

Supporting information

Visible-light driven fumarate production from CO₂ and pyruvate by the photocatalytic system with dual biocatalysts

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1. Enzymatic activity of malate dehydrogenase decarboxylating type (EC 1.1.1.38 code: MDH-73-01 obtained from *Sulfolobus tokodaii*)

One activity unit of malate dehydrogenase decarboxylating type convert 1.0 μmol of NADH to NAD^+ in the presence of 10 mM sodium pyruvate, 0.3 mM NADH, 10 mM sodium bicarbonate and 10 mM MgCl_2 in 50 mM 1,4-piperazinediethanesulfonic acid-KOH buffer per min at pH 6.5 at 37 °C according to the data sheet provided by Thermo Stable Enzyme Laboratory Co., Ltd. The molecular weight of MDH was determined to be 40 kDa based on the SDS-page of electrophoresis.

2. Enzymatic activity of fumarase from porcine heart (EC 4.2.1.2)

One activity unit of fumarase (FUM) from porcine heart (EC 4.2.1.2) convert 1.0 μmol of L-malate to fumarate in potassium phosphate buffer per min at pH 7.6 at 25 °C.

3. The pH dependence of L-malate production from bicarbonate and pyruvate with MDH in the presence of NADH

The reaction mixture consisted of sodium pyruvate (5.0 mM), NADH (5.0 mM), magnesium chloride (10 mM) and sodium bicarbonate (100 mM) in 5.0 mL of 500 mM 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)-NaOH buffer. The reaction vessel is a clear glass vial, and the reaction is a sealed system. The total volume of reaction vessel is 11.0 mL. The gas phase of the reaction vessel and sample solution were replaced by flowing CO_2 gas at a flow rate of 0.1 L min^{-1} for 10 min. The pH of the sample solution was adjusted in the range from 6.7 to 8.2. The reaction was started by adding ME (0.7 units) to above mixture in the agitating water baths set at a temperature of 30.5 °C. The reaction was carried out using the shaking incubator with a thermostatic chamber (EYELA NTS-4000, TOKYO RIKAKIKAI Co. Ltd.). The shaking speed was adjusted to be 80 rpm. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography. At the time of sampling, CO_2 gas was flowed into the gas phase of the reaction vessel. The amount of L-malate was detected using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 \times 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of

eluent solution was adjusted to be 0.5 mL min⁻¹. The electrical conductivity changes in the various L-malate concentrations (0 – 1000 μM) during the ion chromatograph analysis were shown in Figure S1. Inset of Figure S1 shows the relationship between the L-malate concentration and the detection peak area using ion chromatograph.

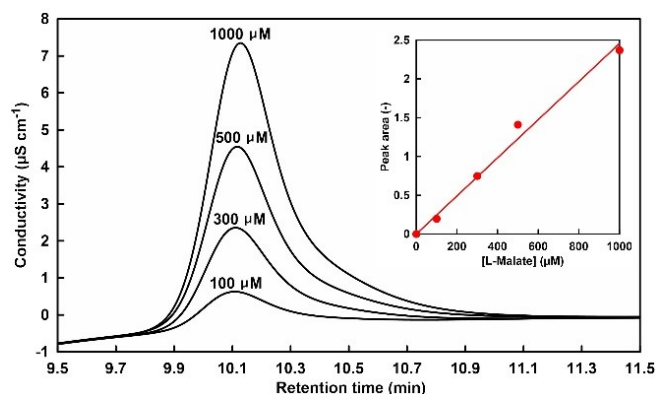


Figure S1. Chromatogram of sodium L-malate (0 - 1000 μM) in 50 mM-HEPES buffer (pH 7.0). Inset: Relationship between the L-malate concentration and the detection peak area.

As shown in the inset of Figure S1, the L-malate concentration and the detected peak area showed a good linear relationship (correlation coefficient: $r^2=0.999$) as following equation (S1).

$$\text{Peak area} = 2.46 \times 10^{-3} \times [\text{L-malate}] (\mu\text{M}) \quad (\text{S1})$$

4. Effect of CO₂ on the visible-light driven NADH regeneration system of triethanol amine (TEOA), zinc meso-tetra(4-sulfonatophenyl) porphyrin tetrasodium salt (ZnTPPS) and pentamethylcyclopentadienyl rhodium bipyridine complex ([Cp*Rh(bpy)(H₂O)]²⁺)

