Journal of Solid-Phase Biochemistry, Vol. 2, No. 2, 1977

PHOTOELECTROCHEMICAL REACTION OF CHLOROPHYLL IMMOBILIZED WITH LIQUID CRYSTAL ON METAL SURFACE

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Accepted May 25, 1977

Chlorophyll was immobilized with liquid crystal on a platinum surface to prepare a photoexcitable electrode. Liquid crystals such as *N*-(*p*-methoxybenzylidene)-*p*-butylaniline were found to be effective on the photoexcitation of the immobilized chlorophyll. Such a chlorophyll–liquid crystal electrode produced photocurrent when it was coupled with a solution of nicotinamide adenine dinucleotide and exposed to light. The electron transfer accompanied by the photoelectrochemical reaction is discussed.

INTRODUCTION

The electron transfer in the primary photosynthetic process is schematically illustrated in Fig. 1. It has been accepted that the primary photosynthetic process consists of photosystems I and II (PS I and PS II) containing chlorophylls as photoexcitable substances. Water is oxidatively decomposed to oxygen at PS II, whereas nicotinamide adenine dinucleotide phosphate ($NADP^+$) is reduced to NADPH at PS I. In the electrochemical point of view, PS I and II might correspond to cathode and anode, respectively. This suggests that a photoexcitable electrode, which undergoes a photoelectrochemical reaction on light irradiation, can be prepared by immobilizing chlorophyll on a metal plate. Photoexcitable electrodes having the function of PS I and II would be fascinating especially in biochemical photoenergy conversion.

In our previous work (1–3), a photoexcitable electrode was prepared by coating a platinum plate with a layer of chlorophyll, a redox compound, and conductive adhesive, although these were a little sophistication. The present investigation aimed at the preparation of simplified and effective photoexcitable electrodes having a similar function as PS I.

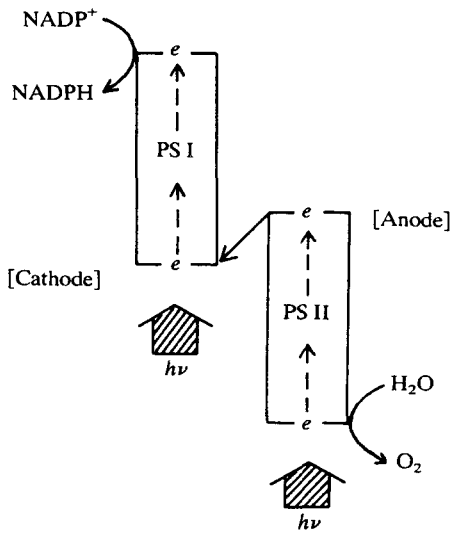


FIG. 1. Schematic comparison of photochemical and electrochemical reactions in primary photosynthesis. PS I and II: photosystems I and II.

It has been well described that chlorophyll is entrapped in the liquid crystal state of phospholipids with carotenoids in thylakoid membranes (4). The liquid crystal can give fluidity and orientation, which might be important in the function of chlorophyll molecules. Therefore chlorophyll was immobilized with liquid crystal onto a platinum plate. These electrodes were found to be photoexcitable. This paper deals with the photoelectrochemical reactions of nicotinamide adenine dinucleotide (NAD^+) at such chlorophyll–liquid crystal electrodes.

EXPERIMENTAL

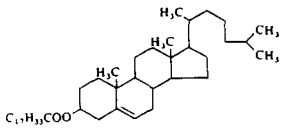
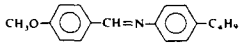
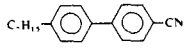
Materials

Chlorophyll was isolated from green spinach leaves according to the procedure described by Jacobs et al. (5), and was then dissolved in petroleum ether to be stored in a cold and dark place. Concentration of chlorophyll was assayed from an 80% acetone solution of chlorophyll from absorbance at 645 and 663 nm (A_{645} and A_{663}) according to the equation (6)

$$C\ (\text{mg/liter}) = 20.2A_{645} + 8.02A_{663} \qquad (1)$$

Cholesteryl oleate (COL), obtained from Tokyo Kasei Company (Tokyo),

TABLE 1. **Specifications of Liquid Crystals**

		Molecular weight	Mesomorphic range (°C)
Cholesteryl oleate		650	17–51
MBBA[a]		267	20–47
HCB[b]		277	28–42

