Supporting Information

Green method of synthesizing L-malate from D-glucose via CO_2 fixation using an ATP-free *in vitro* synthetic enzymatic biosystem

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Table S1. Plasmids used in this study.

Plasmid	Features	Ref
pET28a-ssgdh	Kana^R, *gdh* expression cassette containing GDH protein from *Sulfolobus solfataricus*, C-terminal 6×His tag	[34]
pET28a-sackdga	Kana^R, *kdga* expression cassette containing KDGA protein from *Sulfolobus sacidocaldarius*, C-terminal 6×His tag	[34]
pET28a-taaldh	Kana^R, *aldh* expression cassette containing ALDH protein from *Thermoplasma acidophilum*, C-terminal 6×His tag	[34]
pET28a-pudhad	Kana^R, *dhad* expression cassette containing DHAD protein from *Paralcaligenes ureilyticus*, C-terminal 6×His tag	[37]
pET28a-tkmdh	Kana^R, *mdh* expression cassette containing MDH protein from *Thermococcus kodakarensis*, C-terminal 6×His tag	[35]

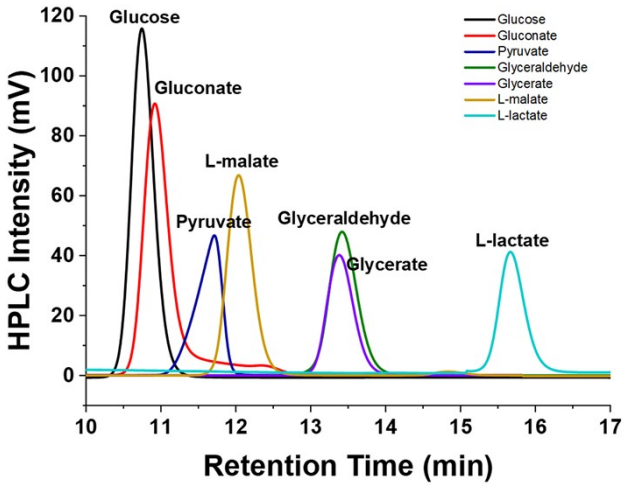

Fig. S1 The High Performance Liquid Chromatography (HPLC) profiles of L-malate and other intermediates. Gluconate, pyruvate, glycerate, glyceraldehyde, and L-malate were measured by the HPLC (SHIMADZU, Japan) equipped with a refractive index detector. Samples were separated on a Bio-Rad Aminex HPLC organic acid column (HPX-87H 300 x 7.8 mm^2) at 35 ^oC with a mobile phase of 5 mM H_2SO_4 solution at a rate of 0.5 mL/min.

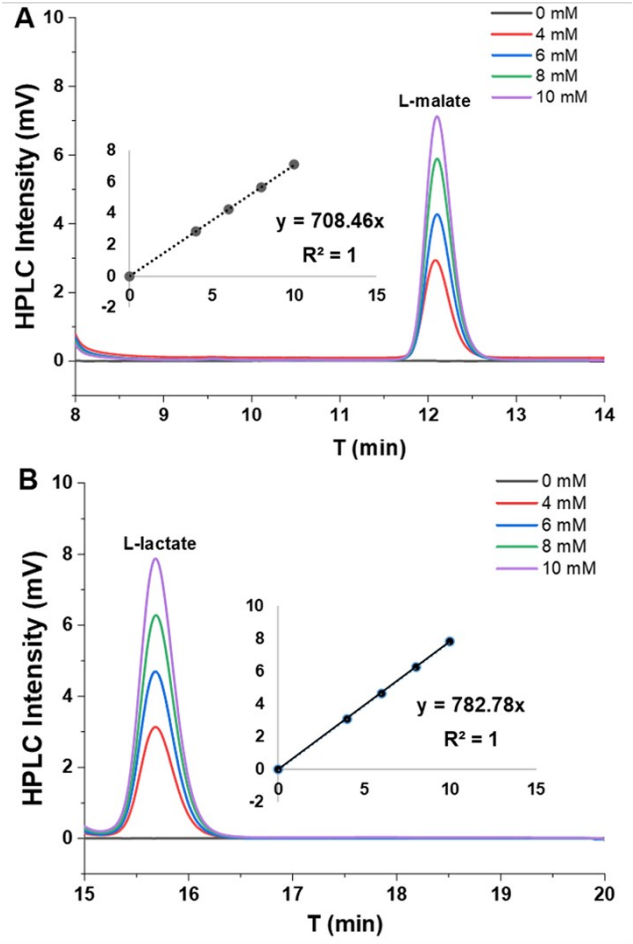

Fig. S2 The standard curves of L-malate (A) and L-lactate (B). The concentration of L-malate and L-lactate from 4-10 mmol/L were determined from the height of the peaks in HPLC equipped with Bio-Rad HPX-87H column and a refractive index detector at 35 ^oC with a mobile phase of 5 mM

H_2SO_4 solution at a rate of 0.5 mL/min.

