Visible-light driven electrochemical biofuel cell with the function of CO₂ conversion to formic acid coupled thylakoid from microalgae and biocatalyst immobilized electrodes

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Visible-light driven electrochemical biofuel cell with the function of CO₂ conversion to formic acid coupled thylakoid from microalgae and biocatalyst immobilized electrodes

Y. Amao, M. Fujimura, M. Miyazaki, A. Tadokoro, M. Nakamura and N. Shuto

Aerobic photosynthesis in green plants, cyanobacteria, and micro-algae has two important sites in the integral-membrane photoactive complexes, photosystems I (PSI) and II (PSII). These systems are assembled into thylakoid membranes. Thylakoid membranes with oxygen-evolution activity owing to PSII can be used in a visible-light driven water photolytic material. In this work, a new visible-light driven electrochemical biofuel-based cell consisting of thylakoid membrane from microalgae *Spirulina platensis* immobilized on a nanocrystalline TiO₂ layer electrode as a photoanode, formate dehydrogenase (FDH)/viologen co-immobilized electrode as a cathode, and CO₂-saturated buffer solution as the redox electrolyte, was developed. The actual short-circuit photocurrent of this cell was estimated to be ca. 50 µA cm⁻². Formic acid and oxygen were produced in this biofuel cell, while generating electricity from irradiated visible light. The ratio of formic acid to oxygen produced in the biofuel cell after continuous irradiation was estimated to be ~ 2. Thus, formic acid and oxygen was produced stoichiometrically in this visible-light driven electrochemical biofuel cell. Thus, a new biofuel cell system with functions of a solar cell and ability of CO₂ conversion was developed.

Introduction

Green plants, cyanobacteria, and micro-algae use an aerobic photosynthesis system to convert solar light energy into chemical materials such as starch. An aerobic photosynthesis system uses a water molecule as an electron donor. In general, an aerobic photosynthesis system consists of two photosystem sites in the thylakoid membrane (TK) photoactive complexes: photosystem I (PSI) and photosystem II (PSII). PSI facilitates the photoreduction of NADP⁺ to NADPH with P700 reaction center complex. PSII serves as the reaction center with P680 which has a strong oxidizing power for oxygen evolution. Thylakoid membranes are an attractive material for visible-light driven electrochemical biofuel cell systems using photosynthetic pigments such as chlorophyll, its derivative, or artificial dyes in assembling the electrode have been reported. We developed the photovoltaic conversion system using light-harvesting antenna complex II (LHCII) isolated from a green plant, spinach, immobilized onto nanocrystalline TiO₂ electrode. As PSII is an attractive material for visible-light driven photolysis of water into oxygen, some studies on the photovoltaic conversion system using purified-PSII assembled onto electrode have been reported.

**Materials and Methods**

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of PSII. We also reported a photovoltaic conversion system based on the combination of oxygen production from water with chloroplast from spinach onto the nanocrystalline TiO$_2$ film electrode by irradiation, and electrochemical reduction of oxygen to water due to the catalytic activity of the platinum electrode. $^{32,33}$ Thus, the chloroplast and TK are attractive materials for assembling a photovoltaic conversion system using a water molecule as a redox electrolyte. In this system, PSII in the chloroplast or TK acts as an oxygen production catalyst with visible light irradiation.

CO$_2$ conversion to organic molecules using visible light irradiation is a lucrative technology for assembling an artificial photosynthesis device. Some studies on the visible-light driven CO$_2$ conversion to organic molecules such as formic acid and methanol, using an enzyme and photo-redox system have been reported. $^{34-37}$ We previously reported visible-light driven enzymatic formic acid production from CO$_2$ using formate dehydrogenase (FDH) via the reduction of methylviologen (MV$^{2+}$) by photosensitization of water-soluble zinc porphyrin or chlorophyll-a. $^{38-43}$ The visible-light driven electrochemical biofuel cell converting CO$_2$ to formic acid involves an FDH and viologen co-immobilized electrode, instead of platinum electrode in the photovoltaic conversion system, combined with chloroplast from Spinach immobilized onto nanocrystalline TiO$_2$ film electrode and platinum electrode.

Since TK of microalgae, that are active in water, are expected to be more stable in aqueous solution than that of green plant, such as Spinach, we focused on TK from microalgae Spirulina platensis. Spirulina platensis thrives at a pH around 8.5 and a temperature around 30 °C. Spirulina grows sufficiently in water, so it is easier to handle as a photosynthetic materials than that of green plant, such as Spinach. As no chloroplast is formed in Spirulina, Spirulina itself acts as a function of chloroplast. For utilization of chloroplast as a photoanode in the photovoltaic conversion system, in the future, chloroplast must be purified from green plants using the centrifugation, but Spirulina directly can be used as a photoanode material. Thus, we devoted to photosynthesis organ in Spirulina in the visible-light driven electrochemical conversion system.

In this work, a new visible-light driven electrochemical biofuel cell consisting of TK from Spirulina platensis immobilized on nanocrystalline TiO$_2$ layer electrode (TK/TiO$_2$) as a photoanode, FDH-viologen (1-carboxyundecanoyl-1'-methyl-4,4'-bipyridinium bromide, iodide; CH$_3$V(CH$_2$)$_9$COOH) co-immobilized electrode as a cathode, and CO$_2$-saturated buffer solution as the redox electrolyte was developed, as shown in Fig. 1.