Pentamethylcyclopentadienyl rhodium bipyridine complex ([Cp*Rh(bpy)(H₂O)]²⁺) was synthesized from (pentamethylcyclopentadienyl)rhodium(III) dichloride dimer ([Cp*RhCl₂]₂), purchased from Tokyo Chemical Industry Co., Ltd., and 2,2'-bipyridine according to a reported literature.¹⁾ A sample solution containing triethanol amine (TEOA) (0.2 M), zinc meso-tetra(4-sulfonatophenyl) porphyrin tetrasodium salt (ZnTPPS), obtained from Frontier Scientific Inc, (10 μM), [Cp*Rh(bpy)(H₂O)]²⁺ (10 μM) and NAD⁺ purchased from Oriental Yeast Co., Ltd, (0.5 mM) in 5.0 mL of 500 mM HEPES-NaOH buffer was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon or CO₂ gas for 10 min. The sample solution was irradiated with

a 250 W halogen lamp (Panasonic) as a visible-light source at 30.5 °C. The concentration of NADH produced was monitored by absorption spectrum change using UV-visible absorption spectroscopy (SHIMADZU, MultiSpec-1500) with the molar coefficient at 340 nm ($\epsilon=6220 \text{ cm}^{-1} \text{ M}^{-1}$).

5. Visible-light driven L-malate production with the system of TEOA, ZnTPPS, $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$, NAD^+ , bicarbonate, pyruvate and MDH

The outline of reaction vessel is shown in Figure S2. An eggplant-shaped flask with a side tube is equipped with a gas-introducing balloon through a stopcock. Total volume of this reaction vessel is 19.84 mL and the balloon volume is 1.0 L. A sample solution containing TEOA (0.2 M), ZnTPPS (10 μM), $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ (10 μM), NAD^+ (0.5 mM), sodium pyruvate (5.0 mM), sodium bicarbonate (100 mM), magnesium chloride (5.0 mM) and MDH (0.7 U) in 5.0 mL of 500 mM HEPES-NaOH buffer was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with CO_2 or argon gas for 10 min. The sample solution was irradiated with a 250 W halogen lamp as a visible-light source at 30.5 °C. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography.

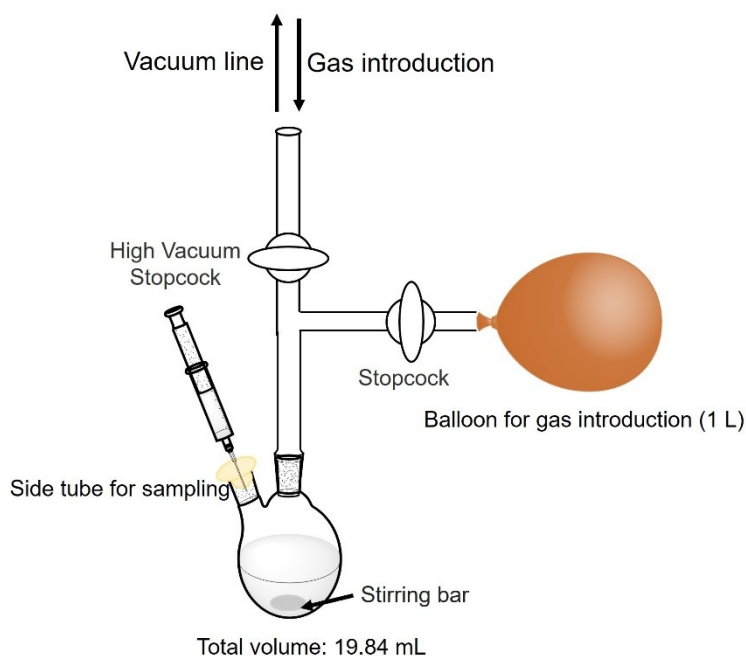


Figure S2. Outline of reaction vessel for visible-light driven redox system.

6. Visible-light driven fumarate production with the system of TEOA, ZnTPPS,

[Cp*Rh(bpy)(H₂O)]²⁺, NAD⁺, bicarbonate, pyruvate, MDH and fumarase (FUM)

The reaction vessel shown in Figure S2 was also used in this reaction. A sample solution containing TEOA (0.2 M), ZnTPPS (10 μ M), [Cp*Rh(bpy)(H₂O)]²⁺ (10 μ M), NAD⁺ (0.5 mM), sodium pyruvate (5.0 mM), sodium bicarbonate (100 mM), magnesium chloride (5.0 mM), MDH (0.7 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES-NaOH buffer was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with CO₂ or argon gas for 10 min. The sample solution was irradiated with a 250 W halogen lamp as a visible-light source at 30.5 °C. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography. The amount of L-malate or fumarate was detected using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 \times 250 mm; composed of 9 μ m polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min⁻¹. The electrical conductivity changes in the various fumarate concentrations (0 – 1000 μ M) during the ion chromatograph analysis were shown in Figure S3. Inset of Figure S3 shows the relationship between the fumarate concentration and the detection peak area using ion chromatograph.

