Electronic Supplementary Material (ESI) for RSC Sustainability.

Supporting information

Phosphate-induced enhancement of fumarate production from CO_2 and pyruvate system of malate dehydrogenase and fumarase

Mika Takeuchi[a] and Yutaka Amao[a,b*]

[a] Graduate School of Science, Osaka Metropolitan University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan

[b] Research Centre of Artificial Photosynthesis (ReCAP), Osaka Metropolitan University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan,

Email: amao@omu.ac.jp

1. Experimental procedure

The outline of the experimental procedure is shown below.

The reaction vessel consists of a glass sample bottle, a screw cap and a septum rubber. The volume of vessel is 13.0 mL. The reaction is carried out using the shacking incubator with a thermostatic chamber (EYELA NTS-4000, TOKYO RIKAKIKAI Co. Ltd.). The reaction temperature is adjusted at 30.5 °C. The shaking speed is adjusted to be 80 rpm.

For fumarate production from L-malate with fumarase (FUM) from porcine heart, add 4.9 mL of L-malate in 500 mM HEPES buffer solution the reaction vessel and then add 0.1 mL of FUM to L-malate aqueous solution by using a syringe.

For L-malate production from pyruvate and bicarbonate with malate dehydrogenase decarboxylating type (ME) from *Sulfobus tokodaii*, add 4.9 mL of pyruvate, bicarbonate, magnesium chloride and NADH in 500 mM HEPES buffer solution the reaction vessel and then bubbling CO_2 gas into the solution for 10 min. Next, add 0.1 mL of ME to the solution by using a syringe.

For fumarate production from pyruvate and bicarbonate with ME and FUM, add 4.8 mL of pyruvate, bicarbonate, magnesium chloride and NADH in 500 mM HEPES buffer solution the reaction vessel and then bubbling CO_2 gas into the solution for 10 min. Next, add 0.2 mL of the mixture of ME and FUM to the solution by using a syringe. The concentration of pyruvate L-malate or fumarate was determined using ion chromatography system.

2. Determination for L-malate and fumarate concentration using ion chromatography

The concentration of L-malate or fumarate was determined 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 x 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 retention time for L-malate was detected at 10.11-10.13 min. The electrical conductivity changes in the various L-malate concentrations (0 – 1.0 mM) were shown in Figure S1(a). The retention time for fumarate was detected at 12.28-12.37 min. The electrical conductivity changes in the various fumarate concentrations (0 – 1.0 mM) were shown in Figure S1(b).

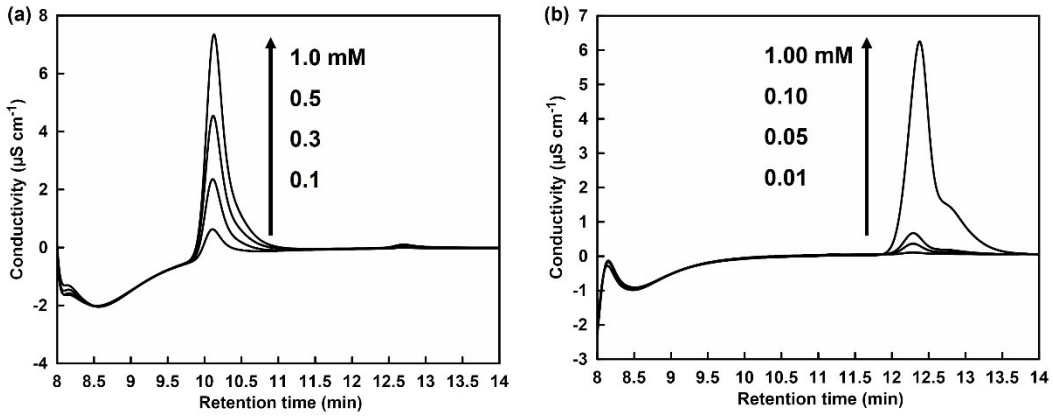

Figure S1. Chromatogram of sodium L-malate (0 - 1.0 mM) (a) and sodium fumarate (0 - 1.0 mM) (b) in 50 mM-HEPES buffer (pH 7.0).

Figure S2 shows the relationship between the L-malate (a) and fumarate (b) concentration and the detection peak area using ion chromatograph.

