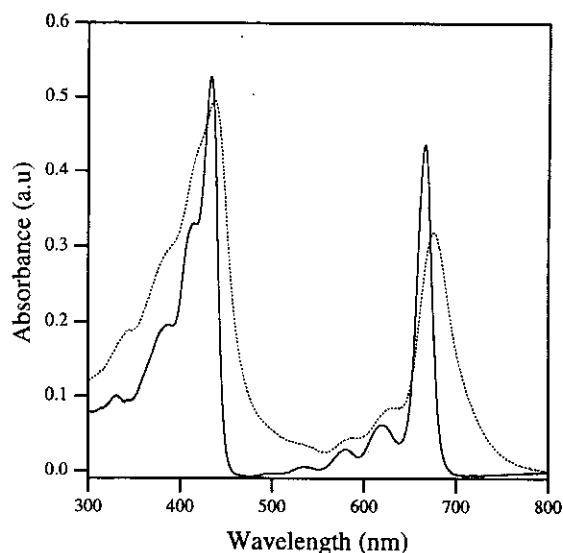


Fig. 3. Absorption spectra of Chl *a*; solid line, absorption spectrum of Chl *a* solution; dotted line, absorption spectrum of Chl *a* LB films (10 layers).

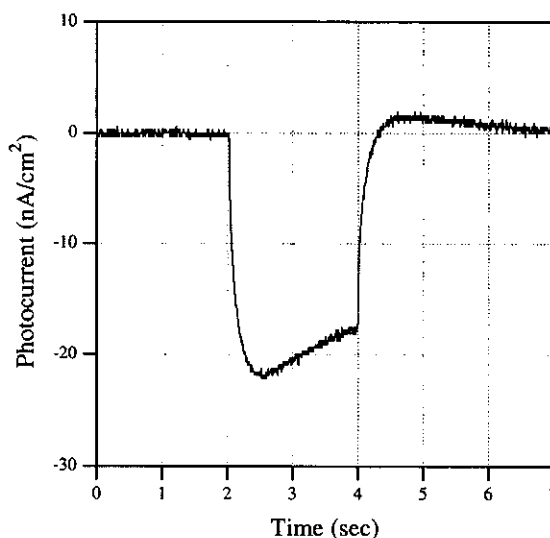


Fig. 4. Typical photoelectric response of Chl *a* LB films (5 layers) relative to the monochromatic light illumination (680 nm).

was investigated with respect to the deposited number of LB layers. In principle, the magnitude of the photocurrent generation increases with an increase in the number of functional molecular layers. As expected, the magnitude of the photocurrent generation increased with an increase in the number of Chl *a* monolayers, up to 20 layers. However, there was no significant change in the magnitude of the photocurrent above 20 layers, as shown in Fig. 5. The excited states of photoactive molecules can be produced in a bulk phase (in this case, Chl *a* LB films) and the generated charge carriers, *i.e.*, electrons, probably diffuse into the interface (in this

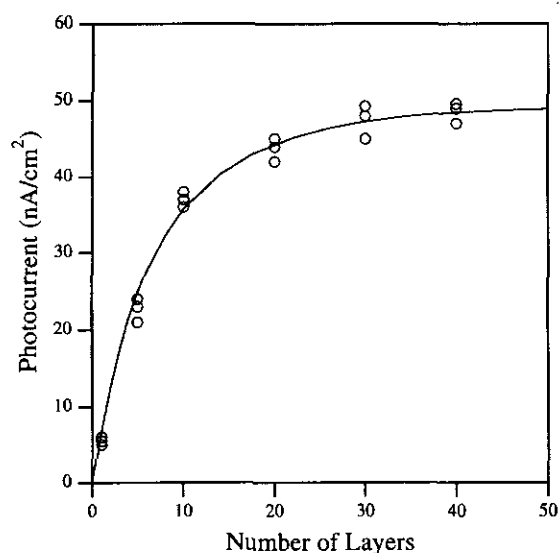


Fig. 5. Photocurrent generation of Chl *a* LB films as a function of number of LB layers.

case, Chl *a*/ITO electrode) [14]. There is also some probability of energy dissipation in another manner during the diffusion process, and thereby the mean diffusion length can have a finite value. As a result, the photocurrent generation would be saturated at a certain thickness of LB films (number of layers). In this work, the mean diffusion length of charge carrier would be about 20 layers. Based on these results, the optimal number of layers in Chl *a* LB film fabrication was determined as 20 layers, which can produce reliable and stable photocurrent generation.