The principle of visible-light driven electrochemical biofuel cell consisting of TK/TiO$_2$, FDH-CH$_3$V(CH$_2$)$_9$COOH and CO$_2$-saturated buffer solution is as follows.

1) Oxygen evolution with PSII in TK/TiO$_2$ occurs with visible-light irradiation.
2) The electron transfers from TK to the conduction band of TiO$_2$.
3) The electron transfers from TK/TiO$_2$ to FDH-CH$_3$V(CH$_2$)$_9$COOH electrode though the external circuit.
4) Single-electron reduction of viologen moiety of CH$_3$V(CH$_2$)$_9$COOH on electrode occurs.
5) CO$_2$ reduction to formic acid with the function of FDH catalytic activity with single-electron reduced viologen moiety as a co-enzyme onto FDH-CH$_3$V(CH$_2$)$_9$COOH electrode proceeds.

**Results and discussion**

Characterization of TK/TiO$_2$

UV-vis absorption spectrum of TK/TiO$_2$ is shown in Fig. 2 (solid line). As shown in Fig. 2, the absorption maxima are observed at 440, 490, 620 and 680 nm. In contrast, the absorption maxima of TK dispersed in a solution also are observed at 440, 490, 620 and 680 nm as shown in Fig. 2 (dash line).

Figure 2. UV-vis absorption spectra of TK/TiO$_2$ (solid line) and TK dispersed in a solution (dash line).
attributed to Mg chlorophyll-\(\alpha\) (MgChl-\(\alpha\)) (Q-band) along with its Soret band at 440 nm. The shoulder band at 490 nm is attributed to carotenoid (Car) absorption.\(^4\),\(^5\) Presence of light harvesting site based on phycobilisomes (PBS) is observed at 620 nm absorption band. Thus, TK is immobilized onto TiO\(_2\) film electrode.

The photosynthetic dyes such as MgChl-\(\alpha\) fully were desorbed by immersing the TK is immobilized onto TiO\(_2\) film electrode in acetone. After immersing the electrode in acetone, the amount of MgChl-\(\alpha\) was determined with the molar coefficient (\(\epsilon_{444} = 8.13 \times 10^3 \, \text{M}^{-1} \, \text{cm}^{-1}\)) using UV-vis absorption spectroscopy. From the result of UV-vis absorption spectroscopy, the amount of adsorbed MgChl-\(\alpha\) in TK/TiO\(_2\) film electrode was estimated to be 14.8 nmol cm\(^{-2}\). The ratio of MgChl-\(\alpha\) contained in PSI and PSII was reported to be 94.5 to 5.5, respectively.\(^4\),\(^5\) It has been reported that 920 molecules of MgChl-\(\alpha\) were contained in one unit of the PSII.\(^4\),\(^5\) Thus, the amount of PSII (0.86 nmol of MgChl-\(\alpha\) cm\(^2\)) in TK/TiO\(_2\) film electrode were estimated to be 2.9 \(\times 10^{12}\) units cm\(^{-2}\) (ca. 5.0 \(\times 10^{-12}\) mol cm\(^{-2}\)). For PSII/benzoquinone polymer-based photo-bioelectrical cell, the amount of PSII onto electrode was estimated to be 1.5 \(\times 10^{-12}\) mol cm\(^{-2}\). In our system, as the TK was immobilized onto the electrode, the amount of PSII onto electrode was about 3.3 times compared with that of PSII/benzoquinone polymer-based photo-bioelectrical cell system.

To make sure the successful fabrication of TK/TiO\(_2\) electrode, surface observation of the electrode was carried out using scanning electron microscopy (SEM). Fig. 3 show the photoimage and SEM image of TK/TiO\(_2\) electrode and bare TiO\(_2\) electrode.

![Figure 3](image)

**Figure 3.** The photoimage, SEM images of TK/TiO\(_2\) electrode and bare TiO\(_2\) electrode.

From the result of SEM observation, it is not clear that TK immobilized onto TiO\(_2\) electrode is formed, but it is estimated from the SEM images of the bare TiO\(_2\) and TK/TiO\(_2\) that a large TK covers the electrode surface. Thus, it was suggested that TK was immobilized onto TiO\(_2\) electrode.

To confirm the successful fabrication of TK/TiO\(_2\) electrode, photoelectrochemical properties of TK/TiO\(_2\) electrode were studied using cyclic voltamogram (CV) under dark and visible-light irradiation.

Fig. 4 shows the representative CV under dark and visible-light irradiation of TK/TiO\(_2\) electrode in the aqueous solution of sodium pyrophosphate buffer (50 mM, pH 7.4) containing 0.1 mM KCl at the scan rate of 10 mV s\(^{-1}\) (vs. Ag/AgCl (1 M KCl)). The reversible redox curve is identifiable in all CV curves at a central potential of around 0.35 V for thylakoid electrochemistry. From CV curves in Fig. 4, the anodic current based on water oxidation of TK/TiO\(_2\) electrode was observed with light irradiation. Thus, TK was immobilized onto TiO\(_2\) electrode and TK/TiO\(_2\) has sufficient oxygen production activity.

