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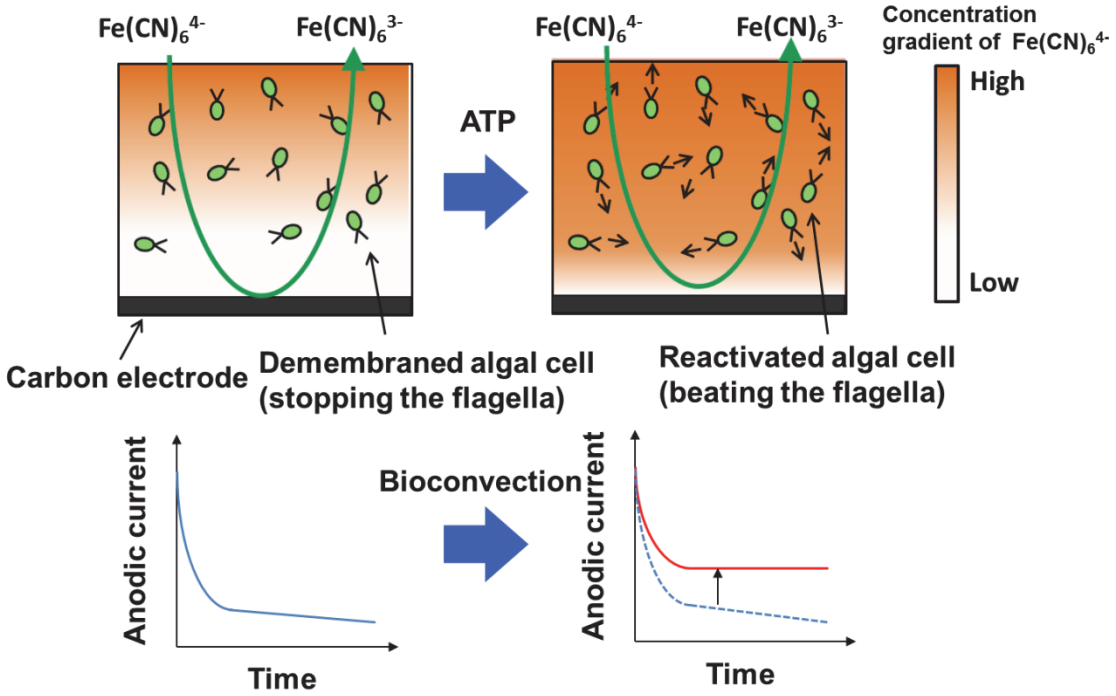
Graphic Abstract



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COMMUNICATION

Electrochemical monitoring systems of demembranated flagellate algal motility for ATP sensing

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The ATP-induced behavior of the unicellular flagellate alga *Chlamydomonas reinhardtii* was recorded as changes in the redox currents for a coexisting redox marker. The ATP concentration was estimated using the presented compact electrochemical system, which is based on monitoring the motility of the flagellates.

Introduction

Monitoring motility is an important method used to elucidate the mechanisms and to characterize the behavior of flagellate algae.[1] In addition, monitoring of the collective flagellar movements could be a rapid and sensitive biosensing technique. We recently developed an electrochemical monitoring system for convection, which is generated by the fluid flow of algal flagellar movement.[2-4] In this system, a solution containing the flagellate alga *Chlamydomonas reinhardtii* and the redox marker ($[Fe(CN)_6]^{4-}$) is stirred by convection due to collective flagellar movement. A diffusion-limited oxidation current for the redox marker is observed using an electrode in the solution. The current increases with the convection rate, which is a function of the algal population and the average intensity of the flagellar movement. This system was applied to monitor the motility,[2] negative gravitaxis,[2] and phototaxis[3] of the flagellate. These activities can be modified or inhibited by different chemicals. We also monitored the local convection generated by the algal flagellar movement using scanning electrochemical microscopy.[4] The convection of the individual flagellate alga *Volvox carteri,* which was entrapped on a glass plate, was monitored in the presence of the redox marker.