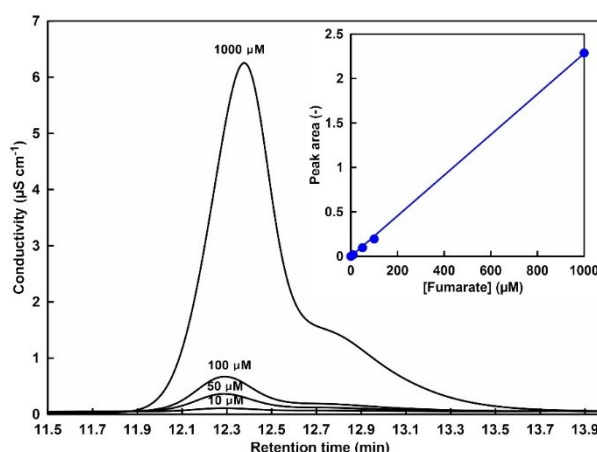


Figure S3. Chromatogram of sodium fumarate (0 - 1000 μ M) in 50 mM-HEPES buffer (pH 7.0). Inset: Relationship between the fumarate concentration and the detection peak area.

As shown in the inset of Figure S3, the L-malate concentration and the detected peak area

showed a good linear relationship (correlation coefficient: $r^2=0.999$) as following equation (S2).

$$\text{Peak area} = 2.28 \times 10^{-3} \times [\text{fumarate}] (\mu\text{M}) \quad (\text{S2})$$

Figure S4 shows a chart of an ion chromatogram sampled from the reaction solution after 5h irradiation.

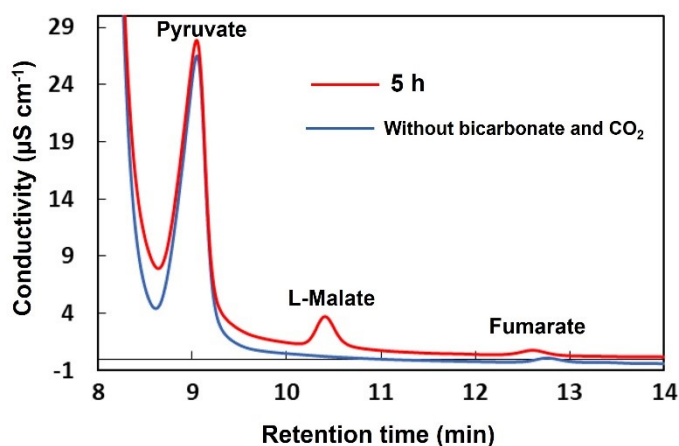


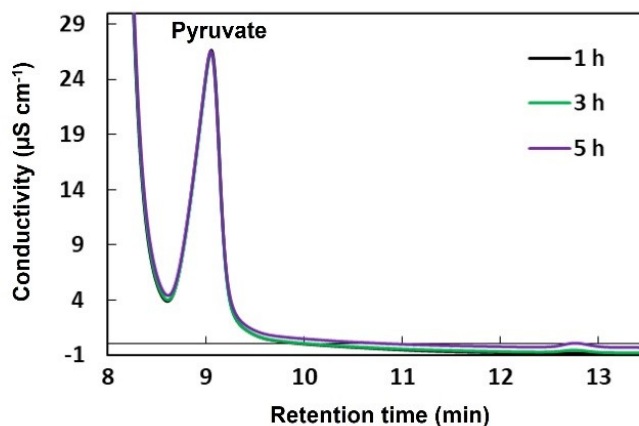
Figure S4. A chart of an ion chromatogram sampled from the reaction solution containing TEOA, ZnTPPS, [Cp*Rh(bpy)(H₂O)]²⁺, NAD⁺, pyruvate, bicarbonate, MDH and FUM in HEPES buffer under the condition of CO₂ filled gas phase after 5 h irradiation.

7. Visible-light driven fumarate production with the system of TEOA, ZnTPPS, [Cp*Rh(bpy)(H₂O)]²⁺, NAD⁺, pyruvate, MDH and FUM without bicarbonate and CO₂

The reaction vessel shown in Figure S2 was also used in this reaction. A sample solution containing TEOA (0.2 M), ZnTPPS (10 μM), [Cp*Rh(bpy)(H₂O)]²⁺ (10 μM), NAD⁺ (0.5 mM), sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), MDH (0.7 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES-NaOH buffer was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 10 min. The sample solution was irradiated with a 250 W halogen lamp as a visible-light source at 30.5 °C. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography. The amount of L-malate or fumarate was detected using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size:

7.8 × 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min⁻¹.

