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Evaluation of Sensor Performance for Harmful Compounds by Using Photo-Induced Electron Transfer from Photosynthetic Membranes to Electrodes

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Abstract: The effect of harmful compounds such as KCN, phenol, and herbicides on the photocurrent of photosynthetic membranes (so-called chromatophores) was investigated using carbon paste electrodes. The photocurrent-time curve of photo-induced electron transfer from chromatophores of the purple photosynthetic bacterium *Rhodobacter sphaeroides* to the electrode via 2,5-dichloro-1,4-benzoquinone (DCBQ) was composed of two characteristic phases: an abrupt increase in current immediately after illumination (I_0), and constant current over time (I_c). Photo-reduction of DCBQ exhibited Michaelis-

Menten-like kinetics, and reduction rates were dependent on the amount of DCBQ and the photon flux intensity. The I_c decreased in the presence of KCN at concentrations over 0.05 μM (= μmol dm^{-3}). The I_0 decreased selectively following addition of phenol at concentrations over 20 μM. The I_c was affected by terbutryn only at concentrations over 10 μM. In contrast, DCMU and atrazine had no effect on either I_0 or I_c. The utility of this electrode system for the detection of harmful compounds is also discussed.

Keywords: *Rhodobacter sphaeroides*; Chromatophore; Photo-induced electron transfer; Michaelis-Menten type kinetics; Carbon paste electrode; Cyanide; Phenol

1. Introduction

Whole effluent toxicity (WET) testing is an integrated approach for detecting and addressing toxicity in surface waters and is recommended by the United States Environmental Protection Agency (US EPA). WET testing is adopted to assess and regulate the comprehensive effects of all constituents of an effluent, in contrast to conventional methods, which typically estimate the toxicity of single constituents [1]. The duration of the test may range from as short as 40 minutes up to 7 days, depending on the organisms used and whether acute or chronic effects are of interest. Practical monitoring programs should involve rapid, simple, and low-cost screening procedures for the detection of harmful compounds in aquatic and soil environments.

The photosynthetic reaction of photosynthetic bacteria can also be affected by harmful compounds in waste effluents. *Rhodobacter sphaeroides* (*R. sphaeroides*) is a typical purple bacterium, and the photocurrent generated in this organism's reaction center has been extensively investigated as a potential biosensor for classes of PSII-inhibiting herbicides [2]. *R. sphaeroides* are both simple and inexpensive to culture. In addition, photosynthetic membranes (so-called chromatophores, which are inside-out bimolecular lipid membrane vesicles) can be readily prepared from *R. sphaeroides*. We previously developed a system using a rotating-disk electrode for the measurement of photo-induced electron transfer from chromatophores of *R. sphaeroides* through an exogenous mediator [3]. Though rotating-disk electrodes are useful for kinetic investigations of photo-induced electron transfer, carbon paste electrodes (CPEs) are preferable for detecting toxic substances, given that the electrodes can be modified with chromatophore vesicles [4].

In the present work, we established a CPE system for measuring photo-induced electron transfer from *R. sphaeroides* chromatophore vesicles to an exogenous mediator. The effect of harmful compounds such as cyanide, phenol, and typical agricultural chemicals on electron transfer in this system was also investigated.

2. Experimental Section

2.1. Preparation of Chromatophore Vesicles and Reagents

The purple photosynthetic bacterium used in this study, *R. sphaeroides* strain NBRC 12203, was obtained from the culture collection department of the National Institute of Technology and Evaluation

(Tokyo, Japan). Bacterial cultivation and chromatophore vesicle preparation followed previously described procedures [3]. The amount of *R. sphaeroides* chromatophores corresponding to a concentration of bacteriochlorophyll (BChl) *a* of 5 μM was adopted in the present work.