In order to use light-sensitive materials with the photoelectric conversion property as the photoreceptor, the possibility of a linear relationship between the incident light intensity and the photocurrent generation should be validated. In desirable light sensors, including biological photoreceptors such as the retina, light sensing is performed in a quantum conversion mode that can guarantee the linear sensitivity of a sensor against any incident photon flux. Since the photocurrent is a result of the energy conversion from light to electricity, photocurrent generation, in principle, should be proportional to the number of photon to be absorbed. As shown in Fig. 6, the photocurrent of the Chl *a* LB films was generated proportionally to the incident light intensity. Compared with other light-sensitive pigments, the electron ejection from an excited state of Chl *a* shows relatively high efficiency. Accordingly, the prepared Chl *a* LB films would appear to have potential as a component of an artificial photoreceptor.

In order to confirm whether the photocurrent generation of Chl *a* LB films reflect the light-energy conversion mechanism, the action spectrum of the Chl *a* LB films should also be analyzed. Using a series of monochromatic light illuminations, the action spectrum was obtained and is shown together with the absorption

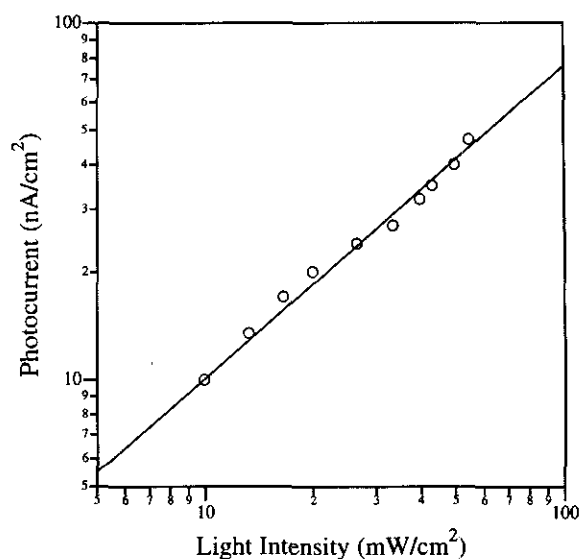


Fig. 6. Photocurrent generation of Chl *a* LB films (20 layers) as a function of light intensity.

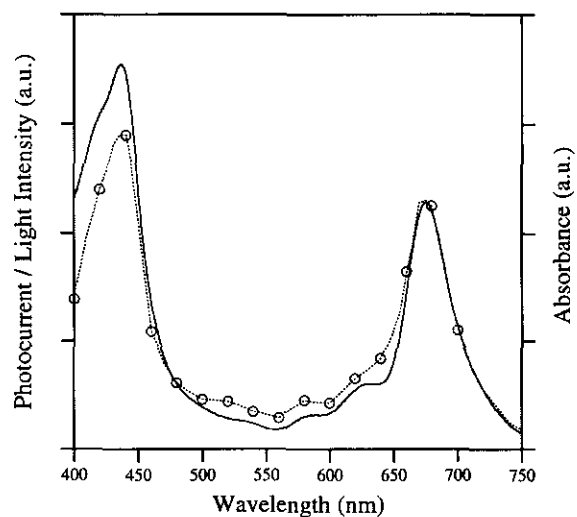


Fig. 7. Action spectrum of Chl *a* LB films; solid line, absorption spectrum; dotted line, action spectrum.