**Oxygen production activity of TK/TiO\(_2\) under visible-light irradiation**

To confirm the oxygen evolved complex (OEC) activity of TK/TiO\(_2\) in CO\(_2\)-saturated buffer solution under visible-light irradiation, oxygen production of TK/TiO\(_2\) under 100 mW cm\(^{-2}\) light irradiation in the presence of dichloroindophenol (DCPIP) as a sacrificial oxidant is studied in the nitrogen-saturated (closed circle) or CO\(_2\)-saturated (open square) 50 mM sodium pyrophosphate buffer (pH 7.4) as shown in Fig. 3. In all experiments, to prevent degradation of TK and direct excitation of TiO\(_2\) by near UV light, wavelengths of less than 390 nm were blocked with a cut-off filter (SCF-500-39L Sigma Koki). In both cases, oxygen production was observed with irradiation time. This result indicates that CO\(_2\) has no effect on oxygen production activity of TK/TiO\(_2\). In contrast, no oxygen production was observed in the absence of DCPIP (×) or dark condition (open circle). Thus, the oxygen was produced with the photocatalytic function of TK/TiO\(_2\) under visible-light irradiation.
irradiation. Oxygen production activity of TK/TiO$_2$ was estimated to be 30 nmol cm$^{-2}$ min$^{-1}$. The turnover number of PSII in TK/TiO$_2$ in the presence of DCPIP was estimated to be $1.5 \times 10^4$ min$^{-1}$.

From these results, it was suggested that TK/TiO$_2$ has sufficient oxygen production activity even in CO$_2$-saturated buffer solution as an electrode. Furthermore, oxygen production from TK/TiO$_2$ was observed even under visible-light while applying a bias equal to or lower than the oxidation potential of water. Thus, the successful fabrication of TK/TiO$_2$ electrode was made sure from these experimental results.

Characterization of FDH-CH$_3$V(CH$_2$)$_9$COOH electrode

UV-vis absorption spectrum of CH$_3$V(CH$_2$)$_9$COOH/ITO electrode is shown in Fig. 6 (solid line). As shown in Fig. 6, the absorption maximum is observed at 270 nm. In contrast, the absorption maximum of CH$_3$V(CH$_2$)$_9$COOH in a methanol solution also is observed at 265 nm as shown in Fig. 6 (dash line).

From the result of SEM observation, it is not clear that FDH immobilized onto CH$_3$V(CH$_2$)$_9$COOH/ITO electrode is formed, but it is estimated from the SEM images of the bare ITO, CH$_3$V(CH$_2$)$_9$COOH/ITO, and FDH-CH$_3$V(CH$_2$)$_9$COOH electrodes that a large FDH covers the electrode surface.

To confirm the successful fabrication of FDH-CH$_3$V(CH$_2$)$_9$COOH electrode, electrochemical properties of FDH-CH$_3$V(CH$_2$)$_9$COOH electrode were studied using CV. Figure 8 shows the CV of CH$_3$V(CH$_2$)$_9$COOH/ITO (dotted line) and FDH-CH$_3$V(CH$_2$)$_9$COOH (solid line) electrodes in aqueous solution of the CO$_2$ saturated sodium pyrophosphate buffer (50 mM, pH 7.4).
Figure 8. Representative CV of CH$_3$V(CH$_2$)$_9$COOH/ITO (dotted) and FDH-CH$_3$V(CH$_2$)$_9$COOH electrodes. Buffer solution: CO$_2$ saturated sodium pyrophosphate buffer (50 mM, pH 7.4) containing 0.1 mM KCl; Scan rate: 10 mV s$^{-1}$ vs. Ag/AgCl (1 M KCl) for 2 h. The colour of surface of CH$_3$V(CH$_2$)$_9$COOH/ITO or FDH-CH$_3$V(CH$_2$)$_9$COOH electrodes was changed to blue with potential application, indicating the single-electron reduction of viologen moiety onto the electrode.

From CV curves in Fig. 8, the cathodic current in FDH-CH$_3$V(CH$_2$)$_9$COOH electrode was increased compared with that of CH$_3$V(CH$_2$)$_9$COOH/ITO electrode. Thus, TK was immobilized onto TiO$_2$ electrode and TK/TiO$_2$ has sufficient oxygen production activity. These results indicate the catalytic current based on the CO$_2$ reduction to formic acid with FDH on the electrode. From the observation of colour changes of CH$_3$V(CH$_2$)$_9$COOH/ITO or FDH-CH$_3$V(CH$_2$)$_9$COOH electrodes surfaces, the viologen moiety of CH$_3$V(CH$_2$)$_9$COOH/ITO electrode was more single-electron reduced compared with that of FDH-CH$_3$V(CH$_2$)$_9$COOH electrode. By immobilized FDH on the electrode, it is shown that single-electron reduced viologen moiety on the electrode acts as a co-enzyme for FDH and is used for CO$_2$ reduction to formic acid.

Figure 9 shows the time dependence of absorption changes at 570 nm due to the single-electron reduced viologen moiety on CH$_3$V(CH$_2$)$_9$COOH/ITO (closed circle) and FDH-CH$_3$V(CH$_2$)$_9$COOH (open circle) electrodes under the bias application of -550 mV potential vs. Ag/AgCl (1 M KCl).

