Electrochemical System for the Simultaneous Monitoring of Algal Motility and Phototaxis

ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 2006

Impact Factor: 5.64 · DOI: 10.1021/ac051755x · Source: PubMed

2 AUTHORS, INCLUDING:

Isao Shitanda
Tokyo University of Science
97 PUBLICATIONS **406** CITATIONS

Anal. Chem. **2006,** *78,* 349−353

Electrochemical System for the Simultaneous Monitoring of Algal Motility and Phototaxis

Isao Shitanda and Tetsu Tatsuma*

Institute of Industrial Science, University of Tokyo, Komaba, Meguro-ku, Tokyo 153-8505, Japan

A compact electrochemical system equipped with dual electrodes was developed for the simultaneous monitoring of motility and phototaxis of flagellates. Photoinduced behavior of unicellular flagellate alga *Chlamydomonas reinhardtii* in the presence of diltiazem, azide, or ethanol was recorded as changes in the redox currents for a coexisiting redox marker. The system responded to the chemicals in significantly different ways; it allows qualitative and semiquantitative evaluation of influences of chemical species on the algal cells. Therefore, the present system is potentially applicable to not only aquatic risk assessment but also screening of newly found and synthesized compounds in terms of hazard probability and severity. Additionally, it unveiled some new and interesting behaviors of the algae.

Flagellates collect environmental information, such as light intensity, gravity, and chemical compositions around them and move to more suitable environments. Monitoring of their movements has been an important issue to characterize their behavior and to elucidate its mechanisms.[1] In addition, the monitoring of flagellate movement could be a rapid and sensitive bioassay technique regarding aquatic risk assessment for environmental water and industrial, agricultural, and domestic wastewater. It would also be effective in screening and evaluation of chemicals in terms of toxicity. In view of this, we recently developed compact monitoring systems for algal flagellar movement.[2] A solution containing flagellate alga, *Chlamydomonas reinhardtii,* and a redox marker is stirred by collective flagellar movement. A diffusion-limited current for the redox marker is observed at an electrode immersed in the solution. The current increases with the stirring rate, which is a function of algal population and average intensity of flagellar movement. This system was applied to monitoring of negative gravitaxis of the algae. Since this activity is inhibited by some organic compounds and heavy metal ions, these species can be detected by the system. However, in these toxicity tests, it was not easy to distinguish changes in gravitaxis from those in motility and other changes in electochemical reactions (e.g., reactions of redox-active species in the sample solution).

In the present study, we developed a new system for simultaneous monitoring of algal phototaxis and motility. In contrast to gravitaxis, phototaxis is easy to activate and inactivate just by turning light on and off, respectively. Therefore, responses based on phototaxis will easily be distinguished from other responses. In addition, the present monitoring cell is equipped with two electrodes at the near and far edges of the cell regarding the light source (Figure 1). Changes in the algal motility are expected to shift the currents at both electrodes (Table 1). On the other hand, changes of the phototaxis activity will give rise to different current changes at each electrode (Table 1).

Monitoring methods for phototaxis based on solution turbidity have been developed elsewhere.[3−7] The solution turbidity increases as the population of algal cells increases; however, not only chemicals that have no effect on the phototaxis, but also strong toxins that inhibit both phototaxis and motility will cause no significant changes in the turbidity distributions. Therefore, these species could not be distinguished. Additionally, these bioassays are also not suitable for turbid or colored samples.

In the case of high-speed microcinematographic analysis of flagellate algae,[1,8,9] the measurement could suffer from long measurement time, large errors, and low reproducibility, since the motility of a single algal cell is visually monitored.

In the present study, we developed the electrochemical monitoring system mentioned above and showed that the system responded to three different chemicals, namely, diltiazem, azide, and ethanol, in significantly different ways. On the basis of the responses, we found some interesting behaviors of the algae that have not been known to date. Since the present method allows qualitative and quantitative evaluation of influences of chemical species on the algal cells, it would be a powerful tool for not only aquatic risk assessment but also screening of newly found and synthesized chemicals, including herbicides.