[a] MBBA: *N*-(*p*-Methoxybenzylidene)-*p*-butylaniline.
[b] HCB: 4'-Heptyl-4-cyanobiphenyl.

was purified by recrystallization. *N*-(*p*-Methoxybenzylidene)-*p*-butylaniline (MBBA) and 4'-heptyl-4-cyanobiphenyl (HCB) were purchased from Fuji Film Company, Ltd. (Tokyo). The specifications of these liquid crystals are presented in Table 1.

Preparation of the Chlorophyll–Liquid Crystal Electrodes

The chlorophyll stock solution was exposed to air for evacuation of petroleum ether. Chlorophyll and a liquid crystal were dissolved in petroleum ether and benzene (9:1). The chlorophyll–liquid crystal electrode was prepared by spreading 0.1 ml of the resulting solution homogeneously on both surfaces of a platinum plate ($2 \times 4\ cm^2$). The chlorophyll–COL, chlorophyll–MBBA, and chlorophyll–HCB electrodes are noted as E(Chl–COL), E(Chl–MBBA), and E(Chl–HCB), respectively.

Photoelectrochemical Measurements

A two-compartment glass cell partitioned by a porcelain diaphragm was employed for measurements. The chlorophyll–liquid crystal electrode was installed in the cell to face the light source. A platinum plate ($2 \times 4\ cm^2$) was used as a counterelectrode. Each compartment of the cell contained 20 ml of electrolyte which was initially deoxygenated with bubbling N_2. A supporting electrolyte of 0.03 M phosphate buffer was used at pH 7.0. The measurements were carried out at 30 ± 0.5°C. The chlorophyll–liquid crystal electrode was illuminated through a heat absorber with a reflector lamp (100 V, 500 W). The experimental setup is profiled in Fig. 2.

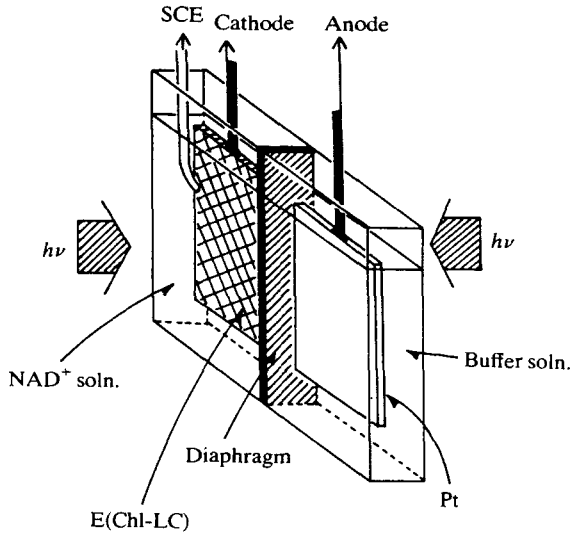


FIG. 2. The experimental set up of the chlorophyll–liquid crystal electrode.

Measurements of electrode potential, current, and current–potential characteristics were conducted with Hokuto Denko Model HA-101 potentiostat, Model HB-107A function generator, and Riken Denshi Model F-3D X-Y recorder, Toa Electronics Model EPR-2T recorder. Electrode potential was referred to a saturated calomel electrode (SCE).

RESULTS

Photoresponse of the Chlorophyll–Liquid Crystal Electrodes

A chlorophyll–MBBA electrode, E(Chl–MBBA), which contained 1.4×10^{-8} mol chlorophyll/cm^2 ([MBBA]/[Chl] = 1.0), was immersed in 1 mM NAD^+ at pH 7.0, 30°C. The electrode exhibited a steady potential of 26 mV vs. SCE in the dark. The potential shifted drastically to more positive potential on light irradiation as shown in Fig. 3. When the light was off, the initial electrode potential in the dark was recovered within 30 sec. The photoresponse in electrode potential was repeated by turning the light on and off. In order to determine the photocurrent, the following electrochemical system was assembled.

