

Supporting information

Effect of water-soluble zinc porphyrin on the catalytic activity of fumarase for L-malate dehydration to fumarate

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1. Determination for fumarate concentration using ion chromatography

The concentration of 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. The retention time for fumarate was detected at 12.28–12.37 min. The electrical conductivity changes in the various sodium fumarate concentrations (0 – 1000 μM) during the ion chromatograph analysis were shown in Figure S1(a). Figure S1 (b) shows the relationship between the sodium fumarate concentration and the detection peak area using ion chromatograph.

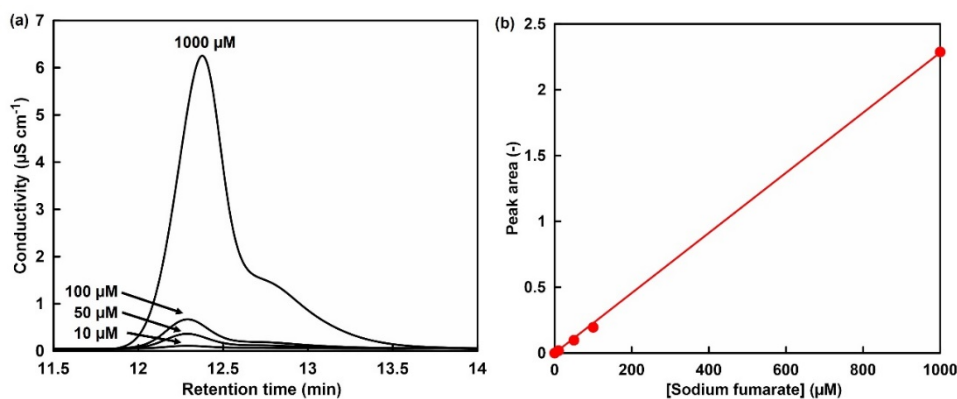


Figure S1. Chromatogram of sodium fumarate (0 – 1000 μM) in 50 mM-HEPES buffer (pH 7.0) (a). Relationship between the fumarate concentration and the detection peak area (b).

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

$$\text{Peak area} = 2.28 \times 10^3 [\text{fumarate}] (\mu\text{M}) \quad (\text{S1})$$

2. Fumarate synthesis from L-malate with fumarase (FUM) in the presence of water-soluble zinc porphyrin

Figure S2 shows a chart of an ion chromatogram sampled from the reaction solution of L-malate (1.0 mM), FUM (0.5 units; 1.3 nM) and water-soluble zinc porphyrin (10 μM) in 5.0 mL of 500 mM HEPES buffer (pH 7.8) during the incubation. The following four zinc porphyrins were used for the experiment; zinc tetrakis(4-methylpyridyl)porphyrin (ZnTMPyP^{4+}), zinc tetrakis(4-*N,N,N*-trimethylaminophenyl)porphyrin (ZnTMAP^{4+}), zinc tetra(4-carboxyphenyl)porphyrin (ZnTCPP^{4+}), zinc tetra(4-sulfonatophenyl)porphyrin (ZnTPPS^{4+}).