There has been considerable recent interest in adenosine-5-triphosphate (ATP) sensing system, owing to their great promise for a wide range of future clinical and environmental monitoring devices. It is important to design a novel biosensing device having high selectivity that can be fabricated using a simple, inexpensive procedure. Various methods, such as fluorescence, have been developed to detect ATP.[5,6] Recently, aptamer- or quantum dot-based electrochemical biosensors for the determination of ATP have been developed.[7-16]

In the present study, we demonstrate a new ATP-sensing system by monitoring algal flagellar movement (Fig. 1). The solution contains a redox marker and a demembranated flagellate algal cells of *Chlamydomonas reinhardtii*, which is known to stop its flagellar movement by demembranation (Fig. 1(a)).[16] Under diffusion-controlled conditions, the rate of the electrode reaction (i.e. oxidation of ferrocyanide as the redox marker) is determined by the diffusion rate of the marker. When ATP reactivates the flagellar movement of the algal cell, the algal cell swam in the solution (Fig. 1(b)). In this case, the oxidation current increases as the diffusion layer thickness decreases due to the convection induced by the flagellar movement.[2-4] Thus, the velocity of the flagellar movement and the density of the algal cells near the electrode surface were expected to be reflected by the oxidation current. Thus, ATP can be detected by monitoring this flagellar motility induced by ATP by using our electrochemical system.

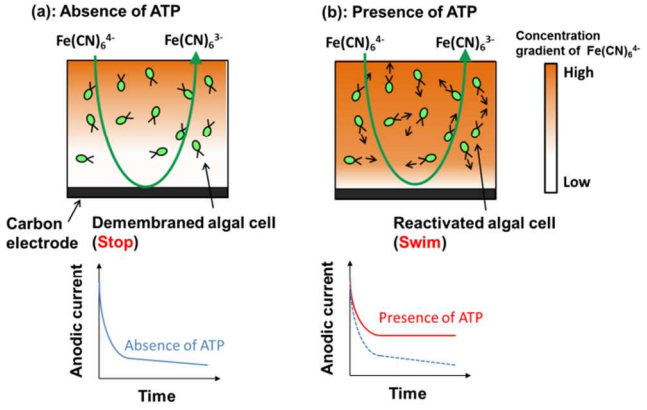

Fig. 1 Schematic illustration of the electrochemical monitoring of ATP-induced reactivated algal flagellate motility

Experimental

We cultivated the wild-type *Chlamydomonas reinhardtii* strain IAM-C9 (Institute of Applied Microbiology Culture Collection, Japan) in Tris-acetate-phosphate medium for 3 or 4 days. Then, we collected the algae by centrifugation, and the algae were resuspended in a solution containing 30 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5 mM magnesium sulfide ($MgSO_4$), 1 mM ethylene glycol tetraacetic acid (EGTA), 50 mM potassium acetate, pH 7.3, to demembranate the flagellate.[17] The demembranated flagellate algae were isolated by centrifugation, suspended in HMDEK buffer solution (30 mM HEPES, 5 mM MgSO4, 1 mM EGTA, and 100 mM potassium acetate), and kept on ice until use. When we reactivated the flagella, 5 μL of the demembranated flagellate alga solution was added to 95 μL of HMDEKP solution (HMDEK and 1% polyethylene glycol) containing ATP and 1.125 mM potassium ferrocyanide (redox marker, $K_4[Fe(CN)_6]$). It should be noted that 1 mM of ferrocyanide ions was not toxic to the algae.