E(Chl–MBBA) | 1 mM NAD^+ | Phosphate buffer | Pt

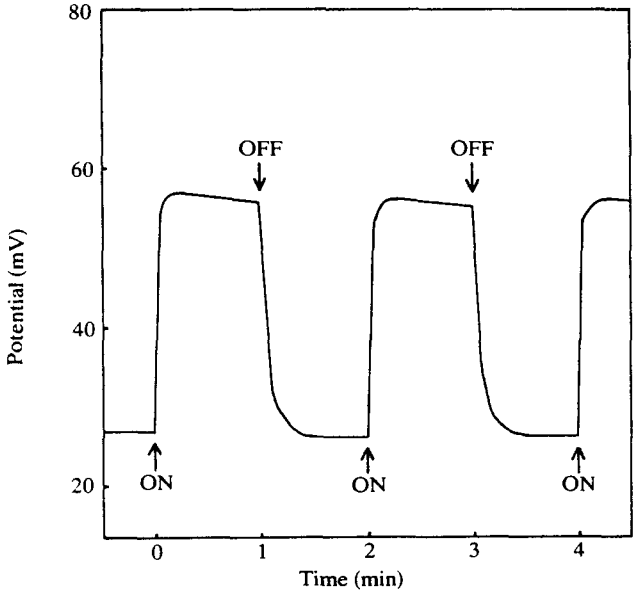


FIG. 3. Photoresponse of E(Chl–MBBA). The E(Chl–MBBA) contained 1.4×10^{-8} mol chlorophyll/cm^2 at a MBBA fractional ratio of 1.0. The measurement was made at 30°C for E(Chl–MBBA)|1 mM NAD^+, pH 7.0 phosphate buffer|pH 7.0 phosphate buffer|Pt. Light was imposed on the E(Chl–MBBA) at an intensity of 30,000 lux.

The potential of E(Chl–MBBA) was controlled at −0.2 V vs. SCE with the potentiostat. Cathodic current of 12 μA was recorded in the dark. A sharp increase in cathodic current occurred on light irradiation as presented in Fig. 4. When the irradiation was stopped, the current immediately decreased to the level in the dark. Photocurrent was prominently dependent on the controlled potential of E(Chl–MBBA) and is defined by the difference between the current on light irradiation and that in the dark. Figure 5 presents the photocurrent derived at different electrode potentials. The photocurrent increased with cathodic polarization of E(Chl–MBBA).

Effect of Liquid Crystals on the Photoresponse

Several chlorophyll–liquid crystal electrodes, such as E(Chl–MBBA), E(Chl–HCB), and E(Chl–COL), were prepared in order to compare the effects of various liquid crystals on the photoresponse. All the electrodes were found to be photoresponsive. An increase in photocurrent was

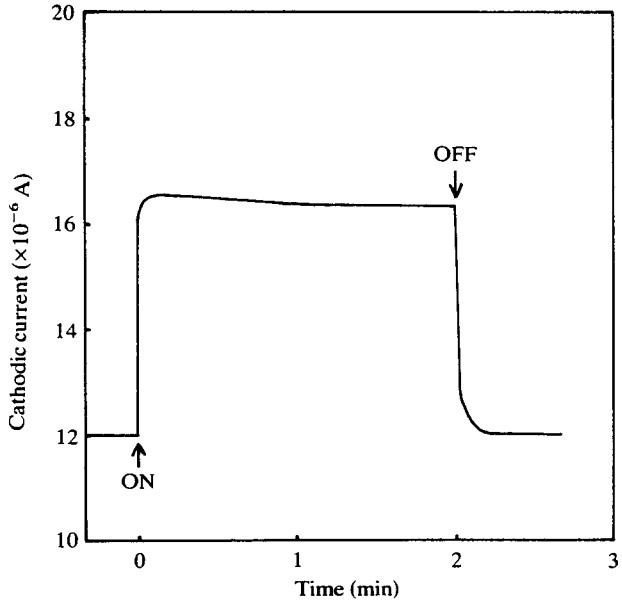


FIG. 4. Photocurrent of E(Chl–MBBA) at a controlled potential. E(Chl–MBBA)|1 mM NAD^+, pH 7.0 phosphate buffer|pH 7.0 phosphate buffer|Pt. The potential of the E(Chl–MBBA) was controlled at −0.2 V vs. SCE in the dark. The electrode was exposed to light at an intensity of 30,000 lux.

