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Properties of Semiconductor Electrodes Coated with Living Films of Cyanobacteria

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Abstract

Intact *Phormidium* sp. cells, immobilized on a SnO_2 semiconductor electrode, are capable of transferring electrons to SnO_2 in a light-dependent reaction. Drying a "wet" algal electrode at 50°C for 60 min increases photocurrent output capacity by 100-fold. We have studied the effect of various parameters on photocurrent generation. The magnitude of the photocurrent increased with increasing light intensity and depended on the nature of the electrolyte solution. The output, about 8 μA 10 μg Chl^{-1} cm^{+2}, was obtained using 50 m*M* H_3BO_3–Na_2CO_3–KCl buffer as an electrolyte, an irradiance (>460 nm) of 250 J/m^2, and potentiostatic conditions (the algal working electrode was poised at +0.6 V vs a saturated calomel electrode). The yield was more than doubled upon addition of an electron carrier, such as methyl viologen, benzyl viologen, or Vitamin K_3, to the electrolyte solution. Maximum photocurrent was obtained at around pH 8 and 45°C, which are optimal conditions for growth of the cyanobacterium. Furthermore, DCMU, an inhibitor of photosynthetic electron flow, drastically decreased the yield, as did heat treatment of the electrode at 110°C for 15 min. The photocurrent action spectrum peak coincided well with the absorption peak of the light-harvesting pigment, phycocyanin. These results support the idea that electron transfer can occur across algal cell walls from the source of the light-induced reactions located within the lamellar membranes to the semiconductor electrode.

Index Entries: Immobilized algal cells; thermophilic blue-green alga; Cyanobacterium, *Phormidium* sp.; photocurrent generation, with immobilized algae; semiconductor electrodes, coated with immobilized algae; electrodes, coated with immobilized algae; electron transfer, by immobilized algae.

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Introduction

Electrons generated during the primary light-induced, charge-separation steps in chloroplasts can be transferred to an electrode, especially when the electrode is poised at a potential of more than +0.2 V vs the saturated calomel electrode (SCE)†. Allen and Crane (*1*) first reported that coulombic outputs were produced upon irradiation of thylakoid membrane suspensions layered on a platinum working electrode. Recently, we demonstrated photoresponses from electrodes composed of immobilized chloroplasts coated on tin–oxide transparent electrodes (SnO_2 OTE) (*2, 3*). However, the chloroplast activity deteriorated rapidly upon aging and warming when the electrode was exposed to light. On the other hand, bacterial chromatophores as well as higher plant photosystem-I (PS-I) particles are known to contain relatively stable reaction center complexes. Janzen and Seibert (*4*) and Seibert et al. (*5, 6*) have demonstrated that bacterial reaction center and chromatophore films dried on SnO_2 electrodes are capable of generating steady-state photocurrents in a photoelectrochemical cell when exposed to red light. Gross and coworkers have developed a "photosynthetic solar cell" that consists of two half-cells separated by a Metricel filter upon which PS-I particles are deposited (*7, 8*). Both approaches have led to longer lasting photoeffects. In 1980, we reported on a "living algal electrode" that was prepared using intact thermophilic algal cells of *Mastigocladus laminosus* in place of higher plant chloroplasts (*9*). This algal electrode functioned as a stable photoanode even under continuous illumination, though the photoconversion efficiency was very low. However, activity lasted for at least 20 d. We report here that the cyanobacterium, *Phormidium* sp., can work much better than *M. laminosus* as an algal electrode and that a significant enhancement of the photocurrent yield can be attained by drying the electrode at moderate temperature. We also have studied the effect of various parameters on photocurrent generation in order to elucidate the properties of the *Phormidium* electrode.

Materials and Methods

Cultivation of Blue-Green Algae

The filamentous blue-green alga, *Phormidium* sp., isolated from the Matsue hot springs, was cultured as described previously (*9*). One can use Fitzgerald-modified culture medium (*10*) to cultivate the algae, though better growth is obtained using Matsue hot spring water as the basal medium. Late logarithmic phase cells were collected by filtration utilizing four layers of nylon cloth. After washing with distilled water, the cells were suspended in distilled water. This *Phormidium* sp. is

†Abbreviations: PS-I, photosystem I; PS-II, photosystem II; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethyl urea; CCCP, *meta*-chlorocarbonyl cyanide phenylhydrazone; DPIP, 2,6-dichlorophenol indophenol; LIP, light induced potential; MV, methyl viologen; BV, benzyl viologen; MB, methylene blue; PMS, phenazine methosulfate; SCE, saturated calomel electrode; SnO_2 OTE, tin-oxide optically transparent electrode; TPB, tetraphenylboron; U_{fb}, flat-band potential.

apparently an organism with remarkably porous membranes. Redox dyes readily penetrate the cell, enabling us to measure both PS-I and Photosystem II (PS-II) activity in the intact cells. This phenomenon is also true for the filamentous cyanobacterium, *Mastigocladus laminosus*.