Figure S5 shows a chart of an ion chromatogram sampled from the reaction



solution with the irradiation.

Figure S5. A chart of an ion chromatogram sampled from the reaction solution containing TEOA, ZnTPPS, [Cp*Rh(bpy)(H₂O)]²⁺, NAD⁺, pyruvate, MDH and FUM in HEPES buffer with the irradiation.

As shown in Figure S5, no pyruvate consumption, L-malate and fumarate production were observed with the irradiation.

8. Fumarate production from pyruvate and ¹³C-labeled bicarbonate with ME and FUM in the presence of NADH

The fumarate production from pyruvate and ¹³C-labeled bicarbonate with ME and FUM in the presence of NADH was investigated. The reaction mixture consisted of sodium pyruvate (5.0 mM), NADH (5.0 mM), magnesium chloride (5.0 mM) and sodium ¹³C-bicarbonate (100 mM) in 5.0 mL of 500 mM in HEPES buffer (pH 7.6). The reaction was started by adding ME (0.7 units) and FUM (0.5 units) to above mixture in thermostatic chamber set at a temperature of 30.5 °C. ¹H-NMR and ¹³C-NMR of the sample solution after 5 h incubation were measured. The ¹H-¹³C heteronuclear multiple bond coherence (HMBC) of the standard solution of fumarate (a) and the sample solution (b) were shown in Figure S6.

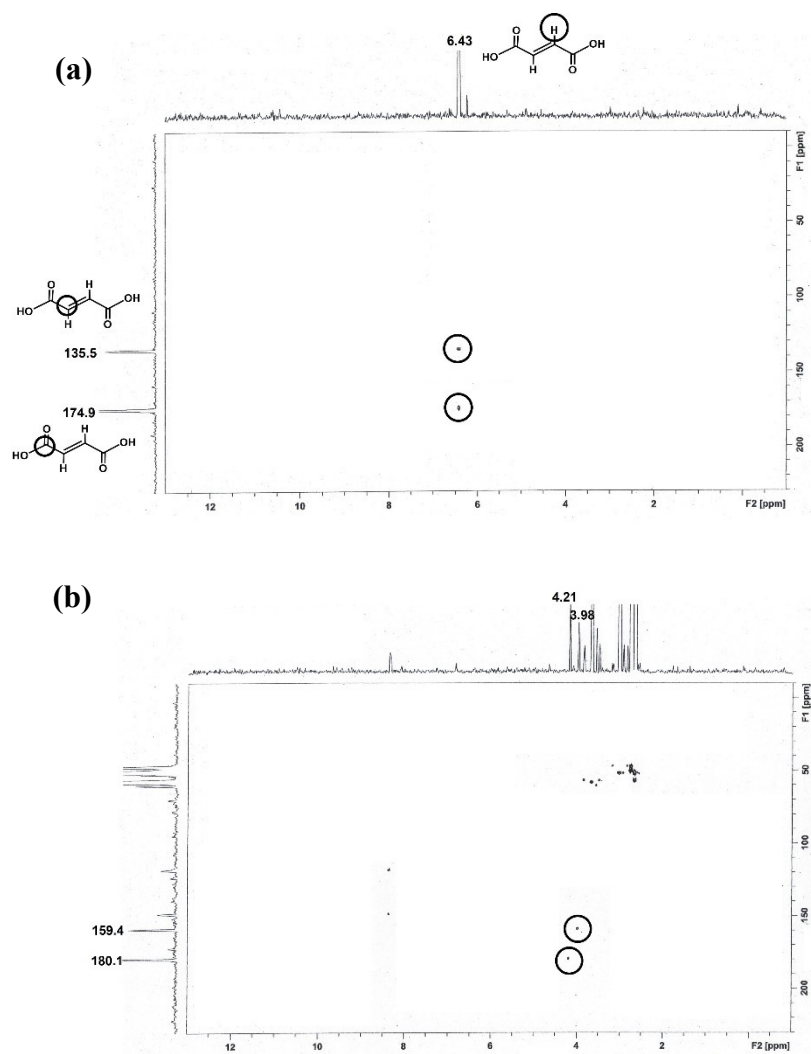


Figure S6. HMBC of the standard solution of fumarate (a) and the sample solution (b).

Reference

1. U. Kölle, B.-S Kang, P. Infelta, P. Comte and M. Grätzel, *Chem. Ber.*, 1989, **122**, 1869.