EXPERIMENTAL SECTION

Incubation of Algae. Unicellular alga *C. reinhardtii* strain IAM-C9 (Institute of Applied Microbiology Culture Collection, Japan) was used throughout. The algae were grown in Tris-acetate-phosphate (TAP) medium[10] (pH 7.5) in a 300-mL culture bottle at 25 °C. The culture bottle was aerated through a membrane

* Corresponding author. Phone: +81-3-5452-6336. Fax: +81-3-5452-6338. E-mail: tatsuma@iis.u-tokyo.ac.jp.

(1) Josef, K.; Saranak, J.; Foster, K. W. *Cell Motil. Cytoskeleton* **2005**, *61*, 83.
(2) Shitanda, I.; Takada, K.; Sakai, Y.; Tatsuma, T. *Anal. Chem.* **2005**, *77*, 6715.
(3) Nultsch, W.; Throm, G. *Arch. Microbiol.* **1975**, *103*, 175.
(4) Nultsch, W.; Throm, G. *Arch. Microbiol.* **1977**, *112*, 179.
(5) Nultsch, W.; Throm, G. *Arch. Microbiol.* **1979**, *123*, 93.
(6) Stavis, R. L.; Hirschberg, R. *J. Cell Biol.* **1973**, *59*, 367.
(7) Morel, L. N. *Photochem. Photobiol.* **1987**, *45*, 119.
(8) Ruffer, U.; Nultsch, W. *Cell Motil. Cytoskeleton* **1987**, *7*, 87.
(9) Ruffer, U.; Nultsch, W. *Cell Motil. Cytoskeleton* **1991**, *18*, 269.
(10) Gorman, D. S.; Levine, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *54*, 1665.

10.1021/ac051755x CCC: $33.50
Published on Web 11/25/2005

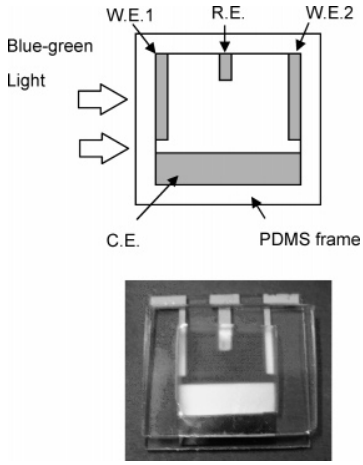

Figure 1. Schematic illustration (top) and a photograph (bottom) of the electrochemical cell. The cell equipped with two working, reference, and counter electrodes (W.E., R.E., and C.E., respectively) is irradiated with blue-green actinic light.

Table 1. Expected Responses of the Electrochemical Cell (shown in Figure 1) to Changes in the Algal Characteristics

	change in the oxidation current[a]	
change in algal characteristics	W.E.1	W.E.2
activation of flagellar motility	+	+
inactivation of flagellar motility	−	−
enhancement of positive phototaxis	+	−
enhancement of negative phototaxis	−	+

[a] +, an increase; −, a decrease.

filter and periodically illuminated by dim fluorescent light (12 h illumination, 12 h dark).

Preparation of the Electrochemical Cell. The configuration of the electrochemical cell used is shown in Figure 1. A Pyrex glass plate (25 × 25 mm) was used as the substrate of the electrochemical cell. The four electrodes were prepared by evaporation of gold onto the substrate. The centered gold electrode was plated with silver and electrolyzed in a 0.1 mM HCl solution at 0.4 mA cm^{-2} for 30 min to obtain a Ag|AgCl reference electrode. A poly(dimethylsiloxane) frame was bonded onto the substrate by an oxygen plasma etching technique. Internal dimensions of the prepared cell were 10 × 15 × 4 mm.

Electrochemical Measurements. Suspensions of the algal cells at logarithmic growth phase (4−6 days after passage) were centrifuged (300*g*, 3 min), then the algal cells were resuspended in the TAP medium (5 × 10^7 cell mL^{-1}). A mixture of the suspension and an equal amount of 2 mM potassium ferrocyanide (the redox marker) solution was poured into the cell (500 μL).