From results of Fig. 9, the viologen moiety of CH$_3$V(CH$_2$)$_9$COOH/ITO electrode was more single-electron reduced compared with that of FDH-CH$_3$V(CH$_2$)$_9$COOH electrode. As the single-electron reduction of viologen moiety onto FDH-CH$_3$V(CH$_2$)$_9$COOH electrode was accomplished, the electrochemical CO$_2$ reduction to formic acid was attempted. Fig. 10 shows the time dependence of formic acid produced using FDH-CH$_3$V(CH$_2$)$_9$COOH electrode in CO$_2$ saturated sodium pyrophosphate buffer (50 mM, pH 7.4) containing 0.1 mM KCl under the application of -550 mV potential vs. Ag/AgCl (1 M KCl).
By using FDH-CH$_3$V(CH$_2$)$_9$COOH electrode, the amount of formic acid was increased with increasing bias application time. On the other hand, no formic acid production was observed with CH$_3$V(CH$_2$)$_9$COOH/ITO electrode. Moreover, formic acid production was observed with CH$_3$V(CH$_2$)$_9$COOH/ITO electrode in addition of FDH into CO$_2$ saturated sodium pyrophosphate buffer under bias application. No formic acid production also was observed with only FDH immobilized ITO electrode under bias application. From these results, both violagen as a co-enzyme and FDH as a catalyst are required for electrochemical CO$_2$ reduction to formic acid.

Voltage response of TK/TiO$_2$ with visible-light irradiation

Fig. 11 shows the voltage response of TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH on 100 mWcm$^{-2}$ irradiation. In all experiments, to prevent degradation of TK and direct excitation of TiO$_2$ by near UV light, wavelengths of less than 390 nm were blocked with a cut-off filter. The voltage increased on irradiation and decreased under dark conditions. The light and dark voltages were estimated to be 570 and 0.1 mV, respectively. In the TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH, the electrolyte consisted of only the CO$_2$-saturated 50 mM sodium pyrophosphate buffer (pH 7.4) containing 0.1 mM KCl. The photovoltage was estimated to be 569 mV under the irradiation.

Current response of TK/TiO$_2$ with visible-light irradiation

Fig. 12 shows the current response of TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH on 100 mWcm$^{-2}$ irradiation. The current increased on irradiation and decreased under dark conditions. The light and dark currents were estimated to be 55 ± 10 and 5.0 ± 5 μA cm$^{-2}$, respectively. In the TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH, the electrolyte consisted of only the CO$_2$-saturated 50 mM sodium pyrophosphate buffer (pH 7.4). The photocurrent was estimated to be 50 ± 5 μA cm$^{-2}$ under the irradiation. For TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH, in contrast, no oxygen reduction process is needed and CO$_2$ reduction to formic acid was observed on irradiation cycles with 100 mWcm$^{-2}$ light. The Nafion 115 membrane was estimated to be 1.3 x 10$^6$ Ω cm$^{-2}$ in this system using electrochemical procedure. The ionic conductivity of Nafion 115 membrane was estimated to be 1.5 x 10$^3$ Ω$^{-1}$ cm$^{-1}$. These results suggested that some impedance loss in this cell will occur. In the case of TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH without Nafion 115 membrane, however, the oxygen produced from TK/TiO$_2$ acted as a scavenger for the reduced form of violagen moiety in FDH-CH$_3$V(CH$_2$)$_9$COOH. Thus, the cell performance drastically was decreased and no formic acid production was observed under irradiation. As there were some impedance loss using Nafion 115 membrane in the cell, to prevent oxygen transferring to FDH-CH$_3$V(CH$_2$)$_9$COOH side, Nafion 115 membrane was needed to the cell.

Dark and light current and potential curves for TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH under dark and irradiation cycles with 100 mWcm$^{-2}$ light.

The ohmic resistance of Nafion 115 membrane was estimated to be 1.3 x 10$^6$ Ω cm$^{-2}$ in this system using electrochemical procedure. The ionic conductivity of Nafion 115 membrane was estimated to be 1.5 x 10$^3$ Ω$^{-1}$ cm$^{-1}$. These results suggested that some impedance loss in this cell will occur. In the case of TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH without Nafion 115 membrane, however, the oxygen produced from TK/TiO$_2$ acted as a scavenger for the reduced form of violagen moiety in FDH-CH$_3$V(CH$_2$)$_9$COOH. Thus, the cell performance drastically was decreased and no formic acid production was observed under irradiation. As there were some impedance loss using Nafion 115 membrane in the cell, to prevent oxygen transferring to FDH-CH$_3$V(CH$_2$)$_9$COOH side, Nafion 115 membrane was needed to the cell.

Dark and light current and potential curves for TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH using linear polarization

Fig. 13 shows dark and light current and potential (J-V) curves for TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH, respectively.

The ohmic resistance of Nafion 115 membrane was estimated to be 1.3 x 10$^6$ Ω cm$^{-2}$ in this system using electrochemical procedure. The ionic conductivity of Nafion 115 membrane was estimated to be 1.5 x 10$^3$ Ω$^{-1}$ cm$^{-1}$. These results suggested that some impedance loss in this cell will occur. In the case of TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH without Nafion 115 membrane, however, the oxygen produced from TK/TiO$_2$ acted as a scavenger for the reduced form of violagen moiety in FDH-CH$_3$V(CH$_2$)$_9$COOH. Thus, the cell performance drastically was decreased and no formic acid production was observed under irradiation. As there were some impedance loss using Nafion 115 membrane in the cell, to prevent oxygen transferring to FDH-CH$_3$V(CH$_2$)$_9$COOH side, Nafion 115 membrane was needed to the cell.

Dark and light current and potential curves for TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_9$COOH with 100 mWcm$^{-2}$ light.
For TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH, $V_{oc}$ and $J_{sc}$ in light current were estimated to be 600 mV and 54.5 μAcm$^{-2}$, respectively. In dark current, $V_{oc}$ and $J_{sc}$ were estimated to be 598 mV and 10.0 μAcm$^{-2}$, respectively. Thus, actual $J_{sc}$ in photocurrent was estimated to be 44.5 μAcm$^{-2}$.