2,5-Dichloro-1,4-benzoquinone (DCBQ; Wako Pure Chemical Industries Ltd.) was used as the exogenous mediator. Potassium cyanide (KCN; Wako Pure Chemical Industries Ltd.), phenol (Wako Pure Chemical Industries Ltd.), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; Sigma Aldrich), 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine (atrazine; Sigma Aldrich), and 2-*N*-tert-butyl-4-*N*-ethyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine (terbutryn; Sigma Aldrich) were used as the inhibitor. The pH of the aqueous solution was adjusted to 8.0 with 50 mM phosphate buffer.

2.2. Fabrication of R. sphaeroides chromatophore-entrapped and DCBQ-embedded CPEs

Mediator-modified CPEs were prepared according to a previously described method [5]. The geometric surface area of the electrode was 0.07 cm^2. The amount of mediator was expressed as the fraction of mediator in the carbon paste mixture, (mediator)m% (w/w). Aliquots of *R. sphaeroides* chromatophore suspension were placed drop-wise onto the surface of the CPE. After the solvent evaporated, the electrode surface was covered with a 20-μm-thick dialysis membrane (No. 20/30; Union Carbide Co.), which was fixed with a nylon net. Electrodes prepared in this way (abbreviated as *R. sph*.-mediator-CPEs) were kept in 50 mM phosphate buffer (pH 8.0) at 25 ± 1°C in the dark and were used as working electrodes within 1 day.

2.3. Electrochemical Measurements

All electrochemical measurements were carried out using a single-compartment cell containing a three-electrode configuration at 25 ± 1 °C under deaerated conditions created by passing argon gas through a 50 mM phosphate buffer solution (pH 8.0). A silver/silver chloride wire in saturated KCl (SSE) and a platinum wire served as the reference and counter electrodes, respectively.

Amperometric measurements were carried out by applying a potential of 0.50 V vs. SSE (the diffusion-controlled region for oxidation of the reduced DCBQ) to the *R. sph*.-DCBQ-CPE using a BAS CV27 potentiostat (Bioanalytical Systems Inc., West Lafayette, IN) in connection with a model F-35Fm X-Y recorder (Riken Denshi Co., Ltd.).

Light at wavelengths longer than 660 nm was supplied from the bottom of the light source (LG-PS2, Olympus Optical Co., Ltd.) through a R66 glass filter (HOYA Candeo Optronics, Saitama, Japan), and the light intensity was measured using a light meter (PM10; Coherent, Inc., Santa Clara, CA).

The effect of potentially harmful test compounds on the photocurrent was examined 10 min after addition of compound-containing solution to the buffer, a time sufficient to allow the reaction between the test compound and the chromatophore to reach equilibrium.

3. Results and Discussion

3.1. Characteristics of Photo-Induced Electron Transfer from R. sphaeroides chromatophores to CPEs through an Exogenous Mediator

Curve 1 in Figure 1(A) shows the amperometric response measured using an *R. sph.*-DCBQ (5×10^{-3} [w/w])-CPE, followed by light illumination between points 'a' and 'b.' The photon flux intensity, [P], was 500 μmol m^{-2} s^{-1}. An abrupt increase in the positive current (I_0) was observed immediately after illumination (point a), followed by a gradual decrease to a constant current value (I_c) by 3 min, with the positive current decreasing to the background level once the light was turned off (point b). This photocurrent was not observed in the absence of *R. sphaeroides* chromatophores (curve 2) or DCBQ (curve 3) upon illumination. The photocurrent is anodic, meaning that electrons are transferred predominantly from the *R. sphaeroides* chromatophores to the CPE via interaction with DCBQ. The I_0 and I_c values were obtained through repeated measurements (n = 5), with an accuracy of ± 2 or ± 3% using the same *R. sph.*-DCBQ-CPE, and with an accuracy of ± 9 or ± 7% with different *R. sph.*-DCBQ-CPEs prepared using the same procedure.