spectrum in Fig. 7. Prior to plotting, the raw data of the photocurrent were divided by the incident light intensity to verify if the response behavior of the Chl *a* LB films reflects the photoelectric conversion mechanism with the given light energy. The action spectrum of Chl *a* LB films was found to be fairly consistent with the absorption spectrum in the visible light region, with two peaks at around 430 nm and 680 nm, respectively. This result seems to suggest that the photoinduced excitation of Chl *a* LB films is the initial process of the charge carrier generation mechanism, which in turn generates the electron flux across the Chl *a* LB films-electrode interface. Consequently, it would appear that the photocurrent generation of Chl *a* LB films mainly

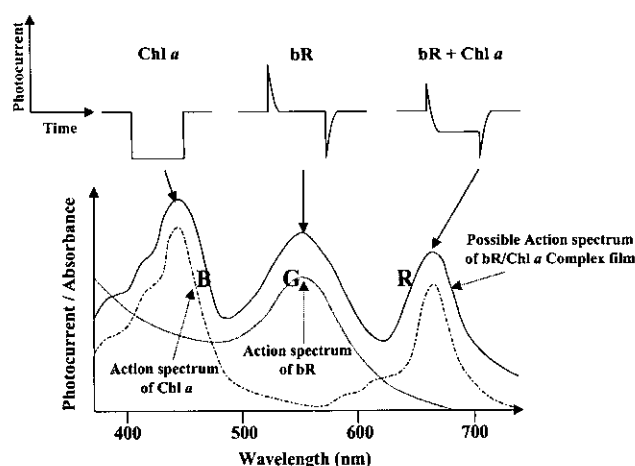


Fig. 8. Possible application of Chl *a* LB films as component of biomolecular photoreceptor for color recognition.

rely on the photoelectric conversion, although the other mechanism, such as thermal energy conversion accompanied by light irradiation, might also contribute to the photocurrent generation.

Possible Application as Color Recognition Device

As mentioned above, the absorption and action spectra of the Chl *a* LB films show a broadband with the peaks at around 678 and 438 nm, respectively. This property can be applied to the organization of a light-sensing device, such as an artificial photoreceptor for color recognition. The current authors previously developed a biomolecular photoreceptor with a color recognition function using complex LB films consisting of bacteriorhodopsin (bR) and flavin [15]. Although this photoreceptor has certain advantages over the current CCDs, such as a better spectral sensitivity, there are still some problems that need to be resolved for producing the more reliable results, such as difficulties in obtaining an accurate differentiation between a greeny-blue (520-540 nm) and yellow (570-620 nm) color. To realize a more reliable color recognition function, possible approaches can be suggested; one is a hardware modification, and the other is to employ a more appropriate algorithm for self-adaptation and learning, such as an artificial neural network (ANN). The former can be achieved by using other light-sensitive biological pigments that can generate a different action spectrum from that of bR or flavin; a possible candidate is Chl *a*.

Fig. 8 shows the possible action spectrum of an artificial photoreceptor using bR/Chl *a* complex LB films and the corresponding signal shapes in the visible light region. The typical response of Chl *a* LB films was found to be an anodic current generation, with a pulse-like signal shape. Of course, the bR signal would not be readily detected in this configuration since the proton-electron coupling would also exist at the bR-Chl *a* interface and the *in vitro* quantum yield of Chl *a* would be

much higher than that of bR. However, it would be possible to distinguish the signals from one another by adjusting the number of layers of the Chl *a* and bR LB films in this configuration, thereby resulting in the desirable photoelectric response with respect to the magnitude of the photocurrent and the signal shape.

CONCLUSION

Molecular films of chlorophyll *a* (Chl *a*) were successfully prepared using the Langmuir-Blodgett (LB) technique. The absorption spectrum of the Chl *a* films was fairly consistent with that of the solution state, with slight red-shifts by 10-15 nm, and two absorption peaks were found at around 678 and 438 nm, respectively. The photoelectric response of the Chl *a* LB films was anodic with a typical pulse-like signal shape. The optimal fabrication conditions of the Chl *a* LB films were determined as a 20 mN/m of surface pressure and 20 layers based on considering the photocurrent generation. From the good agreement between the action spectrum of the Chl *a* LB films and the absorption spectrum, it was confirmed that the photocurrent generation of the Chl *a* LB films mainly rely on the photoelectric conversion mechanism. The possible application of the proposed system as a constituent of an artificial color recognition device was suggested through combining with the photoelectric conversion property of another light-sensitive biological pigment, bacteriorhodopsin (bR).