$V_{oc}$ and $J_{sc}$ values in light and dark current obtained by LP measurements were in good agreement with the result of current and voltage responses with the cycles of dark/irradiation of 100 mWcm$^{-2}$ light.

Fig. 14 shows the photocurrent density and photopower density as a function of applied potential in TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH. Here, photocurrent was calculated by subtracting dark current from light current. Photopower density is calculated by $P = J \times V$.

From these results, the maximum of photopower of TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH was estimated to be 5.3 μWcm$^{-2}$ (V=286 mV), respectively. The data of photocurrent, photovoltage and photopower obtained from the cycles of dark/irradiation of 100 mWcm$^{-2}$ light and LP measurements were summarised in Table 1.

Table 1. J–V characteristics for TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH obtained from the cycles of dark/irradiation of 100 mWcm$^{-2}$ light and LP measurements

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Formic acid production in the TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH on visible-light irradiation

Next, we focused on formic acid production from CO$_2$ in the TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH on irradiation with visible-light. Fig. 16 shows the time dependence of photocurrent of TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH with continuous 100 mWcm$^{-2}$ irradiation. In all experiments, to prevent degradation of TK and direct excitation of TiO$_2$ by near UV light, wavelengths of less than 390 nm were blocked with a cut-off filter. The volumes of electrolyte and gaseous phase in the anode and cathode sides were 0.5 mL, respectively. The distance between the working and counter electrode was 0.5 cm.

The photocurrent generated during a continuous irradiation was estimated to be 55 ± 10 μAcm$^{-2}$. Fig. 17 shows the time dependence of formic acid and oxygen production in the TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH on continuous visible-light irradiation. The amounts of formic acid and oxygen were increased with increasing irradiation time. After 3 h irradiation, the amounts of formic acid and oxygen produced in the TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_3$COOH were estimated to be 30.0 and 12.5 nmol, respectively.
In Fig. 17, the time dependence of the amount of hydrogen carbonate (HCO$_3^-$) also is indicated. Before irradiation, the concentration of HCO$_3^-$ in a sample CO$_2$ saturated sodium pyrophosphate buffer was estimated to be 7.0 mM (3.5 μmol) by an ion chromatography system. In solution, FDH catalyzes the reduction of HCO$_3^-$ to formic acid rather than directly CO$_2$ reduction. Therefore, the decrease in HCO$_3^-$ and the increase in formic acid are interlocked. From the results of Fig. 17, as the amount of HCO$_3^-$ decreases, the amount of formic acid produced increases with irradiation time.

Figure 16. Time dependence of photocurrent generation in the visible-light driven electrochemical biofuel cell consisting of TK/TiO$_2$ and CH$_3$V(CH$_2$)$_3$COOH electrodes under continuous irradiation with 100 mWcm$^{-2}$ light.

Figure 17. Time dependence of formic acid and oxygen production in the visible-light driven electrochemical biofuel cell consisting of TK/TiO$_2$ and CH$_3$V(CH$_2$)$_3$COOH electrodes under continuous irradiation with 100 mWcm$^{-2}$ light. Inset: Time dependence of the amount of HCO$_3^-$ in the visible-light driven electrochemical biofuel cell.

By using bare TiO$_2$/FDH-CH$_3$V(CH$_2$)$_3$COOH, no formic acid and oxygen production were observed under continuous 100 mWcm$^{-2}$ irradiation with a cut-off filter (less than 390 nm). On contrast, formic acid production was observed by using bare TiO$_2$/FDH-CH$_3$V(CH$_2$)$_3$COOH under the UV-light irradiation (Xe-lamp). No formic acid production was observed in the absence of CO$_2$. TK/TiO$_2$/FDH (without CH$_3$V(CH$_2$)$_3$COOH), or TK/TiO$_2$/CH$_3$V(CH$_2$)$_3$COOH (without FDH) under continuous 100 mWcm$^{-2}$ irradiation. Thus, all of TK, TiO$_2$, FDH and CH$_3$V(CH$_2$)$_3$COOH are required to develop the visible-light driven electrochemical biofuel cell with the function of CO$_2$ conversion to formic acid.

In addition, as a result of investigating the influence of the distance between the TK/TiO$_2$ and CH$_3$V(CH$_2$)$_3$COOH electrodes to the cell performance, photocurrent was observed, but the reduction efficiency of CO$_2$ decreased under the distance more than 1.5 cm between the TK/TiO$_2$ and CH$_3$V(CH$_2$)$_3$COOH electrodes.

Although highly phoeoelectrochemical conversion efficiency using the purified-photosynthetic protein such as PSI, PSII immobilized electrode was achieved, the stability against visible-light irradiation for a long period was hardly improved. As the membrane-bound proteins exert their function in the lipid bilayer membrane, in general, their stabilities decrease due to purified from the membrane. Activities of these membrane-bound proteins were reduced even in micelles formed with surfactants. In contrast, by using TK directly for visible-light driven electrochemical biofuel cell, photosynthetic proteins, PSI, PSII work in almost the same environment as nature, thus, PSII stably was functioned onto the TiO$_2$ electrode. Thus, a new biofuel cell system with functions of solar cell and CO$_2$ conversion was developed using TK/TiO$_2$/FDH-CH$_3$V(CH$_2$)$_3$COOH.