Preparation of the Immobilized Algal Electrode

SnO_2 OTE (Matsuzaki Shinku Ltd., Tokyo) functions as a semiconductor in the same way as noted previously (*3*). The procedure used to prepare immobilized algal electrodes has been described earlier (*3*). The algal cells are suspended in an equal volume of 8% sodium alginate solution of hot spring water, then the suspension is spread on the surface of a SnO_2 OTE and the electrode plate dipped for 5 min in a 50 m*M* $CaCl_2$ solution buffered at pH 8.0. Finally, the electrode is washed with hot spring water. Thus, one obtains an algal electrode entrapped inside a wet calcium alginate matrix, about 250 μm thick. A freshly prepared electrode was incubated in culture medium for the indicated period of time and used as a "wet" electrode. Wet electrodes were allowed to dry in an electric oven at 50°C, then set up in the cell assembly for photoelectrochemical measurements. Dried algal electrodes were about 40 μm thick. It should be noted that the SnO_2 OTE is suitable for use as a support material because it does not catalyze Chl photodestruction, as is the case with platinum. In addition, Seibert et al. (*5*) noted that substitution of a semiconductor for platinum electrodes was advantageous because Dember effects (*4*) in the former represented a small fraction of the total photoeffects.

Photoelectrochemical Measurements

The photoelectrochemical cell and the apparatus for photocurrent measurements has been described earlier (*3*). The algal/SnO_2 electrode served as the working electrode and the illumination area was 36 cm^2, unless otherwise stated. The basal electrolyte was buffered with 50 m*M* borate containing 50 m*M* sodium carbonate and 50 m*M* potassium chloride, unless otherwise noted. The potential of the SnO_2 electrode was controlled by a Model NPGS-301 Nikko Keisoku potentiostat. A platinum plate and a SCE served as the counter electrode and the reference electrode, respectively. A 300 W projector lamp was used as the light source in combination with a color filter (Toshiba Kasei, Y-46) that cuts off below 460 nm to eliminate photoresponses attributable to SnO_2 itself. The light intensity absorbed by the algal electrode was determined wih a Model TC-3 Ritsu Ohyokogaku thermopile radiometer. Photocurrents were measured at 45°C with a Model PM-18 Toa Electronics, DC-Microvolt ammeter and recorded with a Model 057 Hitachi XY recorder. Algal cell density deposited on an SnO_2 OTE glass plate varies with each preparation, hence the photocurrent value is expressed on the basis of 10 μg Chl cm^{-2} of the SnO_2 OTE.

Photosystem Activity Measurements

Algae/calcium alginate film was detached from the SnO_2 OTE surface and cut into pieces that then were suspended in the reaction mixture for assays. The assays for PS-I and PS-II activity were performed by procedures similar to those used with

intact cells. PS-I activity (Mehler reaction) was assayed by the method of Epel et al. (*11*), with a slight modification. The assay system was as follow: 3.0 mL of 50 m*M* potassium phosphate (pH 8.0), 0.15 mL of 100 m*M* ascorbate–5 m*M* DPIP, 0.15 mL of 2 m*M* methyl viologen, 0.03 mL of 300 μ*M* DCMU, 0.05 mL of 100 m*M* sodium azide, and immobilized cells (equivalent to about 40 μg of Chl) in a total volume of 10 mL . The mixture was illuminated under constant stirring, and oxygen uptake was measured with a Model 570 YSI dissolved oxygen meter. PS-II activity was assayed by determining the photoreduction of DPIP in accordance with the method of Katoh and San Pietro (*12*), but modified slightly. The assay mixture contained 2.0 mL of 50 m*M* potassium phosphate buffer (pH 8.0), 0.3 mL of 1 m*M* DPIP, and immobilized cells (equivalent to about 10 μg Chl) in a total volume of 3.0 mL. The mixture was stirred with illumination and centrifuged to obtain a supernatant. The decrease in absorbance of the supernatant at 610 nm was recorded (molar extinction coefficient: 21×10^3). Throughout the assay procedure, illumination of 150 J/m^2 and a temperature of 40°C were maintained. The rate of the dark control was subtracted in all cases. Oxygen evolution was measured with a Model 570 YSI dissolved oxygen meter without addition of any Hill oxidant.

For Chl determination, the cells were ground with a Type VI3, Edmund Bühler Vibrogen and treated with 80% acetone. The Chl a/acetone extract was assayed spectrophotometrically by the method of MacKinney (*13*). All the chemicals were of special grade from Wako Pure Chemical Industries and used without further purification.

Results

Algal Cells Immobilized with Calcium Alginate

Figure 1 shows the time course of three photochemical activities and of Chl accumulation in a wet algal electrode stored under continuous illumination in culture medium. Apparent low activities with some fluctuations are likely the result of the diffusion resistance of calcium alginate gel to the reagents. As shown by the Chl accumulation curve, the algae remain alive and grow in the alginate matrix under these conditions. The specific activities of PS-I, PS-II, and oxygen evolution were almost unchanged throughout the light period.

Wet electrode photocurrent–potential curves are similar to those reported before for the algal electrode (*9*). The anodic photocurrent rises from around +0.2 V vs SCE and increases linearly with the potential imposed. Since *Phormidium* sp. cells immobilized in the alginate matrix can multiply in the light, the wet electrode, cultured for 5 d, gives twice the photocurrent on an area basis compared to the same electrode when freshly coated (data not shown). This is not true for polyvinyl alcohol-immobilized algae, because the cells were killed during the process of drying *in vacuo*.

Light Intensity

We examined the dependence of the photocurrent on the incident light intensity under the condition where the working electrode was poised at +0.6 V vs SCE.