observed for these electrodes with cathodic polarization. The photocurrent and response time of these electrodes are listed in Table 2. The measurements were made for the following system:

$$\text{E(Chl–LC)} \mid 1\ \text{mM}\ NAD^+ \mid \text{Phosphate buffer} \mid \text{Pt}$$

The chlorophyll–liquid crystal electrode contained 1.9×10^{-8} mol/cm^2 chlorophyll. The fractional ratio of liquid crystal to chlorophyll was maintained at 1.0. The potential of the chlorophyll–liquid crystal electrode was

TABLE 2. **Comparison of Various Chlorophyll–Liquid Crystal Electrodes**[a]

Electrode	Photocurrent (× 10^{-6} A)	Response time (sec)
E(Chl)	14	84
E(Chl–MBBA)	50	44
E(Chl–HCB)	32	66
E(Chl–COL)	43	78

[a] See the text for details.

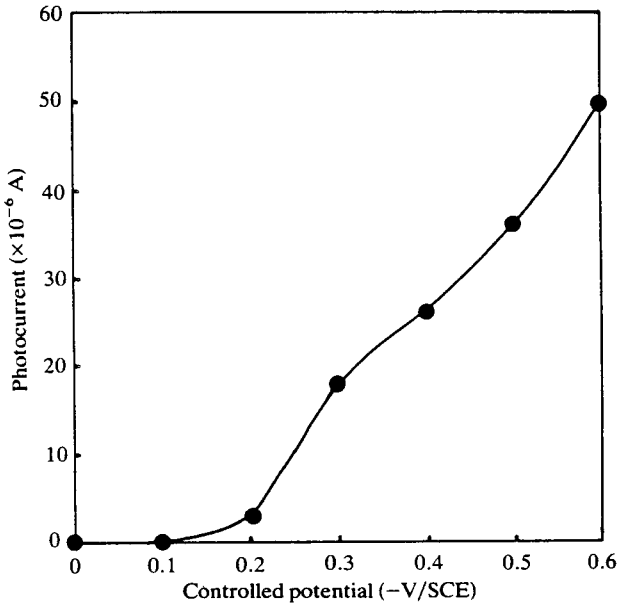


FIG. 5. Photocurrent at various controlled potentials of E(Chl–MBBA). E(Chl–MBBA)|1 mM NAD^+, pH 7.0 phosphate buffer|pH 7.0 phosphate buffer|Pt.

controlled at −0.6 V vs. SCE with the potentiostat, and then the electrode was irradiated at an intensity of 30,000 lux. The response time is defined as the time required for attaining 90% of photocurrent. These chlorophyll–liquid crystal electrodes exhibited an increase in the photocurrent as compared with that of the chlorophyll electrode, E(Chl), containing no liquid crystal. The incorporation of MBBA caused a marked generation of photocurrent in comparison with that of HCB and cholesteryl oleate. MBBA also promoted the photoresponse as given in Table 2.