Electrochemical measurements were carried out in a dark box. A light-emmiting diode (LED) (E1L51-YC1A2-03, Toyota Gosei) emitting a blue-green light (λ_{max} = 550 nm), namely, an actinic light for the algae, was used for phototaxis tests. The light intensity was ~100 μW cm^{-2} at working electrode 1 (W.E.1). The electrochemical cell was irradiated with a red light, which has no effect on phototaxis but maintains cell motility,[11,12] using a fluorescent lamp (ETA15ELR13-SHG, NEC) and a filter (no. 20, Tokyo Butai Shomei, λ > 600 nm) before (for more than 10 min) and during the measurements. The potential of the working electrodes was polarized at +0.3 V vs Ag|AgCl, and anodic currents for ferrocyanide oxidation were monitored with a digital dual potentiostat (ALS-1202).

Two different toxicity tests were carried out. In the first one, the algal cells that had been exposed to a sample solution for 10 min were irradiated with the actinic light. In the other one, the algal cells that had been irradiated with the actinic light for 10 min were exposed to a sample solution. Responses of the electrodes (W.E.1 and W.E.2) to diltiazem (LD_{50}(rat) = 560 mg kg^{-1}), sodium azide (LD_{Lo} (human-man) = 786 mg kg^{-1}), and ethanol were examined.

RESULTS

Responses to the Actinic Light. *C. reinhardtii* has two flagella at the front edge of the cell and swims by beating them like the breast stroke.[1,11] Collective flagellar beatings give rise to bioconvection, which agitates the solution in the electrochemical cell. As the stirring effect enhances, the diffusion layer thickness (*d*) decreases on the electrode surface. Under diffusion-controlled conditions, in which the rate of the electrode reaction (i.e., oxidation of ferrocyanide as the redox marker) is determined by the diffusion rate of the marker, the oxidation current (*i*) is anticipated to increase as the diffusion layer thickness decreases (eq 1 derived from the Fick's law).

$$i = nFAD\frac{C}{d} \quad (1)$$

where *n*, *F*, *A*, *D*, and *C* are the number of electrons involved (*n* = 1), the Faraday constant, electrode surface area, the diffusion coefficient of ferrocyanide ion, and bulk concentration of ferrocyanide ion, respectively. The current is, therefore, a function of algal population and average intensity of flagellar movement.[2] It should be noted that 1 mM ferrocyanide ion is not toxic to the algae.

Figure 2 shows typical current responses of the electrodes at +0.3 V vs Ag|AgCl upon irradiation of the actinic light. As can be seen, the oxidation current at W.E.1 started to decrease at 60−90 s and turned to increase at around 150 s. Finally, the steady state current, which was larger than that before the irradiation, was reached in ~400 s. The oxidation current at W.E.2 also decreased and then increased; however, the final current was smaller than that before the irradiation. After the measurement, it was visually observed that most algal cells had gathered around W.E.1, and some others, around W.E.2. On the other hand, in the absence of algal cells, the oxidation currents at W.E.1 and 2 appeared to remain essentially constant, even when the actinic light was irradiated. When ferricyanide was used as a redox marker (electrode potential was −0.1 V vs Ag|AgCl), the cathodic currents changed in a similar manner, as did the anodic currents in the case of ferrocyanide.

Responses to the Actinic Light in the Presence of Chemicals. On the basis of the present system, photoinduced behavior

(11) Yoshimura, K.; Shingyoji, C.; Takahashi, K. *Cell Motil. Cytoskeleton* **1997**, *36*, 236.
(12) Govorunova, E. G.; Altschuler, I. M.; Hader, D. P.; Sineshchekov, O. A. *Photochem. Photobiol.* **2000**, *72*, 320.