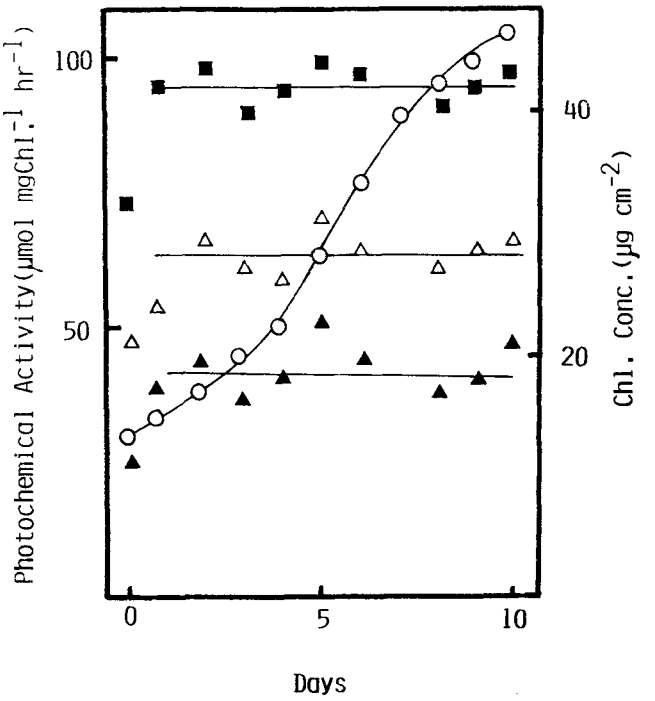


Fig. 1. Time courses of photochemical activities (PS-I, PS-II, and oxygen evolution) and Chl accumulation of the algal cells immobilized on an SnO_2 OTE with calcium alginate under continuous illumination. Freshly prepared algal electrodes were used from day 0: ○, chlorophyll; △, PS-I; ▲, PS-II; ■, oxygen evolution.

The photocurrent output increased with increasing light intensity and did not saturate at 500 J/m^2 (data not shown) similar to the case of the chloroplast electrode (400 J/m^2) (*3*). In general, the photosynthetic capacity of C_3-plants saturates at light intensities of less than 200 J/m^2 because of limiting dark reactions. Our findings suggest that, by imposing potentiostasis (voltage clamp) on an irradiated algal electrode, electrons driven by the photosynthetic light reactions are transferred to the SnO_2 anode independently of the normal limiting dark reactions.

Time Course

Figure 2 is a profile of the photocurrent output as a function of time with the working electrode poised at +0.6 V and irradiated with 150 J/m^2 light. The photocurrent was steady for the first hour, but dropped rapidly thereafter. However, no pigment bleaching occurred on the algal electrode during the 3 h run, as determined by measuring the absorption spectrum of the electrode. Interestingly, after incubating the same electrode in culture medium under fluorescent lamps (Mitsubishi FL-20SPG, 2000 lux) for 24 h, the electrode was able to function as actively as a fresh electrode.

Drying Treatment

Figure 3 reveals the relationship between the drying period and the photocurrent output from the dried electrode. The outputs in the figure are shown in terms of

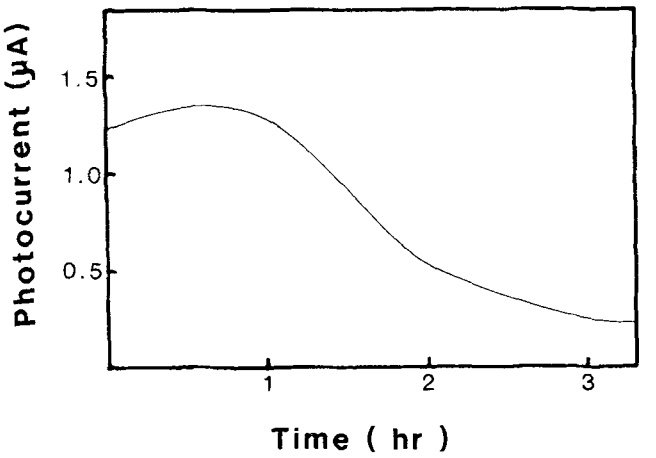


Fig. 2. Time course of the photocurrent output of a wet algal electrode poised at +0.6 V vs SCE. Light passed through a Y-46 filter (150 J/m^2) and illuminated the electrode continuously. Electrolyte: H_3BO_3–Na_2CO_3–KCl (50 m*M*, pH 8.0, 45°C). Illumination area of the electrode, 36 cm^2.

enhancement ratios. The specific photocurrent measured with a 60 min-treated electrode was 5.1 μA 10 μg Chl^{-1} cm^{+2}. This represents about a 100-fold increase in output in comparison to that observed with a wet electrode. It should be noted that this large enhancement could result from increases in algal cell contact with the SnO_2 electrode from the drying treatment. Drying decreases the thickness of a wet electrode from 250 to 40 μm. Figure 4 shows cyclic voltammograms for a