From these results the subsequent characterization was focused on the E(Chl–MBBA). In Fig. 6, the photocurrent of E(Chl–MBBA) is presented at various contents of MBBA. The chlorophyll content of the E(Chl–MBBA) was fixed at 1.9×10^{-8} mol/cm^2, and the measurements were made for the above-mentioned system. The potential of the various E(Chl–MBBA) was controlled at −0.6 V vs. SCE in the dark. An increase of current on the light irradiation was plotted versus the content of MBBA. The photocurrent increased with an increase in the MBBA content, reaching the maximum. When the fractional ratio of MBBA to chlorophyll exceeded 1.0, the photocurrent was rather suppressed, and decreased with

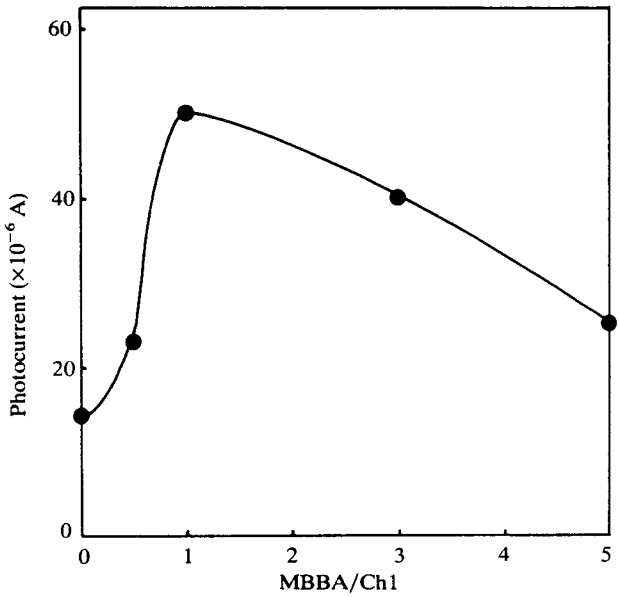


FIG. 6. Effects of MBBA on the photocurrent. E(Chl–MBBA)|1 mM NAD^+, pH 7.0 phosphate buffer|pH 7.0 phosphate buffer|Pt. The content of chlorophyll was maintained at 1.9×10^{-8} mol/cm^2. The potential of E(Chl–MBBA) was controlled at −0.6 V vs. SCE.

the MBBA content. At a fractional ratio (MBBA/Chl) of 30, a stable chlorophyll–MBBA electrode could not be prepared.

The effect of the chlorophyll content on the photocurrent is presented in Fig. 7. The fractional ratio of MBBA to chlorophyll was fixed at 1.0, and the other conditions were the same as those just described. As shown in Fig. 7, the maximum photocurrent was obtained at an appropriate chlorophyll content. It is to be noted that excess chlorophyll depressed the photocurrent.

DISCUSSION

A marked photoresponse was observed for the chlorophyll–MBBA electrode, E(Chl–MBBA). The potential of this electrode shifted to a more positive potential on light irradiation. Furthermore, an increase in cathodic current resulted from the exposure to light. These facts indicate that the photoexcitation of chlorophyll immobilized with MBBA on the platinum

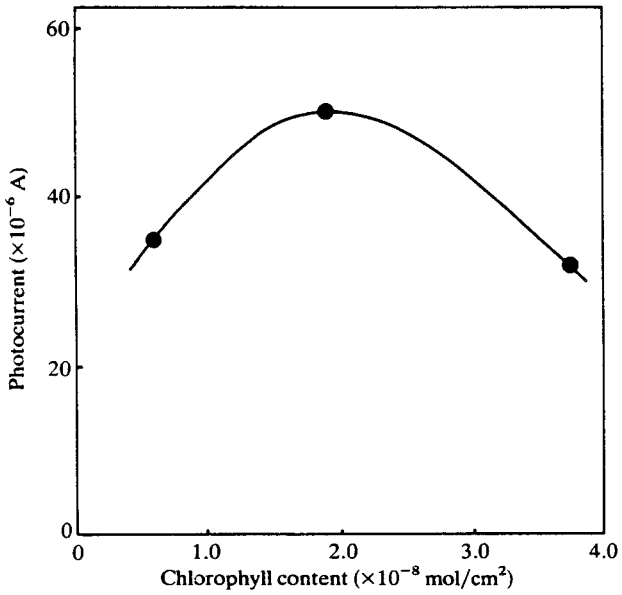


FIG. 7. Dependence of photocurrent on the chlorophyll content. E(Chl–MBBA)|1 mM NAD^+, pH 7.0 phosphate buffer|pH 7.0 phosphate buffer|Pt. The MBBA fractional ratio (MBBA/Chl) was maintained at 1.0. The potential of E(Chl–MBBA) was controlled at −0.6 V vs. SCE.