On the contrary, formic acid and oxygen were not produced under dark conditions. The mechanism of photocurrent generation and formic acid and oxygen production in TK/TiO$_2$/FDH-CH$_3$V(CH$_2$)$_3$COOH on continuous irradiation is as follows. Initially, the PSII in TK/TiO$_2$ was excited by irradiation with visible light, thus producing oxygen. Then, an electron was transferred from the TK to the conduction band of TiO$_2$, followed by transfer to the viologen moiety, and lastly to the FDH moiety in FDH-CH$_3$V(CH$_2$)$_3$COOH electrode. Finally, CO$_2$ in the electrolyte was reduced to formic acid by the catalytic activity of FDH onto FDH-CH$_3$V(CH$_2$)$_3$COOH electrode. The ratio of formic acid to oxygen production in TK/TiO$_2$/FDH-CH$_3$V(CH$_2$)$_3$COOH on continuous irradiation was estimated to be ~2. Thus, formic acid and oxygen were produced stoichiometrically in this visible-light driven electrochemical biofuel cell. The turnover number of PSII in TK/TiO$_2$ in this visible-light driven electrochemical biofuel cell was estimated to be ca. 2777 h$^{-1}$.

On the other hand, the amount of FDH in FDH-CH$_3$V(CH$_2$)$_3$COOH was calculated using enzyme activity assay and was estimated to be ca. 97.5 nmol cm$^{-2}$. The turnover number of FDH in FDH-CH$_3$V(CH$_2$)$_3$COOH was calculated to be ca. 0.3 h$^{-1}$. In CO$_2$ saturated 50 mM sodium pyrophosphate buffer containing 0.1 mM KCl, FDH catalyzes the reduction of HCO$_3^-$ to formic acid. Before irradiation, the concentration of HCO$_3^-$ in a sample CO$_2$ saturated sodium pyrophosphate buffer was estimated to be 3.5 μmol by an ion chromatography system. As the volume of aqueous media was 0.5 mL, the conversion yield of CO$_2$ to formic acid in TK/TiO$_2$/FDH-CH$_3$V(CH$_2$)$_3$COOH was estimated to be ca. 0.85%.
Mechanistic pathway for electron transfer in TK/TiO$_2$/FDH-CH$_3$V(CH$_2$)$_3$COOH

Fig. 18 shows the mechanistic pathway for visible-light driven electron transfer in TK/TiO$_2$/FDH-CH$_3$V(CH$_2$)$_3$COOH.

In this Figure, C.B and V.B represent the conduction and valence band of TiO$_2$. P700 and P700* represent lower and excited energy states of PSI respectively, P680 and P680* represent the lower and excited states of PSII respectively and Q represents quinone. OEC represents the oxygen-evolved complex, V represents the viologen moiety of FDH-CH$_3$V(CH$_2$)$_3$COOH. The band position values also were mentioned in the figure. The process starts at PSI, where the reaction center chlorophyll molecules in P680 harvest visible-light energy for charge separation. An electron from P680, the lower energy state in PSI is excited to the higher energy state, P680*. After subsequent transfer within PSII, the electron transfers to P700 in PSI, via intrinsic electron mediators such as quinones. A second electron excitation occurs at PSI and the electron moves to the excited state, P700*. After sequential electron transfer in PSI, the electron reaches the mediators such as ferredoxins in PSI, and then to the conduction band (C.B) of TiO$_2$ at a lower redox potential. From TiO$_2$, the electron transfers through the external circuit and reaches the FDH-CH$_3$V(CH$_2$)$_3$COOH electrode, where it reduces viologen moiety of CH$_3$V(CH$_2$)$_3$COOH and then CO$_2$ reduction to formic acid with the catalytic activity of FDH. The electron in PSII, is regenerated by the oxidation of water to oxygen with OEC as the redox potential of water oxidation is higher than that of P680. Thus, the cycle completes, producing oxygen at the working electrode (TK/TiO$_2$) and CO$_2$ reduction at the counter electrode (FDH-CH$_3$V(CH$_2$)$_3$COOH).

This scheme PSI alone cannot complete the cycle of electron transfer because of the absence of a sacrificial electron donor in the system. PSI cannot accept electrons from water attributed to unfavorable energy level alignment, as the water oxidation potential is lower than that of P700, the lower energy state of PSI as explained in Fig. 18. The electron at PSI has to move from PSI to PSII. Simultaneously, PSI alone will also not work with TiO$_2$ because the conduction band of TiO$_2$ is higher potential than that of quinones in the electron transfer chain in PSI, disabling the direct electron transfer from PSII to TiO$_2$. From the Fig. 18, the photovoltaic system using the combination of thylakoid membrane including PSI and PSII, and FDH-based electrodes would be operated without any bias voltage.

As mentioned above, all of TK, TiO$_2$, FDH and CH$_3$V(CH$_2$)$_3$COOH are needed to develop the visible-light driven electrochemical biofuel cell with the function of CO$_2$ reduction to formic acid. Especially, PSII in TK with the function of water to photolysis into oxygen, and acquires electrons and protons with visible-light irradiation is the most important photoanode material. As the PSI purified from micro-algae is very unstable, however, stability against light irradiation for a long period and on the electrode are serious problems for the utilization of visible-light driven electrochemical biofuel cell. Therefore, direct utilization of TK assembled the photosynthetic proteins give important approach for device application of visible-light driven electrochemical biofuel cell.