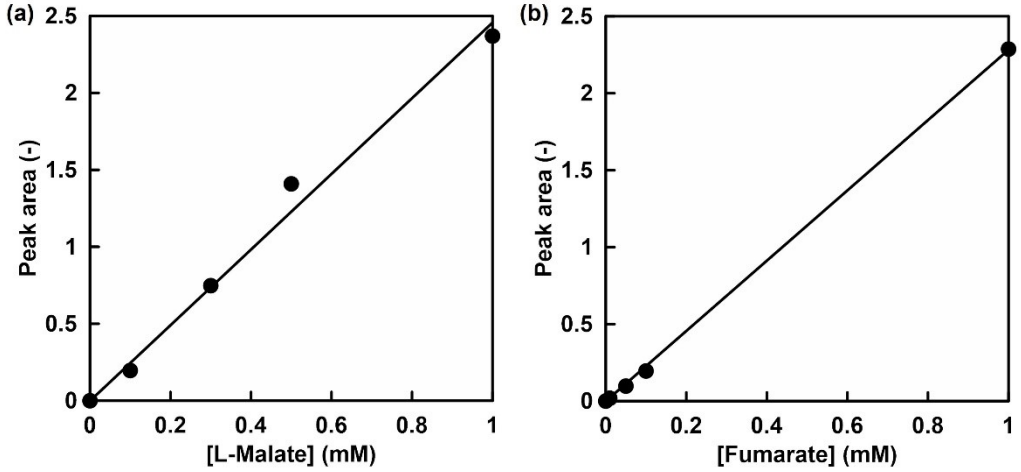

Figure S2. Relationship between the L-malate (a) and fumarate (b) concentration and the detection peak area.

As shown in Figure S2(a), the L-malate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S1).

$$\text{Peak area} = 2.46 \times [\text{L-malate}](\text{mM}) \quad \text{(S1)}$$

As shown in Figure S2(b), the fumarate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S2).

$$\text{Peak area} = 2.28 \times [\text{fumarate}](\text{mM}) \quad \text{(S2)}$$

3. Determination for pyruvate concentration using ion chromatography

The concentration of pyruvate was determined 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 x 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 retention time for pyruvate was detected at 8.71-9.20 min. The electrical conductivity changes in the various pyruvate concentrations (0 - 10 mM) were shown in

Figure S3(a). Figure S3(b) shows the relationship between the pyruvate concentration and the detection peak area using ion chromatograph.

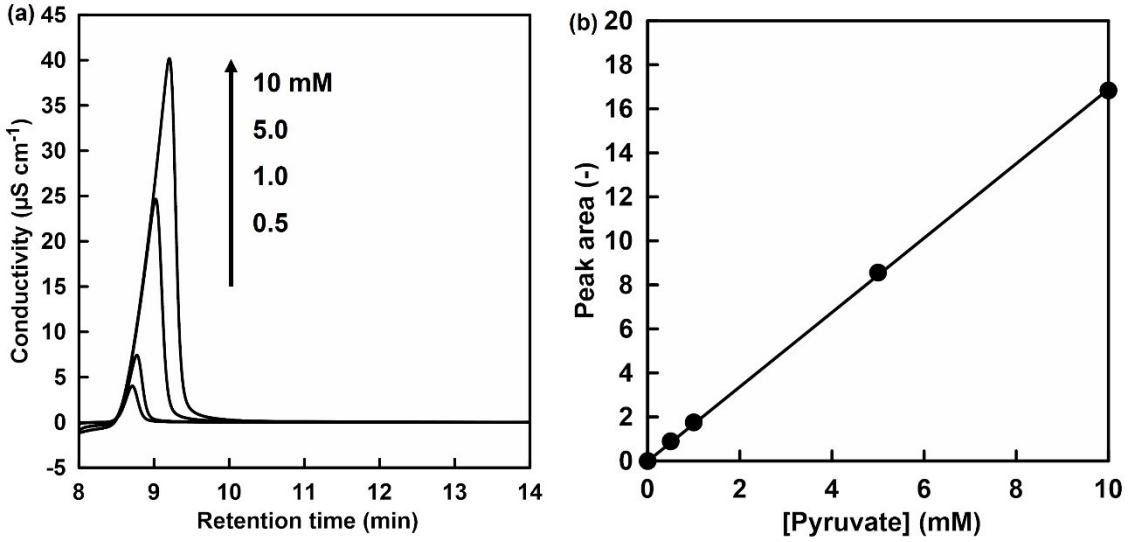

Figure S3. Chromatogram of sodium pyruvate (0 - 10 mM) in 50 mM-HEPES buffer (pH 7.0) (a). Relationship between the pyruvate concentration and the detection peak area (b).

As shown in Figure S3(b), the pyruvate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S3).

$$\text{Peak area} = 1.69 \times [\text{Pyruvate}](\text{mM}) \quad \text{(S3)}$$