surface promotes the electron transfer from the platinum to NAD^+ molecules in a solution. The postulated scheme of the electron transfer is illustrated in Fig. 8. The potential of the electrode is controlled with a potentiostat in such a way that electrons are driven from the platinum to the layer of chlorophyll and liquid crystal. According to polarographic data, NAD^+ in a solution cannot accept electrons from the platinum at such a potential. In other words, there might exist an energy barrier at the chlorophyll–solution interface. However, electrons may pass over the energy barrier due to the photoexcitation of chlorophyll. The electrons are considered to be excited enough to flow into a solution. As illustrated in Fig. 8, the electron transfer may be continued if chlorophyll is consecutively regenerated in the liquid crystal layer.

Liquid crystals were found effective on the photocurrent generation of immobilized chlorophyll. The role of liquid crystal is considered to relate to the mobility and orientation of chlorophyll molecules, which are required for the photoexcitation and electron transfer.

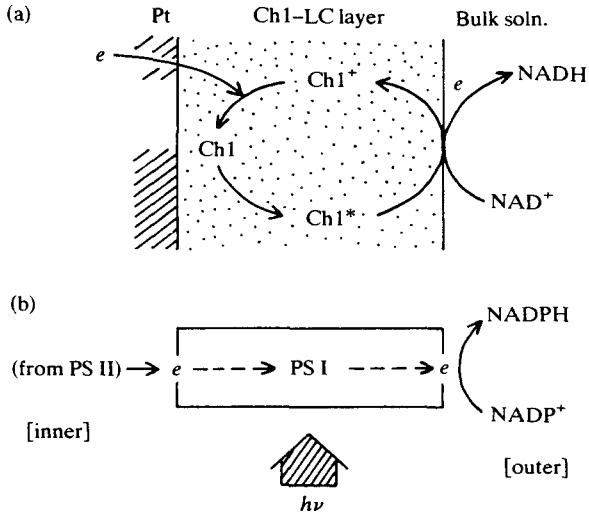


FIG. 8. A postulated scheme of electron transfer. (a) Electron transfer at a chlorophyll–liquid crystal electrode. (b) A simplified electron transfer of PS I in primary photosynthesis.

Evidence derived from electron microscopy, x-ray diffraction, and birefringence as well as circular dichroism studies strongly indicates that the photosynthetic system is composed of highly organized thylakoid membranes. This high degree of orderliness and lamellar organization of thylakoid membranes has led to the suggestion that crystalline lattice containing chlorophyll molecules and other compounds may be involved in the photosynthetic system, and that such an organized structure of light-sensitive pigments may possess photoconductive properties resembling those of organic semiconductors. Such orderliness is accomplished by phospholipids in the liquid crystal state.

Liquid crystals contained in the chlorophyll–liquid crystal electrodes are thought to play roles similar to those of phospholipids in thylakoid membranes. However, a chlorophyll–liquid crystal layer in the electrodes is a multimolecular layer, whereas the thylakoid membranes are believed to be composed of bimolecular layers. Electron transfer through the chlorophyll–liquid crystal layer should be one of the most important processes in the photoresponse of the electrodes. In fact, excess liquid crystal depressed the photocurrent. The chlorophyll–liquid crystal electrodes responded on light irradiation at an appropriate ratio of liquid crystal to chlorophyll.

A marked photoresponse of the chlorophyll–liquid crystal electrodes suggests that a new photoelectrochemical system composed of the chloro-

phyll–liquid crystal electrode is promising in the development of biochemical photoenergy conversion.

CONCLUSION

The photoexcitable chlorophyll–liquid crystal electrode was prepared by coating a platinum plate with a thin layer of chlorophyll–liquid crystal composite. Liquid crystals were effective on the photoexcitation of the immobilized chlorophyll. The photoelectrochemical reaction was promoted at the chlorophyll–liquid crystal electrode as PS I in photosynthesis.

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