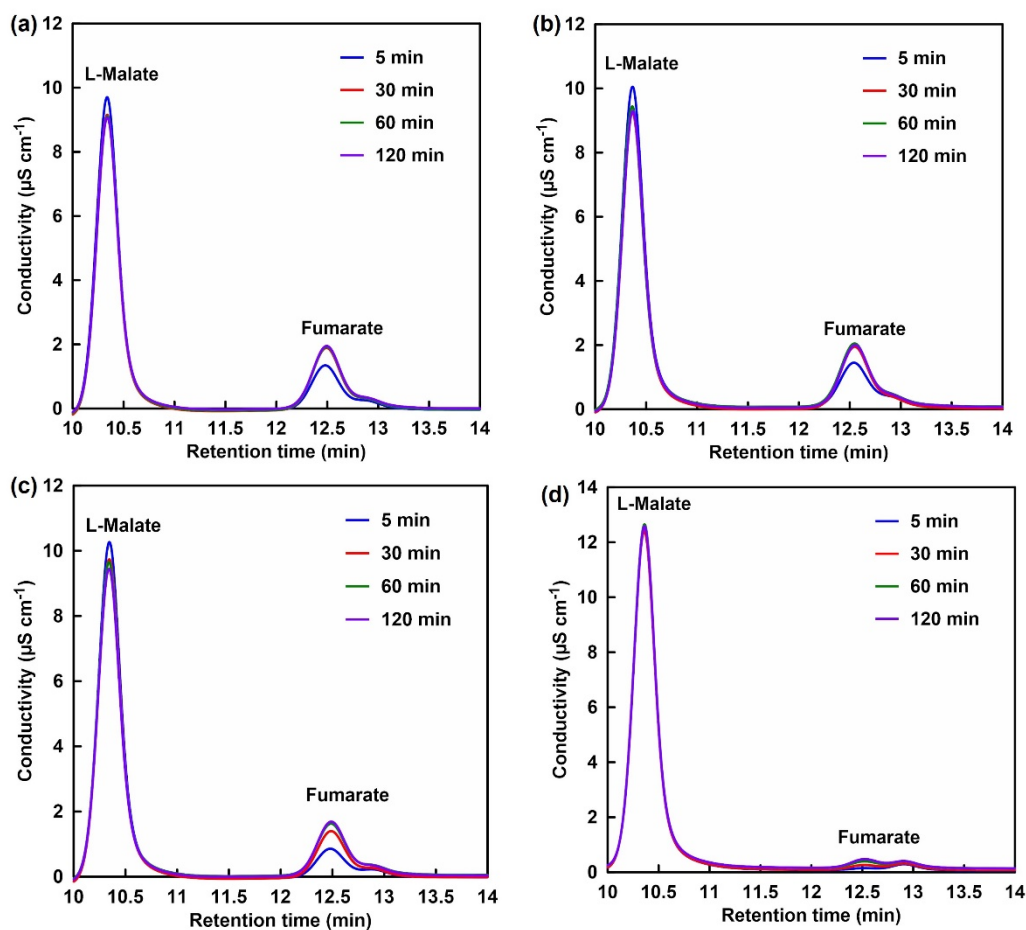


Figure S2. A chart of an ion chromatogram for L-malate or fumarate concentration in the solution of L-malate (1.0 mM), FUM (0.5 units; 1.3 nM) and water-soluble zinc porphyrin (10 μM) in 5.0 mL of 500 mM HEPES buffer (pH 7.8). (a): ZnTMPyP^{4+} , (b): ZnTMAP^{4+} , (c): ZnTCPP^{4+} , (d): ZnTPPS^{4+}

3. Fumarate synthesis from L-malate with fumarase (FUM) in the presence of water-soluble metal-free porphyrin

Figure S3 shows a chart of an ion chromatogram sampled from the reaction solution of L-malate (1.0 mM), FUM (0.5 units; 1.3 nM) and water-soluble metal-free porphyrin (10 μM) in 5.0 mL of 500 mM HEPES buffer (pH 7.8) during the incubation. The following four zinc porphyrins were used for the experiment; tetrakis(4-methylpyridyl)porphyrin ($\text{H}_2\text{TMPyP}^{4+}$), tetrakis(4-*N,N,N*-trimethylaminophenyl)porphyrin ($\text{H}_2\text{TMAP}^{4+}$), tetra(4-carboxyphenyl)porphyrin ($\text{H}_2\text{TCPP}^{4-}$), tetra(4-sulfonatophenyl)porphyrin ($\text{H}_2\text{TPPS}^{4-}$).

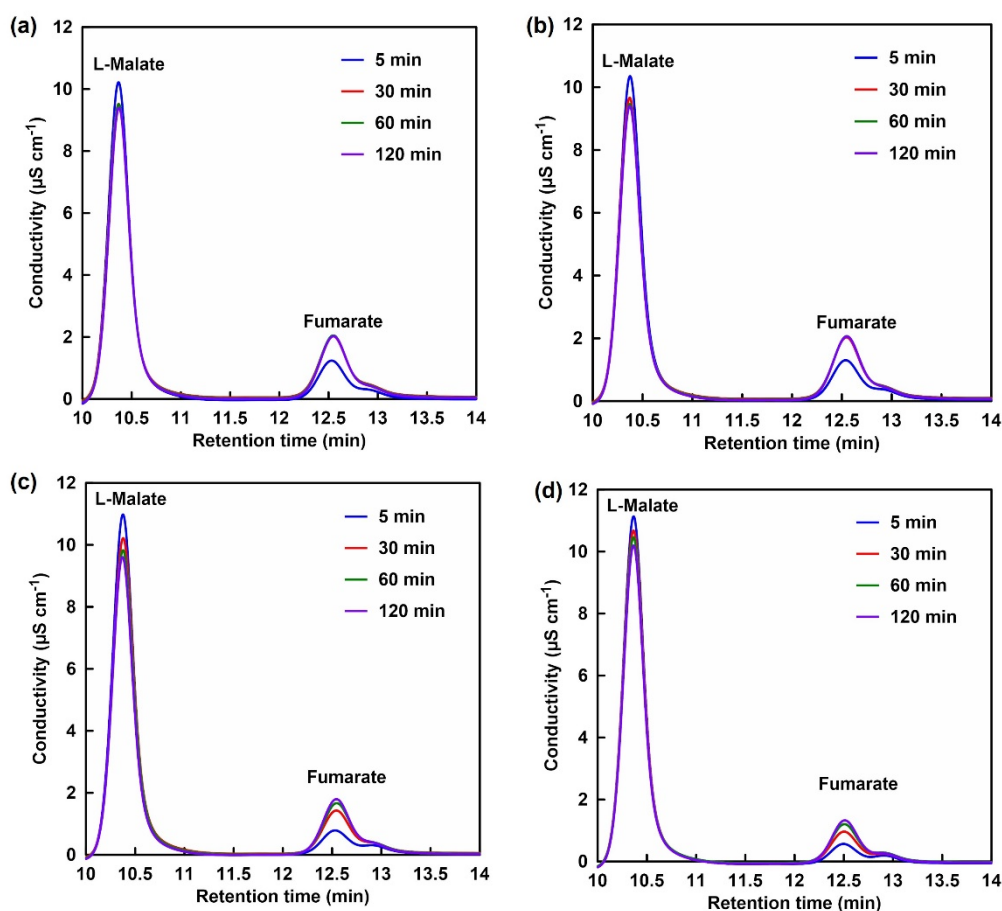


Figure S3. A chart of an ion chromatogram for L-malate or fumarate concentration in the solution of L-malate (1.0 mM), FUM (0.5 units; 1.3 nM) and water-soluble metal-free porphyrin (10 μM) in 5.0 mL of 500 mM HEPES buffer (pH 7.8). (a): $\text{H}_2\text{TMPyP}^{4+}$, (b): $\text{H}_2\text{TMAP}^{4+}$, (c): $\text{H}_2\text{TCPP}^{4-}$, (d): $\text{H}_2\text{TPPS}^{4-}$