The configuration of the electrochemical cell is shown in Figure 2. The electrode chip was formed by consecutively printing resist ink (TF-200 FRI, Taiyo Ink Mfg., Japan), carbon ink (Jelcon CH-10, Jujo Chemical, Japan), silver ink (ECM-100 AF5000, Taiyo Ink Mfg., Japan), and silver/silver chloride (Ag/AgCl) ink (BAS Inc., Japan) onto a polyimide substrate using a screen-printer (LS-150TV, Newlong Seimitsu Kogyo). The geometric area of the carbon working electrode was 1.77 mm^2. The electrode chip was set on a poly-acrylic frame. The internal dimensions of the cell prepared was 15 × 10 × 3 mm. The solution containing the reactivated flagellate algal cells (6×10^8 cells) and redox marker, mentioned above, was poured into the cell (300 μL). Electrochemical measurements were carried out in a dark box at 4°C. The potential of the working electrode was polarized at +0.4 V vs. Ag/AgCl, and the anodic currents for ferrocyanide oxidation were monitored using a digital potentiostat (ALS-802B).

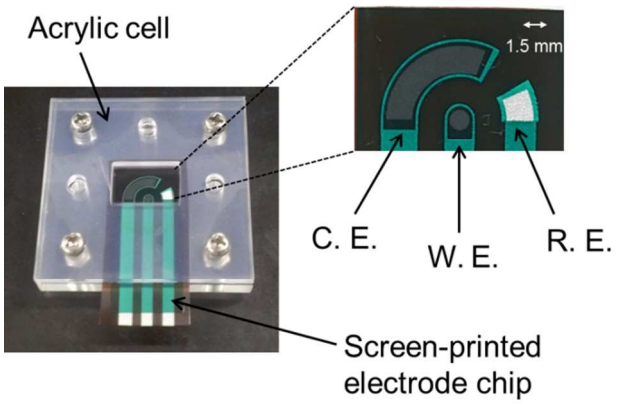

Fig. 2 Photograph of the electrochemical cell. The cell is equipped with screen-printed working, reference, and counter electrodes (W. E., R. E., and C. E., respectively).

Results and discussion

Chlamydomonas reinhardtii (average diameter of the cell body is 10 μm) has two flagella at the front edge of the cell, and swims by beating them, similar to the movement in the breast stroke.[17,18] Collective flagellar beatings give rise to a convection, which agitates the solution in the electrochemical cell.[2,3]

Firstly, the motility of the demembranated algal cells was observed in the presence or absence of 1000 μM ATP (SI Videos 1 and 2). As you can see, the demembraneated algal cells were found to stop its motility in the absence of ATP (SI Video 1). On the other hand, the algal cell swam in the solution in the presence of ATP (SI Video 2).

Figure 3 displays the typical current responses of the electrodes at +0.4 V vs. Ag/AgCl in the presence and absence of 1000 μM ATP. As observed, the oxidation current slightly decreased just after applying the potential and then remained constant in the presence of ATP because the diffusion layer thickness on the electrode surface became quasi steady-state because of convection induced by the algal flagellar movement. The microscopic observation of the algal cells immediately after this experiment indicated good swimming motility. However, the oxidation current started to decrease at 0 s in the absence of ATP, and the algal cells had no swimming motility. The decrease in the oxidation current was due to the increase in the diffusion layer thickness. It is noted that the original values of the detected currents were 1.64 μA and 1.45 μA (at 1000 s) in the presence and absence of ATP, and the current was normalized using the each current value at 0 s.

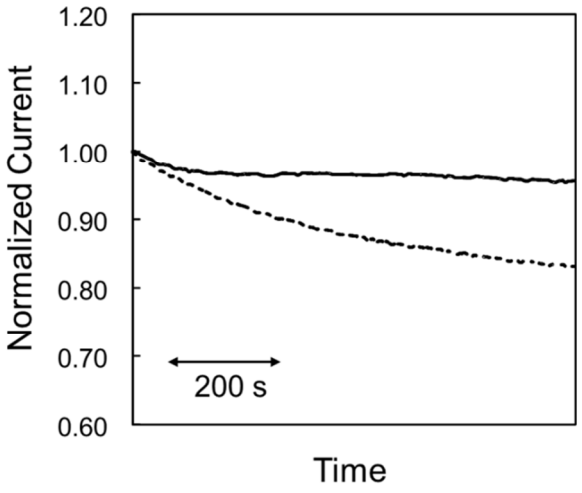

Fig. 3 Changes in the normalized current observed at the working electrodes at +0.4 V vs. Ag/AgCl in the presence (solid line) or absence (dotted line) of 1000 μM ATP.