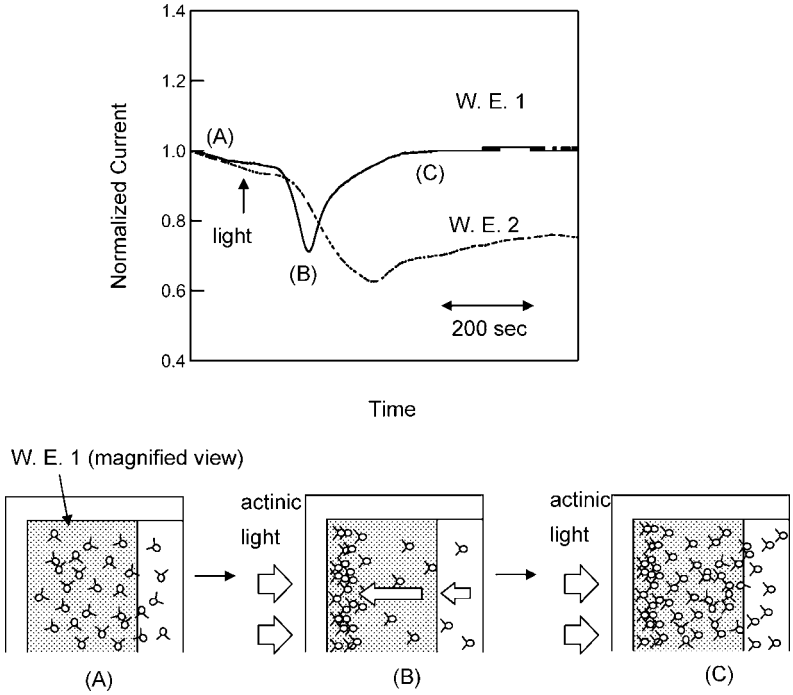

Figure 2. Changes in the normalized current observed at the working electrodes at +0.3 V vs Ag|AgCl in response to the actinic light in a TAP medium (pH 7.0, 20 °C) containing the algal cells (10^7 cells mL^{-1}) and 1 mM potassium ferrocyanide. Schematic illustrations of algae at W.E.1 (magnified view, A−C) are also shown.

of the algae was evaluated in the presence of diltiazem, sodium azide, or ethanol. Diltiazem is known to be a reagent that binds to calcium channels at the flagellar surface and block the flow of Ca^{2+} to inhibit the flagellar movement.[13] Azide ion binds to rhodopsin of the eye spot of *C. reinhardtii* and reverses its phototaxis activity from positive to negative, whereas it does not inhibit the algal motility at 0.1 mM.[14] Changes in the Ca^{2+} concentration (0.0−0.5 mM) in the solution caused no effect on the oxidation current.

Diltiazem (1 mM) was added to the monitoring cell, which was filled with the algal suspension containing the redox marker, and stirred sufficiently. The electrodes were polarized at +0.3 V 5 min after the addition, and the suspension was left for an additional 5 min prior to irradiation with the actinic light. Figure 3a shows typical current responses of the electrodes. The oxidation currents at both electrodes gradually decreased. The decrease basically continued, even after the actinic light was turned on, although a slight and temporal increase in the currents was observed right after the turning on. Microscopic observation revealed that most algal cells had lost swimming motility during the electrochemical measurement.

Figure 3b shows typical current responses of the electrodes upon irradiation with the actinic light in the presence of 1 mM sodium azide. The oxidation currents gradually decreased at both electrodes for the initial 300−400 s, then the current turned to increase at W.E.2, but it was almost constant at W.E.1. After the measurement, it was visually observed that most algal cells had gathered around W.E.2. Microscopic observation proved that the swimming motility of most algal cells had been suppressed to some extent.

(13) Nultsch, W.; Pfau, J.; Dolle, R. *Arch. Microbiol.* **1986**, *144*, 393.
(14) Stavis. R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1824.

Current responses in the presence of 1 mM ethanol are depicted in Figure 3c. The initial and temporary decreases of the oxidation currents, which were observed in the control experiment (Figure 2), were significantly suppressed. On the other hand, the steady-state currents at both electrodes appeared to be larger than those in the control experiment (Figure 2). In the meantime, no change in the oxidation current was observed upon addition of the chemicals into a solution without the algal cells.

Responses to the Chemicals under the Actinic Light. Changes in the oxidation currents in response to the addition of the chemicals were also monitored under the actinic light. Electrochemical measurements were started 10 min after the light was turned on.

Figure 4a shows typical current responses of the electrodes upon an addition of 1 mM diltiazem. The oxidation current at W.E.1 started to decrease about 1 min after the addition of diltiazem; however, in the case of W.E.2, the distinct current decrease was delayed by ~250 s.