Figure 1(B) shows a plot of I_c as a function of (DCBQ)m%. Here, (DCBQ)m% is considered to be proportional to the concentration of DCBQ in the membrane layer of the immobilized *R. sphaeroides* chromatophores [5]. The value of I_c increases with (DCBQ)m% and approaches the maximum current value. The redox reactions appear to follow Michaelis-Menten-like kinetics with respect to (DCBQ)m%. I_c was also appeared to follow Michaelis-Menten-like kinetics with respect to [P]. Based on these results, all experiments in the present work were carried out at a (DCBQ)m% of 5×10^{-3} (w/w) and light intensity of 500 μmol m^{-2} s^{-1}.

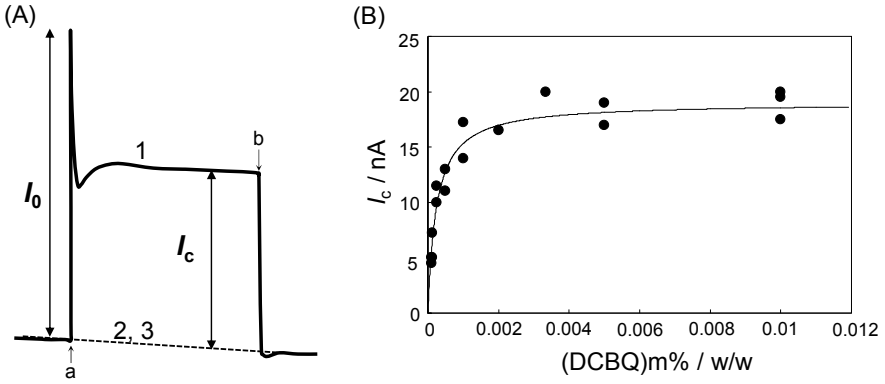

Figure 1. (**A**) Time-course of the amperometric response measured using a *R. sph* (5 μM [BChl])-DCBQ (5×10^{-3} [w/w])-CPE (curve 1), *R. sph* (5 μM [BChl])-CPE (curve 2), or DCBQ (5×10^{-3} [w/w])-CPE (curve 3) in pH 8.0 buffer. Light illumination was applied between points 'a' and 'b.' (**B**) Dependence of (DCBQ)m% (w/w) on the photocurrent (I_c).

3.2. Effect of CN^-, Phenol, or Herbicides on Electron Transfer from R. sphaeroides chromatophores to CPE via DCBQ

Figure 2(A) shows the amperometric response measured using *R. sph.*-DCBQ-CPEs in a pH 8.0 buffer solution in the absence (curve 1) or presence of 5 μM KCN (curve 2) or 100 μM phenol (curve 3) under light illumination between points 'a' and 'b.' Although the I_0 value did not change, I_c decreased following addition of KCN. The inhibition of photo-induced electron transfer is thought to be due to the strong binding of CN^- to cytochromes, such as cytochrome c, resulting in a shift in the redox potential of the Q_i site [6]. The ratio of the I_c value obtained with CN^- to that obtained without CN^-, $I_{c,CN} / I_c$, was plotted as a function of the concentration of CN^- added to the buffer [Figure 2(B)].