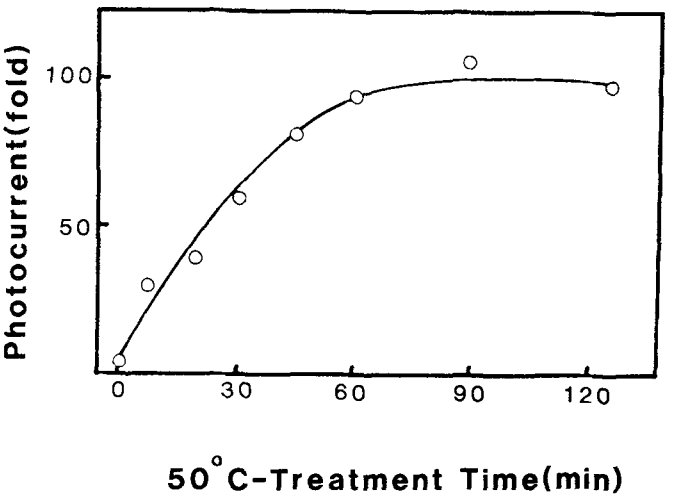


Fig. 3. Relationship between drying time and the photocurrent enhancement. Wet algal working electrodes were dried in an electric dryer at 50°C for different periods of time. The working electrode, poised at +0.6 V vs SCE, was illuminated (200 J/m^2) in an electrolyte consisting of 50 m*M* borate buffer (pH 8.0) at 45°C. The ordinate shows the photocurrent in terms of enhancement ratio (fold) with respect to the value observed with the wet electrode (0 min). The activity of the wet electrode in this case was 58 nA 10μg Chl^{-1} cm^{+2}.

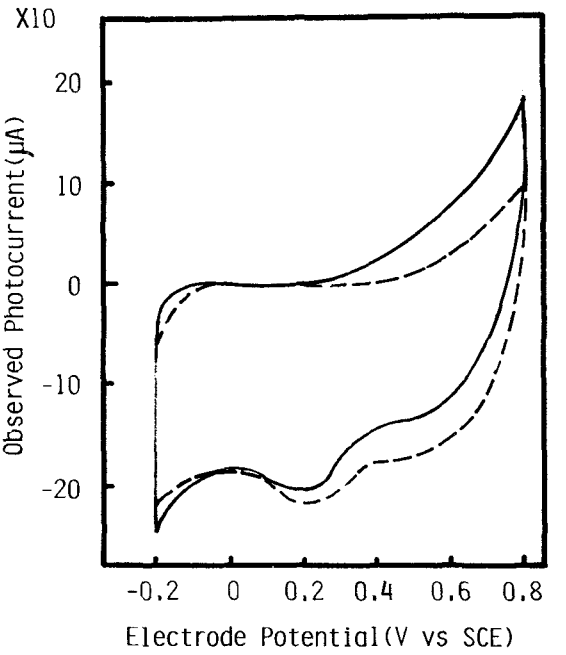


Fig. 4. Cyclic voltammograms for a dried algal electrode. A dried algal electrode dipped in 50 m*M* borate electrolyte (pH 8.0) at 45°C. First, a cyclic voltammogram was recorded repeatedly under dark condition from −0.2 to +0.8 V vs SCE with a scan rate of 1 V/min until the current was steady over a wide range of the scanning potentials. The resulting steady curve was illustrated as a "dark" voltammogram. Then the same algal electrode was illuminated (250 J/m^2 through a Y-46 filter) with the same scan rate to obtain a steady "light" voltammogram. Illumination area of the electrode: 36 cm^2; (——) light; (---) dark.

dried electrode. Above +0.2 V vs SCE, the anodic photocurrent increases with applied potential, though the current value is variable depending upon a scan rate used.

Table 1 exhibits the difference in output capacity between *Phormidium* sp. and *Mastigocladus laminosus* described earlier (*9*). The former was more efficient than

TABLE 1
Photocurrent Outputs by *Phormidium* sp. and by *Mastigocladus* sp.

Mastigocladus : *Phormidium*[a]			μA/10 μg Chl cm^{-2}
10	:	0	1.2
7	:	3	2.3
3	:	7	5.9
0	:	10	6.6

[a]Mixing ratio of two species on a chlorophyll basis. Each dried electrode was illuminated (250 J/m^2) under potentiostatic conditions at +0.6 V vs SCE in 50 m*M* borate buffer (pH 8.0) at 45°C.

the latter. Filamentous *Phormidium* cells are generally much thinner than *Mastigocladus* sp. cells. Hence, the packing density of *Phormidium* on the SnO_2 is probably greater than is the case for *Mastigocladus* cells.

Effect of Electrolyte Nature

Table 2 summarizes the influence of various electrolyte buffers on photocurrent yields. High yield (8.2 μA 10 μg Chl^{-1} cm^{-2}) was attained using 50 m*M* borate buffer (pH 8.0). Fifty m*M* was the optimum concentration required for maximum effect (data not shown). We have reported elsewhere that borate also stimulates photosynthetic ATP accumulation in living algal cells (*14*). These findings support the previous idea that borate may have potent effects on ion transfer through the plasma membrane and cell wall of the algae. It also suggests the uniqueness of this alga and the Matsue hot spring water. This water, used as basal culture medium, contains more than 50 ppm borate as one of its inorganic constituents. This is a unique composition relative to the general nature of hot spring waters. In fact, Matsue hot spring water used as electrolyte also gives satisfactory results (Table 2). On the other hand, Tris buffer and Tricine buffer were rather inhibitory to photocurrent output, while 50 m*M* phosphate buffer induced a solvolysis of the alginate matrix resulting in the lowest yield. Addition of 10 m*M* $CaCl_2$ to the borate buffer leads to an increased output, but this is not true for $MgCl_2$ and $BaCl_2$. Calcium ions probably prevent cells from leaking out of the alginate matrix during the experiments.