Current changes upon an addition of 1 mM sodium azide are shown in Figure 4b. The oxidation current at W.E.1 decreased just after the addition, whereas the current decrease at W.E.2 was preceded by a slight increase for 100−200 s. The decrement for W.E.1 was obviously larger than that for W.E.2.

Incidentally, an addition of 1 mM ethanol gave rise to a sharp but temporal increase in the oxidation currents. As a result of the microscopic observation, it was ascribed to a temporary increase in the convection due to mixing of ethanol and water. For this reason, we concluded that this mode of measurement is not suitable for ethanol.

DISCUSSION

Phototaxis of the Algal Cells. The initial and temporal decrease in the oxidation current observed in response to the light

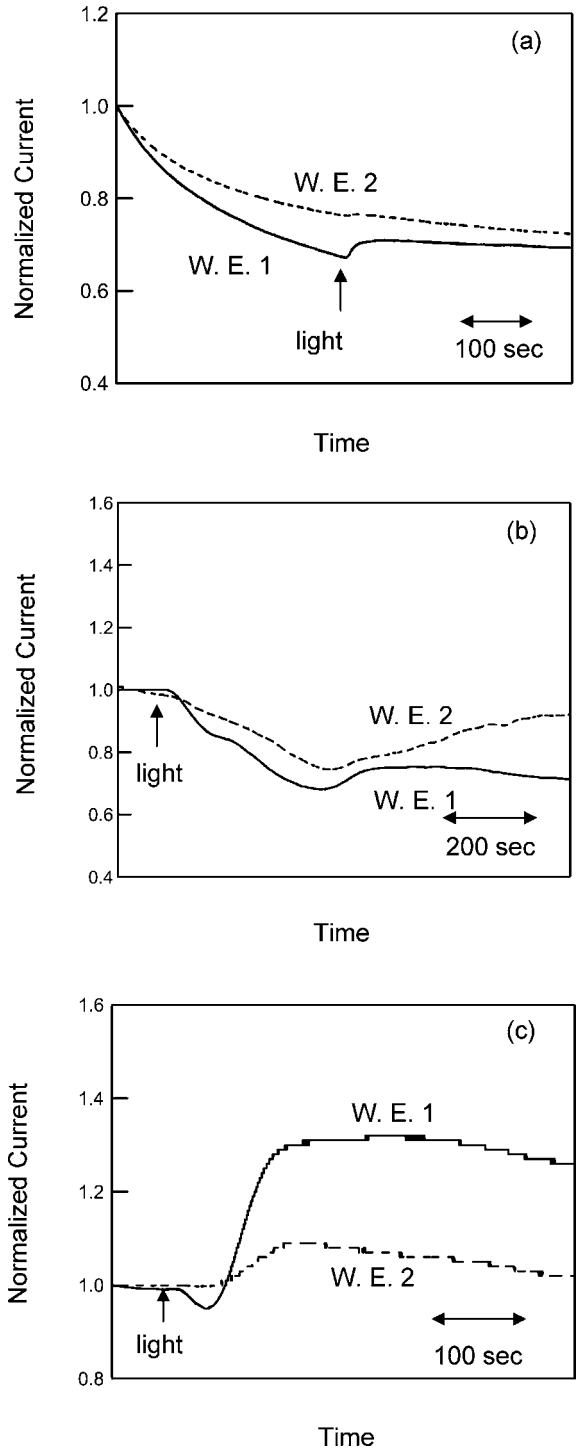

Figure 3. Changes in the normalized current observed at the working electrodes at +0.3 V vs Ag|AgCl in response to the actinic light in a TAP medium (pH 7.0, 20 °C) containing the algal cells (10^7 cells mL^{-1}); 1 mM potassium ferrocyanide; and 1 mM analyte: (a) diltiazem, (b) sodium azide, or (c) ethanol. The light was turned on ~10 min after the addition of an analyte.

irradiation (Figure 2) would be ascribed to a temporary enhancement of the positive phototaxis. Chlamydomonas cells at logarithmic growth phase (incubated for 4−6 days), which were employed in the present work, are known to exhibit positive phototaxis; namely, the algal cells move toward a light source.[15]