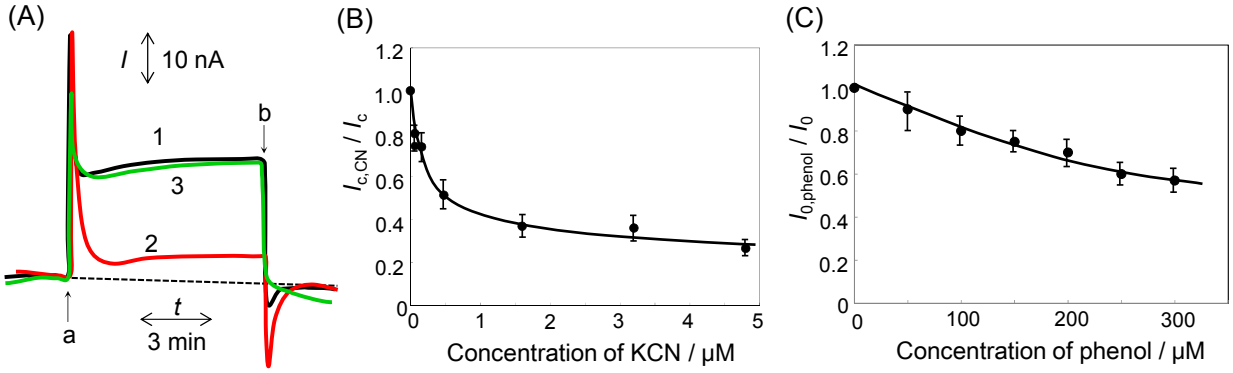

Figure 2. (**A**) Time-course of the amperometric response measured using a *R. sph.* (5 μM [BChl])-DCBQ (5×10^{-3} [w/w])-CPE in the absence (curve 1) or presence of 5 μM KCN (curve 2) or 100 μM phenol (curve 3). (**B**) Effect of KCN concentration on the photocurrent (I_c). (**C**) Effect of phenol concentration on the photocurrent (I_0).

The $I_{c,CN} / I_c$ decreased to ca. 0.8 at a CN^- concentration of 0.01 μM and to ca. 0.3 at CN^- concentrations over 1.5 μM. As reported detection limits of CN^- biosensors are in the range 0.01 to 10 μM [7], the *R. sph.*-DCBQ-CPE system could be used for environmental detection of CN^-.

In contrast to the results obtained with CN^-, the I_0 decreased following addition of phenol. Phenol may impair the proton gradient coupled with ATP synthesis, resulting in the observed decrease in I_0 in the present study [8]. Figure 2(C) shows a plot of the ratio of the I_0 value obtained with phenol to that obtained without phenol, $I_{0,phenol} / I_0$, versus the concentration of phenol. At phenol concentrations of 100 and 300 μM, the resulting $I_{0,phenol} / I_0$ was ca. 0.8 and 0.6, respectively. The detection limit of phenol biosensors is reportedly in the low micromolar range [9], and therefore, the sensitivity of the *R. sph.*-DCBQ-CPE system is not superior to that of previously reported systems. Although the *R. sph.*-DCBQ-CPE system is suitable for the detection of phenol at concentrations over 50 μM, other purple bacteria should be examined for more sensitive detection of this compound.

Triazine-type herbicides block electron transfer from Q_A to Q_B in the electron transport chain of purple bacteria, whereas urea- and phenol-type herbicides do not [10]. Neither I_0 nor I_c were affected by the addition of DCMU or atrazine in the *R. sph.*-DCBQ-CPE system, even at a DCMU or atrazine concentration of 500 μM. In contrast, terbutryn affected I_c only at concentrations over 10 μM. The ratio of the I_c value obtained with terbutryn to that obtained without terbutryn, $I_{c,terbtryne} / I_c$, was ca. 0.8

at terbutryn concentrations over 20 μM. Terbutryn reportedly interferes with electron transport from Q_A to Q_B in purple bacteria more effectively than other triazine-class inhibitors [11]; therefore, the effect on I_c is considered to reflect the re-oxidation of DCBQ reduced not only by cytochromes in the Q_i but also by Q_B in the electron transport chain.

4. Conclusions

The present study demonstrated the detection of photo-induced electron transfer from *R. sphaeroides* chromatophores to an electrode via a mediator using a *R. sph.*-DCBQ-CPE system. Electron transfer in *R. sphaeroides* chromatophores is impacted by harmful compounds such as CN^-, phenol, and terbutryn. The *R. sph.*-DCBQ-CPE system is a particularly sensitive CN^- sensor. By monitoring decreases or increases in I_0 and I_c, qualitative and quantitative analyses are possible. In addition, given that the photocurrent of chromatophore vesicles is more stable than that of live cells [3], the system described herein represents an attractive technology for sensing harmful compounds in wastewater effluents.

Conflicts of Interest

The authors declare no conflict of interest.

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