Effect of KCl Concentration, Temperature, and pH

Concentrated KCl is generally used as a conductor in a vareity of electrochemical cells. The effect of KCl concentration on the photocurrent output was investigated in Fig. 5A and 100 m*M* was found optimal. However, we have found that KCl

TABLE 2
Effects of Various Electrolytes on the Photocurrent Output by a Dried Electrode[a]

	Electrolyte, pH 8.0	Photocurrent, %
A.	50 m*M* H_3BO_3–Na_2CO_3–KCl	100
B.	Matsue hot spring water	55
C.	50 m*M* Tris-HCl-KCl	35
D.	50 m*M* Tricine-NaOH-KCl	18
E.	A + 50 m*M* Tris-HCl-KCl	32
F.	50 m*M* K-phosphate	2
G.	A + 10 m*M* $CaCl_2$	150
H.	A + 10 m*M* $CaCl_2$ + 20 m*M* $MgCl_2$	76
I.	A + 10 m*M* $BaCl_2$	87

[a]The algal electrode was poised at +0.6 V vs SCE at 40°C. Illumination, 250 J/m^2 through a Y-46 filter. The photocurrent was expressed in terms of a relative value compared to the photocurrent yield observed with A. A specific current with A was 8.2 μA 10 μg Chl^{-1} cm^{-2}.

concentrations higher than 50 m*M* exert an inhibitory effect on the photosystem functions of the living algae, especially during long continuous runs. Hence, we have used 50 m*M* KCl as a normal component in the electrolyte. Seibert and Kendall-Tobias (*6*) have reported qualitatively similar results for bacterial chromatophore function. The maximum photocurrent output was noted at around 45°C (Fig. 5B), which is close to the cultivation temperature of the algae. In addition, maximum photocurrent output was found at pH 8.5, which is also close to the pH of the hot spring water employed as basal culture medium (Fig. 5C).

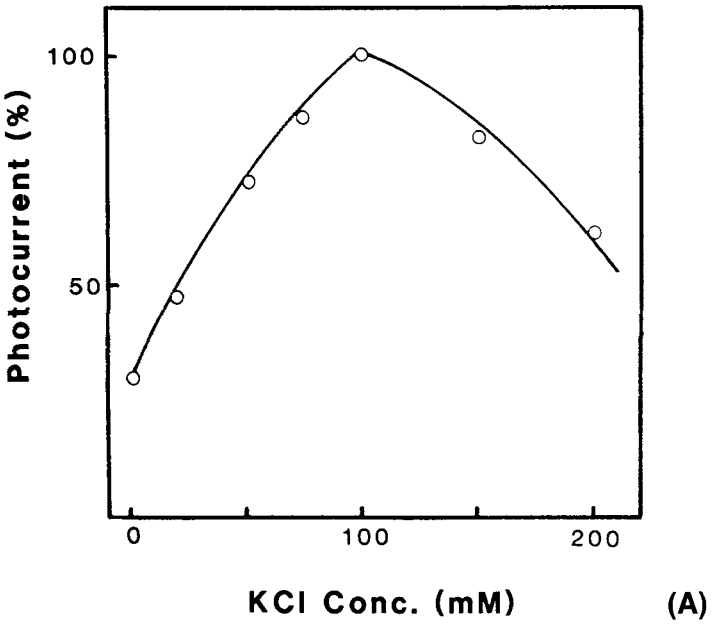
Action Spectrum

A typical photocurrent spectrum is illustrated in Fig. 6, together with the absorption spectrum of an immobilized algal film deposited on an SnO_2 OTE. Both spectra coincide fairly well in the region from 550 to 650 nm, over which phycobiliprotein antenna pigments are known to absorb. Phycocyanin that absorbs around 620 nm contributes to the photocurrent production more strongly than Chl a (680 nm). Fay (*15*), using an intact cyanobacterium, *Anabaena cylindrica,* reported that maximum oxygen evolution occurred in 625 nm light, a wavelength very close to the absorbance peak of phycocyanin.

Effects of Photosynthetic Inhibitors, Heat Treatment, and Electron Mediators

It is important to investigate the effect of various photosynthetic inhibitors in order to confirm whether the observed photocurrent depends upon electrons ejected from either or both of the algal photosystems in light-induced reactions. DCMU is a well-known inhibitor of electron flow from *Q,* the primary electron acceptor of PS-II, to the plastoquinone pool located between PS-II and PS-I. CCCP and TPB act as potent inhibitors of water oxidation on the oxidizing site of PS-II. Water oxidation is also known to be sensitive to heating; hence, an algal electrode was subjected to heat treatment in an electric dryer at 110°C for 15 min. Each inhibitor was added to the electrolyte so as to give the final concentration indicated in Table 3. After standing for 15 min, the photoresponse of the treated electrode was examined under potentiostatic conditions. Twenty μ*M* DCMU completely inhibited electron flow from *Q* to plastoquinone, as confirmed by the fact that no DPIP photoreduction occurs (data not shown). Furthermore, when DCMU was added to the electrolyte of a running (irradiated) algal photocell, the photocurrent output decreased hyperbolically to 6% of the original value over a period of 15 min. Both CCCP and TPB at the indicated concentrations completely inhibit the intact (not-immobilized) algae; however, TPB was not so effective under our conditions. The data in the table support the contention that PS-II and water photolysis contributes to photocurrent generation. Questions about the small inhibitory effect of TPB remain unanswered as yet, but it is at least partly a result of the diffusion resistance of alginate gel to TPB.