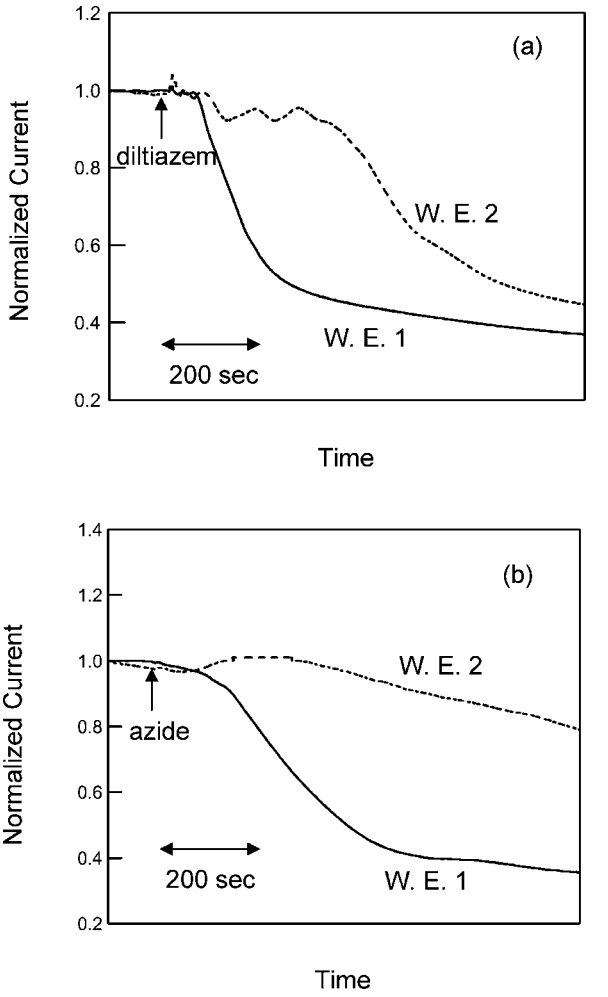

Figure 4. Changes in the normalized current observed at the working electrodes at +0.3 V vs Ag|AgCl in response to an analyte, (a) diltiazem or (b) sodium azide, in the light-irradiated electrochemical cell filled with a TAP medium (pH 7.0, 20 °C) containing the algal cells (10^7 cells mL^{-1}) and 1 mM potassium ferrocyanide. An analyte was added 10 min after turning the light on.

The positive phototaxis is known to be enhanced right after turning the light on.[16−18] Since the cells closer to the light source exhibit more significant phototaxis, most of the cells around W.E.1 go left (in Figure 1) and aggregate at the left wall of the monitoring cell, whereas incoming cells from the right-hand side are fewer. As a result, algal population around W.E.1 decreases temporarily, giving rise to the temporary decrease in the observed current.

The steady-state currents at W.E.1 and W.E.2 under the actinic light were found to be larger and smaller, respectively, than those before irradiation (Figure 2). Since the light irradiation caused no change in the currents in the absence of the algal cells, the electrochemical responses observed in the presence of the cells are ascribed to increased algal population at W.E.1 and decreased population at W.E.2. Namely, the responses reflect the positive phototaxis as expected in Table 1.

(15) Takahashi, T.; Watanabe, M. *FEBS* **1993**, *336*, 516.
(16) Zakes, D. N.; Derguini, F.; Nakanishi, K.; Spudish, J. L. *Biophys. J.* **1993**, *65*, 508.
(17) Hagemann, P.; Bruck, B. *Cell Motil. Cytoskeleton* **1989**, *14*, 501.
(18) Mayer, A. M. *Nature* **1968**, *217*, 875.