Acknowledgements This research was supported by the Brain Science and Engineering Research Program sponsored by the Korean Ministry of Science and Technology (00-J-BS-01-B-24).

REFERENCES

- [1] Miyasaka, T., T. Watanabe, A. Fujishima, and K. Honda (1978) Light energy conversion with chlorophyll monolayer electrode. *In vitro* electrochemical simulation of photosynthetic primary process. *J. Am. Chem. Soc.* 100: 6657-6665.
- [2] Miyasaka, T., T. Watanabe, A. Fujishima, and K. Honda (1979) Highly efficient quantum conversion at chlorophyll *a*-lecithin mixed monolayer coated electrodes. *Nature* 277: 638-640.
- [3] Iida, K., A. Kashiwada, and M. Nango (2000) Construction of Langmuir-Blodgett films from light-harvesting complex I from photosynthetic bacteria. *Colloids Surfaces A* 169: 199-208.
- [4] Iida, K., N. Ohya, A. Kashiwada, M. Mimuro, and M. Nango (2000) Characterization of the light-harvesting polypeptide/bacteriochlorophyll *a* complex isolated from photosynthetic bacteria by the linear dichroism spectra. *Bull. Chem. Soc. Jpn.* 73: 221-229.
- [5] Birge, R. R. (1990) Photophysics and molecular electronic

- applications of the rhodopsin. *Annu. Rev. Phys. Chem.* 41: 683-733.
- [6] Iriyama, K. (1979) Methods of preparing chlorophyll *a* multilayers on glass plates. *Photochem. Photobiol.* 29: 633-636.
- [7] Iriyama, K., M. Yoshiura, and F. Mizutani (1980) Deposition of chlorophyll-*a* Langmuir-Blodgett films onto an SnO₂ optically transparent electrode. *Thin Solid Films* 68: 47-54.
- [8] Ulman, A. (1991) *An Introduction to Ultrathin Organic Films from Langmuir-Blodgett to Self-Assembly*. Academic Press Inc., New York, USA.
- [9] Gaines, L. G. (1966) *Insoluble Monolayers at Liquid-Gas Interfaces*. Interscience Publishers, New York, USA.
- [10] Petty, M. C. (1996) *Langmuir-Blodgett Films: An introduction*. Cambridge University Press, New York, USA.
- [11] Hirsch, R. E. and S. S. Brody (1979) Spectral properties of chlorophyll *a* monolayers: Monolayers of chlorophyll *a* and pheophytin at a gas-water interface. *Photochem. Photobiol.* 29: 589-596.
- [12] Heithier, H., K. Ballschmiter, and H. M \ddot{u} rwald (1983) Liquid-crystalline phase transition of monomolecular layers of chlorophyll *a*. *Photochem. Photobiol.* 37: 201-205.
- [13] Agrawal, M. L., J.-P. Chauvet, and L. K. patterson (1985) Effects of organization on photophysical behavior: lifetime and steady-state fluorescence of chlorophyll *a* singlets in monolayers of dioleoylphosphatidylcholine at the nitrogen-water interface. *J. Phys. Chem.* 89: 2979-2982.
- [14] Jones, R., R. H. Tredgold, and J. E. O'Mullane (1980) Photoconductivity and photovoltaic effects in Langmuir-Blodgett films of chlorophyll-*a*. *Photochem. Photobiol.* 32: 223-232.
- [15] Choi, H. G., W. C. Jung, J. Min, W. H. Lee, and J. W. Choi. (2001) Color image detection by biomolecular photoreceptor using bacteriorhodopsin-based complex LB films. *Biosensors Bioelectronics* in press.

[Received April 18, 2001; accepted June 8, 2001]