Table 3 also shows the effect of electron mediators upon the photocurrent output. The output was more than doubled upon addition of viologen reagents to the electrolyte. BV and MV are able to accept electrons only from P-430 of PS-I (16). The efficiency of electron flow to an SnO_2 OTE from the algal cells that are located



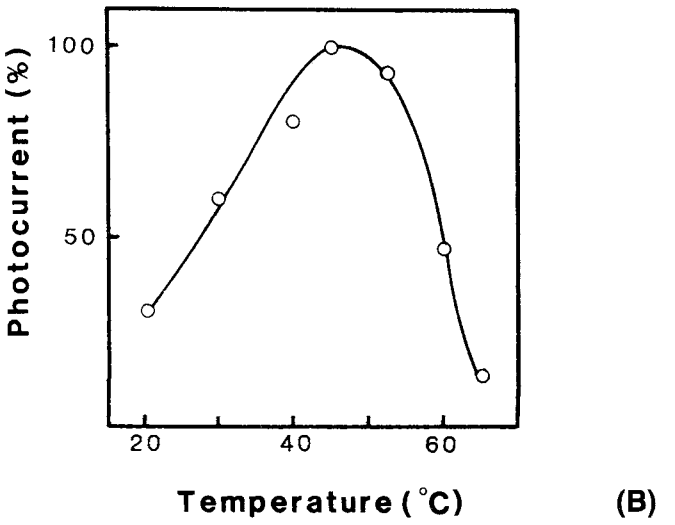


Fig. 5. Dependence of the photocurrent yield on KCl concentration, temperature, and pH of the electrolyte. *A*, Effect of KCl concentration: electrolyte used, 50 mM H_3BO_3–Na_2CO_3 (pH 8.0) at 45°C. Light intensity, 250 J/m^2 with a Y-46 filter. Applied potential, +0.6 V vs SCE. *B*, Effect of temperature: the electrode was in contact with the electrolyte solution adjusted to the temperature indicated for 15 min, then illuminated under the potentiostatic condition at +0.6 V vs SCE. Electrolyte, 50 m*M* H_3BO_3–Na_2CO_3–KCl (pH 8.0).

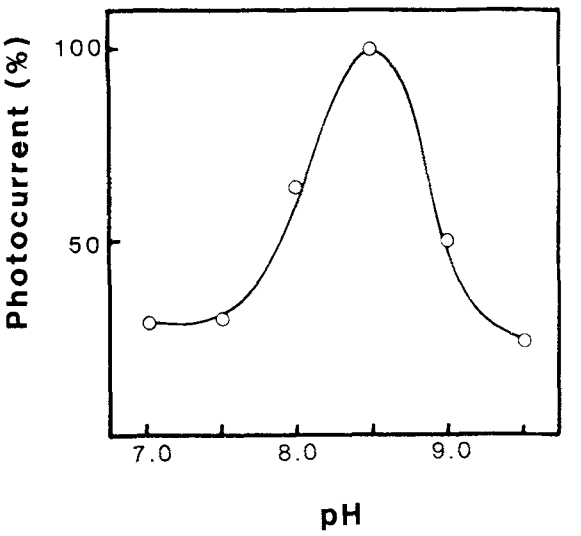


Fig. 5C. Effect of electrolyte pH: The electrode was in contact with the buffer solution adjusted to the pH indicated for 15 min, then illuminated. The maximum value observed in this case (C) was 10.2 μA 10 μg Chl^{-1} cm^{+2}.