Experimental

Materials

*Spirulina platensis* (NIES-39) was purchased from the National Institute for Environmental Studies. Titanium dioxide nano-powder (P25) was purchased from Degussa. Optical transparent conductive glass plate electrode (OTE) (10-15 Ω/square SnO$_2$: fluorine coated) was obtained from Nihon Sheet Glass Co. Ltd. Indium-tin oxide (ITO) nano-particle (particle shape: angular, average particle size: 30 nm) was obtained from C.I Kasei Co., Ltd. FDH from *Candida boidinii* and proton permeable membrane, Nafion™ 115 film, was obtained from Sigma-Aldrich Co. Ltd. The other chemicals used were of analytical grade or the highest grade available.

1-Carboxyundecanoyl-1ʹ-methyl-4,4ʹ-bipyridiniumbromide, iodide (CH$_3$V(CH$_2$)$_3$COOH) was synthesized using a procedure described in the literature. The Nafion™ 115 film was treated with sulfuric acid before use for photochemical biofuel cell.

Isolation of TK from *Spirulina platensis*

*Spirulina platensis* was incubated in SOT culture medium et al., and TK was isolated using a previously described procedure.

Preparation of nanocrystalline TiO$_2$ film electrode

The nanocrystalline TiO$_2$ film on ITO substrate was prepared using a procedure described previously. TiO$_2$ powder was dispersed in concentrated nitric acid aqueous solution (pH=1.0). The viscous suspension was spread onto ITO (1 cm × 3 cm) at room temperature using Scotch tape as a spacer. A thin film was obtained by raking off the excess suspension with a glass rod. After removing the tape, the plate was dried using a hot plate at 80 °C for 30 min, followed by annealing at 450 °C for 30 min under ambient conditions to form a nanocrystalline TiO$_2$ film onto the ITO substrate. The thickness of the film, determined by a micron-sensitive caliper, was approximately 10 µm. The active area of the electrode was 1.0 cm$^2$.

Preparation of TK-immobilized TiO$_2$ film electrode (TK/TiO$_2$)

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TK-immobilized nanocrystalline TiO$_2$ layer on ITO glass substrate was prepared as follows: a nanocrystalline TiO$_2$ film on an ITO glass substrate was dipped into a TK suspension at 4 °C for 24 h. To prevent the TK on the electrode from eluting into the solution, the electrode surface was covered with polyanion complex film consisting of poly-L-lysine and poly-L-lysine immobilized nanocrystalline TiO$_2$ film on ITO glass substrate is defined as TK/TiO$_2$ electrode. The surface structure of TK/TiO$_2$ electrode was observed using scanning electron microscopy (JSM-6500F FE-SEM JEOl). The CV curves under dark and visible-light irradiation of TK/TiO$_2$ electrode in the aqueous solution of sodium pyrophosphate buffer (50 mM, pH 7.4) containing 0.1 mM KCl at the scan rate of 10 mV/s$^{-1}$ (vs. Ag/AgCl (1 M KCl)) were measured using potentiostat (Hokuto Denko HZ-3000). A solar simulator was also used as a light source (A.M. 1.5 100 mW cm$^{-2}$) with a cut-off filter (SCF-505-39L Sigma Koki; less than 390 nm).

Preparation of FDH- and viologen-immobilized electrode (FDH-CH$_3$V(CH$_2$)$_5$COOH)

The FDH- and viologen-immobilized electrode was prepared using a modification of a previously described procedure. The viscous suspension containing indium-tin oxide (ITO) nanoparticle and polyethylene glycol was spread to 1 cm$^2$ onto an ITO glass substrate (1 x 5 cm) at room temperature. An ITO thin layer film was obtained by raking off the excess suspension with a glass rod. The substrate was dried using a hot plate at 80 °C for 30 min and annealed at 450 °C for 30 min under ambient conditions to form an ITO thin layer film electrode. The active area of electrode was 1.0 cm$^2$. To immobilize the 1-carboxyundecanoyl-1'-methyl-4,4'-bipyridinium bromide, iodide (CH$_3$V(CH$_2$)$_5$COOH) onto the ITO thin layer film electrode, the substrate was dipped into methanol solution containing CH$_3$V(CH$_2$)$_5$COOH (300 µM) for 68 h at 25 °C. The electrode was washed with methanol to remove physically adsorbed CH$_3$V(CH$_2$)$_5$COOH. This electrode was defined as CH$_3$V(CH$_2$)$_5$COOH/ITO electrode. Finally, FDH was immobilized onto the CH$_3$V(CH$_2$)$_5$COOH/ITO electrode. The electrode was dipped into sodium pyrophosphate buffer solution (pH 7.4) containing FDH (0.75 µM) for 1 h at 4 °C, followed by washing with sodium pyrophosphate buffer solution (pH 7). To prevent CH$_3$V(CH$_2$)$_5$COOH and FDH on the electrode from eluting into the solution, the electrode surface was covered with polyanion complex film consisting of poly-L-lysine and poly-L-lysine-glutamine. This electrode was defined as FDH-CH$_3$V(CH$_2$)$_5$COOH electrode. The surface structure of FDH-CH$_3$V(CH$_2$)$_5$COOH electrode was observed using scanning electron microscopy. The CV curves FDH-CH$_3$V(CH$_2$)$_5$COOH electrode and CH$_3$V(CH$_2$)$_5$COOH/ITO electrodes in the aqueous solution of CO$_2$ saturated sodium pyrophosphate buffer (50 mM, pH 7.4) containing 0.1 mM KCl at the scan rate of 10 mV/s$^{-1}$ (vs. Ag/AgCl (1 M KCl)) were measured using potentiostat (Hokuto Denko HZ-3000). The concentration of formic acid produced in FDH-CH$_3$V(CH$_2$)$_5$COOH with continuous electrochemical bias application was measured using an ion chromatograph system (Dionex ICS2000). The other details of FDH-CH$_3$V(CH$_2$)$_5$COOH electrode were reported previously.$^{47,48}$