Effects of the Chemicals on Motility and Phototaxis. A Chlamydomonas cell has two flagella as motile organelles and one eye spot as a photoreception organelle. Previously, the photoreceptors in the latter were proposed to be rhodopsins.[19,20] The rhodopsins also function as ion channels. The actinic light causes a conformation change of the rhodopsins and raises cation flux into the cell. The flux changes membrane potential of the cell, and calcium channels at the flagella are controlled by the potential. Calcium concentrations in the flagella are thus regulated, and the movement of each flagellum depends on the calcium concentration. The cell changes its direction by tuning the balance between the calcium concentrations of the flagella.[21−24]

When the algal cells were exposed to diltiazem, the currents at both electrodes decreased (Figures 3a and 4a). This indicated that the cell motility was inhibited (Table 1). This observation is consistent with the fact that diltiazem inhibits the flagellar movement by blocking the calcium channels at the flagella.[13] The delay of the current decrease of W.E.2 in comparison with that of W.E.1 could suggest inversion of the phototaxis from positive to negative (Table 1). This behavior is obvious in Figure 4a, but not in Figure 3a, probably because motility of most algal cells was inactivated before the light irradiation in the latter case. The block of the calcium channels might change the balance between the movements of the two flagella, leading to the inversion of phototaxis.

Incidentally, although we confirmed the immobilization of flagella by means of microscopy, the electrochemical measurement is much more advantageous because it is easy to quantify and average the collective flagellar movements.

The typical current responses for azide in Figures 3b and 4b are indicative of reversed phototaxis and depressed motility (Table 1). It has been reported that 0.1 mM of sodium azide reverses phototaxis of Chlamydomonas cells without significant inhibition of swimming motility.[14] Chlamydomonas cells at logarithmic growth phase exhibit positive phototaxis at normal light intensities, as mentioned above; however, at higher light intensities (e.g., $>600\ \mu W\ cm^{-2}$), they show negative phototaxis.[15] Azide binds to the rhodopsin at the eye spot and accelerates its cyclic photochemical reaction.[25] This acceleration might have the same effect as increased light intensity. Although the swimming motility was not inhibited by 0.1 mM azide in the previous report,[14] it was depressed by 1 mM azide in the present work. This difference might be simply ascribed to the difference in the concentration.

It was found that ethanol inhibited the initial and temporary enhancement of positive phototaxis mentioned above, since the temporary decreases of the oxidation currents were suppressed (Figure 3c). In addition, the increases in the final currents reflect enhancement of the algal motility (Table 1). Ethanol could directly attack the plasma membrane and partially disrupt its structure so that the control of the ion flux and flagellar movement could be disordered.[26] To the best of our knowledge, until now, it has not been reported that the temporary enhancement of positive phototaxis is inhibited by ethanol.

CONCLUSIONS

On the basis of the results mentioned above, the present system proved to be a powerful tool for simultaneous monitoring of algal motility and phototaxis. It would also be useful to elucidate phototaxis mechanisms. Actually, by using this device, we revealed some new characteristics of *C. reinhardtii*. Additionally, the present method allows not only aquatic risk assessment but also screening of newly synthesized chemicals and found biochemical compounds.

ACKNOWLEDGMENT

We are grateful to Dr. K. Takada, Prof. R. Kamiya, Prof. K. Yoshimura, and Dr. N. Okita for valuable discussion and Prof. T. Fujii and H. Kimura for help with the preparation of the monitoring cell. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (Area No. 417, Research No. 14050028 for T.T.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Received for review September 30, 2005. Accepted November 1, 2005.

AC051755X

(19) Foster, K. W.; Smyth, R. D. *Microbiol. Rev.* **1980**, *44*, 572.
(20) Witman, G. B. *Trends Cell Biol.* **1993**, *3*, 403.
(21) Harz, H.; Hagemann, P. *Nature* **1992**, *351*, 489.
(22) Beck, C.; Uhl, R. *Biophys. J.* **1994**, *125*, 1119.
(23) Pazour, J. G.; Sineshchekov, O. A.; Witman, G. B. *J. Cell Biol.* **1995**, *131*, 427.
(24) Yoshimura, K.; Matsuo, Y.; Kamiya, R. *Plant Cell Physiol.* **1992**, *351*, 489.
(25) Sudo, Y.; Iwamoto, M.; Shimono, K.; Kamo, N. *Biochim. Biophys. Acta* **2002**, *1558*, 63.
(26) Blachely, J. D.; Johnson, J. H.; Knochel, J. P. *Am. J. Med. Sci.* **1985**, *289*, 22.