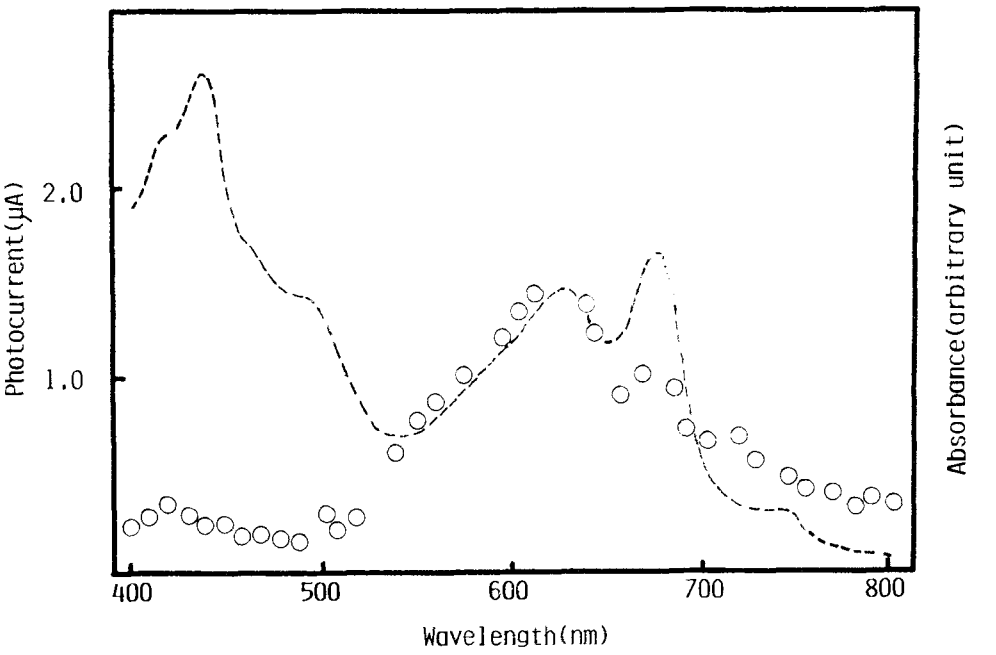


Fig. 6. Comparison of the action spectrum with the absorption spectrum. Open circles show the action spectrum for the anodic photocurrent. The broken line is the absorption spectrum of the dried algae deposited on an SnO_2 OTE. The electrolyte used was 50 m*M* borate buffer (pH 8.0) at 40°C. Ten nanometer half height bandwidth filters (Toshiba Kasei Ltd.) were employed. Electrode potential: +0.6 V vs. SCE.

TABLE 3
Effect of Various Inhibitors, Heat-Treatment, and Electron Mediators on Photocurrent Generation by a Dried Algal Electrode[a]

Treatment	Photocurrent, %
None	100
DCMU (20 μ*M*)	6
CCCP (50 μ*M*)	16
TPB (5 μ*M*)	79
Heat (110°C, 15 min)	3
BV (20 μ*M*)	294
MV (20 μ*M*)	242
Vitamin K_3 (10 μ*M*)	256
FMN (10 μ*M*)	83
MB (10 μ*M*)	45
PMS (20 μ*M*)	100

[a]Electrolyte used, 50 m*M* H_3BO_3–Na_2CO_3–KCl (pH 8.0) at 45°C. Light intensity, 250 J/m^2 through a Y-46 filter. Applied potential, +0.6 V vs SCE. The activity was expressed as a relative value to the photocurrent yield of an untreated algal electrode (None). A specific value of "None" was 8.6 μA 10 μg Chl^{-1} cm^{+2}.

distant from the SnO_2 must be lower than that from the algae in contact with the SnO_2. Hence, with the aid of BV, reducing equivalents from the algal photosystems can be transferred more easily to the SnO_2 OTE, resulting in the increased output. Similar effects are also true for MV. However, it is well known that reduced viologens are rapidly reoxidized by oxygen under aerobic condition (reduced MV: $k = 8.0 \times 10^8$ mol^{-1} s^{-1} at pH 7.0), and thus reduced oxygen or superoxide radicals can cause oxygen poisoning of electrode function. Therefore, we examined various redox agents and found that Vitamin K_3 (2-methyl-1,4-naphthoquinone) works as a stable and promising electron mediator. However, the midpoint redox potential of Vitamin K_3 is around +400 mV. Therefore, Vitamin K_3 in this case could facilitate electron transfer from Q to PS-I working as an exogenous phylloquinone, which is the main phytoquinone located in the thylakoid membrane of this alga. On the other hand, phenazine methosulfate (PMS), flavine mononucleotide (FMN), and methylene blue (MB) did not work well.

Discussion

Photosynthetic organisms generate reducing equivalents through light-induced electron transport reactions and ultimately obtain electrons from water molecules. Living *Phormidium* sp. cells are able to perform light-dependent electron transfer to SnO_2 OTE, especially under the imposed potentiostatic conditions discussed in the previous text. We have examined a wide variety of algae, both unicellular and

filamentous, for their capacity to generate photocurrents in a conventional three electrode system. Two species, *Phormidium* sp. and *Mastigocladus laminosus,* from the Matsue hot springs were found to function well. It is postulated that thermophilic algae from this hot spring are unique in their cell permeability properties compared with other algae and that this results in the large photocurrents observed in this study. Analysis of the Matsue hot spring water reveals that it contains a high concentration of borate (more than 50 ppm), again a unique circumstance. In general, plant growth is depressed in the presence of more than 50 ppm of borate, while our algal cells grow well in the hot spring water. More interestingly, activity of the algae, including photocurrent generation and ATP accumulation (*14*), is maximal when borate buffer (50 m*M*, pH 8.0) was used as reaction medium. Boric acid is a well-known crosslinking agent with *cis*-dihydroxy groups of sugar moieties. Therefore, the finding described above suggests that algal cells, which are sugar-coated, react favorably to the action of borate anion.