Oxygen production activity of TK/TiO$_2$ in the presence of DCPIP under visible-light irradiation

The oxygen production activity of PSII in TK/TiO$_2$ under the continuous visible-light irradiation in the presence of sacrificial reagent DCPIP was studied. The sodium pyrophosphate buffer (50 mM, pH 7.4) containing 0.3 mM of DCPIP was subjected to bubbling nitrogen or CO$_2$ gas for 20 min. The visible-light was irradiated to TK/TiO$_2$ in the sodium pyrophosphate buffer containing DCPIP. A solar simulator (YSS-40, Yamashita Denso) was used as a visible light source (A.M. 1.5 100 mW cm$^{-2}$). To prevent degradation of TK and direct excitation of TiO$_2$ by near UV light, wavelengths of less than 390 nm were blocked with a cut-off filter. The amount of oxygen produced was measured using the optical oxygen monitor (FireStingO2, Pyroscience).

Photocurrent action spectra measurement

A 400 W xenon lamp with a monochromator was used as light source for photocurrent action spectra measurements. The cell was operated in the short-circuit mode. Photocurrent action spectrum of TK/TiO$_2$|FDH-CH$_3$V(CH$_2$)$_5$COOH was measured with a sandwich type cell. The working electrode with TK/TiO$_2$ was gently squeezed together with FDH-CH$_3$V(CH$_2$)$_5$COOH as a counter electrode using spring and irradiated from the working electrode side. The sodium pyrophosphate buffer (50 mM, pH 7.4) containing 0.1 mM KCl was subjected to bubbling CO$_2$ gas for 20 min and then used as the electrolyte. The distance between the working and counter electrode was 0.5 cm. The incident photon-to-current conversion efficiency (IPCE) values were determined between 400 and 800 nm. The IPCE was then calculated according to the following equation:

$$\text{IPCE} = \frac{1240 J_{ph} (\mu A)/P(\mu W)}{\lambda (\text{nm})}$$

where $J_{ph}$ and $P$ are the photocurrent and power of the incident radiation per unit area and $\lambda$ is the wavelength of the monochromatic light.

Characterization of visible-light driven electrochemical biofuel cell

The working electrode with TK/TiO$_2$ electrode was gently squeezed together with a FDH-CH$_3$V(CH$_2$)$_5$COOH electrode as a counter electrode via the proton transparent membrane (PEM), Nafion® 115 using a spring and irradiated from the side of the working electrode. The sodium pyrophosphate buffer (50 mM, pH 7.4) containing 0.1 mM KCl was subjected to bubbling CO$_2$ gas for 20 min, and then used as the electrolyte. The volumes of electrolyte and gaseous phase in the anode and cathode sides were 0.5 mL, respectively. This visible-light driven electrochemical biofuel cell was defined as TK/TiO$_2$ | FDH-CH$_3$V(CH$_2$)$_5$COOH.

A solar simulator was used as a light source (A.M. 1.5 100 mW cm$^{-2}$) with a cut-off filter (less than 390 nm). The cell
was operated in the short-circuit mode. The voltage and current responses were measured using a digital multimeter (model 34401A, Agilent) under dark and irradiation cycles. The actual photocurrent was calculated from the difference in the curves. The active electrode area was 1.0 cm$^2$.

Linear polarization (LP) was used to obtain dark and light current–potential (J–V) curves for the visible-light-driven electrochemical biofuel cells using potentiostat (Hokuto Denko HZ-3000) with sweep rate of 50 mV s$^{-1}$, and the photocurrent was calculated from the difference in the curves. In LP measurements, the potential between the anode and cathode was varied without a reference electrode, while current was monitored, allowing for probing of light and dark J–V characteristics of the visible-light-driven electrochemical biofuel cells. A solar simulator was also used as a light source (A.M. 1.5 100 mW cm$^{-2}$) with a cut-off filter (less than 390 nm) for LP measurements. The active electrode area also was 1.0 cm$^2$ for LP measurements.

The concentration of formic acid produced and HCO$_2$H$_3$ in TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_2$COOH during irradiation was measured using an ion chromatograph system ( Dionex IC2000). The amount of oxygen produced in the side of TK/TiO$_2$ was measured using the optical oxygen monitor.

Conclusions

In this work, development of a TK isolated from Spirulina platensis assembled onto TiO$_2$ film electrode, and its photon-to-current conversion function was studied. A visible-light driven electrochemical cell, TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_2$COOH, which converted CO$_2$ to formic acid, was also developed. The photovoltage and photocurrent of TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_2$COOH were responsive to visible-light on / off. The ratio of formic acid to oxygen production in TK/TiO$_2$ | FDH-CH$_3$V(CH$_3$)$_2$COOH on continuous irradiation was estimated to be ~2. Thus, formic acid and oxygen were produced stoichiometrically in this visible-light driven electrochemical biofuel cell. By using TK directly for visible-light driven electrochemical biofuel cell, PSI and PSII work in almost the same environment as nature, thus, the stable electrochemical cell using photosynthetic organs was accomplished. A new biocatalyst-fuel cell system with functions of a solar cell, reduction of CO$_2$, and lower carbon fuel production was developed in this study.

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