Next, the oxidation currents were measured using different concentrations of ATP because the intensity of the flagellum movement might be altered by the ATP concentration. Here, the acceleration ratio (A_r) of algal swimming motility is defined by

$$A_r = \left(\frac{\Delta i_{ATP}}{\Delta i_{No-ATP}}\right) \times 100 \quad (\%) \qquad (1)$$

where Δi_{ATP} and $\Delta i_{No\text{-}ATP}$ are decrements of the anodic currents at 1000 s after starting the measurement in the presence and absence of ATP, respectively. On the basis of this equation, the responses of the present biosensing system were evaluated with different concentrations of ATP. The dependence of A_r on the ATP

concentration (Figure 4) showed linearity in the concentration range from 250 to 1250 μM. These results indicate that the present biosensing system can measure ATP concentrations within several minutes, although the sensitivity of the present biosensing system requires improvement for future practical use, in comparison with other electrochemical biosensors for ATP determination,[7-16].

In addition, we will investigate the responses for other kinds of nucleotide derivates such as adenosine monophosphate and guanosine triphosphate. Up to now, a screening of chemicals which can reactivate the flagellar activity has not been reported, to the best of our knowledge. The present sensing system would be screening tool of chemicals including nucleotides.

Furthermore, since the flagellar activity is known to be inhibited by some organic compounds and heavy metal ions,[2,3] these species can be detected by the system. In the case of the previous system, a fresh swimming algal cell were used for the experiments.[2,3] On the other hand, we may be able to store the algal cell in the refrigerator prior to use by using present system. Thus, the present system would be a powerful tool for not only screening of chemicals but also aquatic risk assessment.

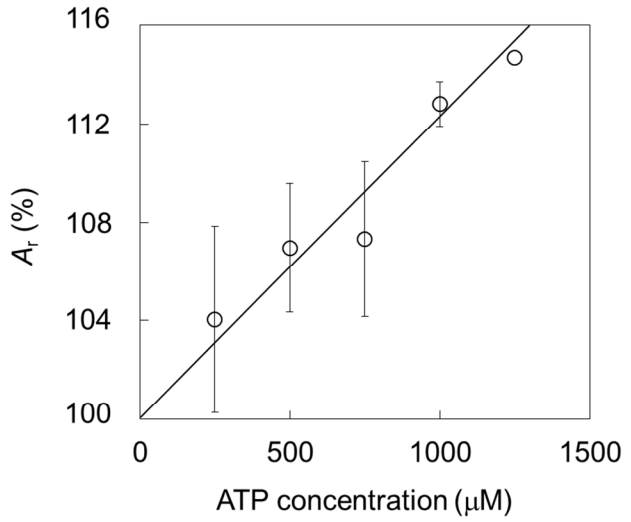

Fig. 4 Dose-response (acceleration ratio *I* vs. ATP concentration) curve of the biosensing system (n = 3).

Conclusions

A new ATP-sensing system based on monitoring flagellar movement was demonstrated for the first time in this study. The convection induced by flagellum movement with the addition of ATP was monitored by our electrochemical monitoring system. The results mentioned above showed that the present biosensing system is a powerful tool for the measurement of ATP.

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Notes and references

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Electronic Supplementary Information (ESI) available: Microscopic observation of the motility of the demembranated algal cells were observed in the presence or absence of 1000 μM ATP See DOI: 10.1039/c000000x/

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