Since the current output of unirradiated algal electrodes is small and SnO_2 is not sensitive to the actinic light, algal light reactions have to participate in photocurrent generation. Figure 7 is a model that can explain the experimental results we have observed. When the light is turned on, electrons are driven from water molecules ($E_0' = +820$ mV or more) to P-430 ($E_0' = -500$ mV or less) (*16*) through the photosystems in the algal cells. The flat-band potential (U_{fb}) of the SnO_2 OTE used in these experiments is around -90 mV on the electrochemical scale (*5*). Especially when the algal photocell system is poised at $+200$ mV or more vs SCE, the SnO_2 conduction band must "bend down" within the bulk of the semiconductor material from the U_{fb}. This potential difference is the driving force for the photocurrent output (*5*). Therefore, electrons extracted from water molecules and delivered to PS-I through PS-II are assumed to be transferred to the SnO_2

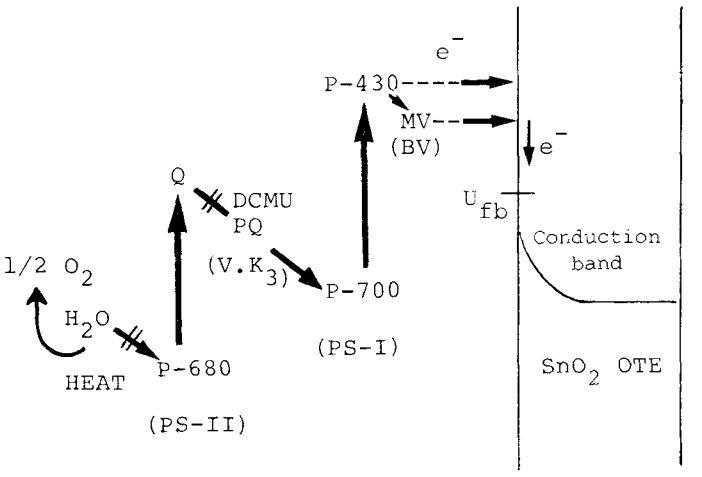


Fig. 7. Postulated electron transfer system. Adsorption of algal cells onto the SnO_2 does not appear to change significantly the flat-band potential (U_{fb}), which is around -90 mV under the conditions we used. Photosystems in the algal cells are depicted with simplicity. Electrons ejected from P-430 of PS-I, mediated by reduced MV or BV if present, must flow into the SnO_2 OTE. See the text for a more detailed explanation.

OTE mainly from P-430 of PS-I. MV ($E_0' = -450$ mV) and BV ($E_0' = -350$ mV) (*16*) must mediate electrons more efficiently from the photosystems to the SnO_2 OTE if the viologens are reduced through P-430 in the light. Several lines of evidence described in this study are in support of the interpretation postulated in Fig. 7. The photocurrent yield was more than doubled in the presence of BV or MV, which are reduced at the reducing side of PS-I. DCMU, an inhibitor of photosynthetic electron flow, drastically decreased the photocurrent output, as did heat treatment of the electrode at 110°C for 15 min. Moreover, the photocurrent spectrum peak coincided well with absorption peak of the light-harvesting pigment, phycocyanin. Effect of Vitamin K_3 ($E_0' = +400$ mV or more) on the photocurrent increase looks somewhat mysterious. Ejection of electrons from reduced Vitamin K_3 into the SnO_2 OTE is difficult because of the inhibition by band bending of the semiconductor conduction band, which results in an energy barrier for electrons more oxidizing than U_{fb}. However, we have recently confirmed that PS-I and oxygen evolution activities of quinone-depleted algal lamellae are completely recovered upon the addition of phytoquinone-extracts from the algae (unpublished results). Thus, Vitamin K_3, which is one of the simplest analogs of the phytoquinones, must work as an exogenous electron mediator, causing an increase of the electron transfer rate from PS-II to PS-I.

However, much can still be learned about this system. What is the entity produced on the lamellae and transmitted to the SnO_2 OTE across the cytoplasm and cell membrane? It is worth noting here that illumination brings about an electric potential difference across the plasma membrane and this is related to photosynthetic charge separation. Tazawa and coworkers have reported changes in membrane potential in eukaryotic algae *Spirogyra* (*17*) and *Chara* (*18*). The magnitude of the light-induced potential (LIP) depended on the number of chloroplasts inside the cell. Their experiments using photosynthetic inhibitors or potentiators clearly proved that LIP is related to both noncyclic and cyclic electron flow in the photosystems. However, there has been no information about the light-induced potential in the prokaryotic alga, *Phormidium* sp. Questions still remain about correlations between the phytoelectric phenomena, LIP, and the algal electrode.

In 1980, Hirano et al. demonstrated that plastoquinone functions not only in photosynthetic, but also in respiratory electron transport, thereby forming a common link between the two energy conservation systems in the thermophilic blue-green alga, *Shynechococcus* sp. (*19*). Similar ideas were noted earlier by Murai et al (*20*) and Yamaoka et al. (*21*) in the blue-green alga *Anabaena variabilis*. If this is also true for our *Phormidium* sp., neither prompt nor complete blocking of current generation could be expected by the addition of DCMU (Table 3). Stored ATP could generate electrons through back electron-transport system and supply them to PS-I for a while even in the presence of DCMU. Investigation of the two energy conversion systems of the *Phormidium* sp. is now in progress.

Photoelectrochemical cells using algal cell-coated electrodes such as described in this study may at some time be considered as solar energy conversion devices or, perhaps more realistically, model systems for artificial devices. Even though much progress has been made on the stability for biological photoelectrodes, efficiencies are still low. The power conversion efficiency is around 0.1% under the present

conditions (unpublished data). In this context, immobilizing the material with more strongly conducting substances should be attempted because significant energy transfer losses occur within the bulk of the calcium alginate gel. The next challenge is to develop more efficient algal species and this may include genetic manipulations.

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