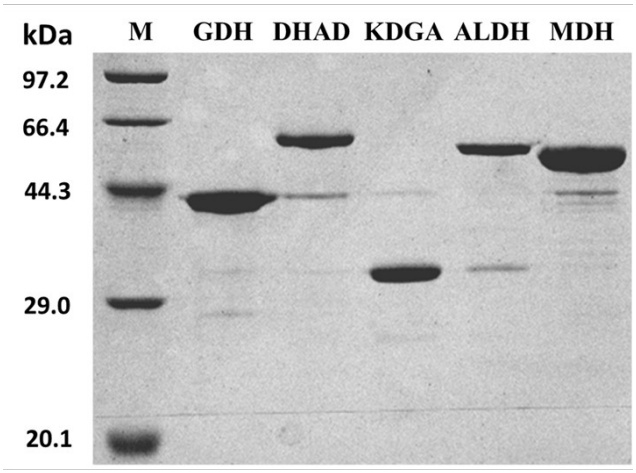

Fig. S3 SDS-PAGE gel analysis of five recombinant enzymes for the one-pot biosynthesis of L-malate from D-glucose and CO_2. M, protein marker. The molecular weights of GDH, DHAD, KDGA, ALDH, and MDH were approximately 41.7, 59.4, 32.5, 54.7, and 50.0 kDa, respectively.

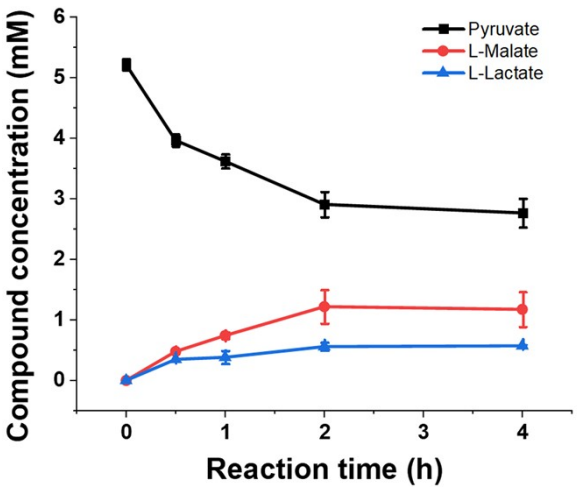

Fig S4 Biocatalytic efficiency of MDH. The concentration profiles of pyruvate (square), L-malate (circle), and L-lactate (triangle). The reaction was performed at 50 °C in 500 mM HEPES-NaOH buffer (pH 7.0) containing 100 mM $NaHCO_3$, 5.21 mM pyruvate, 0.5 mM $MnCl_2$, 2 mmol/L DTT, 15 mmol/L NH_4Cl, 0.01 g/L MDH, and 2.0 mM NADH. Values shown are means of triplicate determinations.

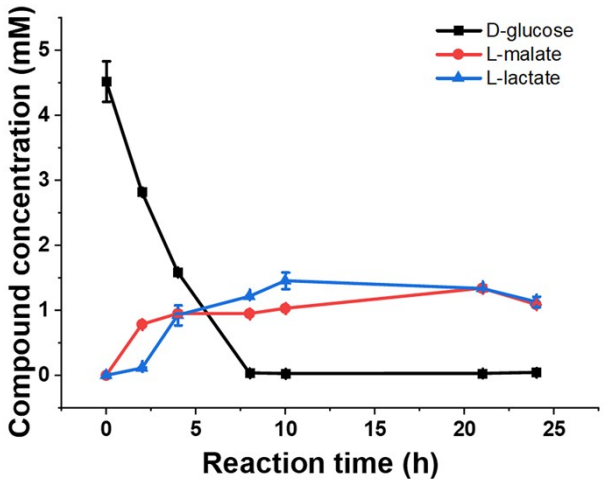

Fig. S5 The proof-of-concept experiment for the production of L-malate from D-glucose via HCO_3^- fixation by an ivSEB in the aerobic state. The concentration profiles of D-glucose (square), L-malate (circle), and L-lactate (triangle). The reaction was performed at 50 °C in the aerobic state in 100 mM HEPES-NaOH buffer (pH 7.0) containing 100 mM $NaHCO_3$, 4.5 mM D-glucose, 5 mM $MgCl_2$, 0.5 mM $MnCl_2$, 2 mM DTT, 50 mM NH_4Cl, 1.0 U/mL GDH, 1.0 U/mL DHAD, 1.0 U/mL KDGA, 1.0 U/mL ALDH, 1.0 U/mL MDH, and 2.0 mM NAD^+. Values shown are means of triplicate determinations.

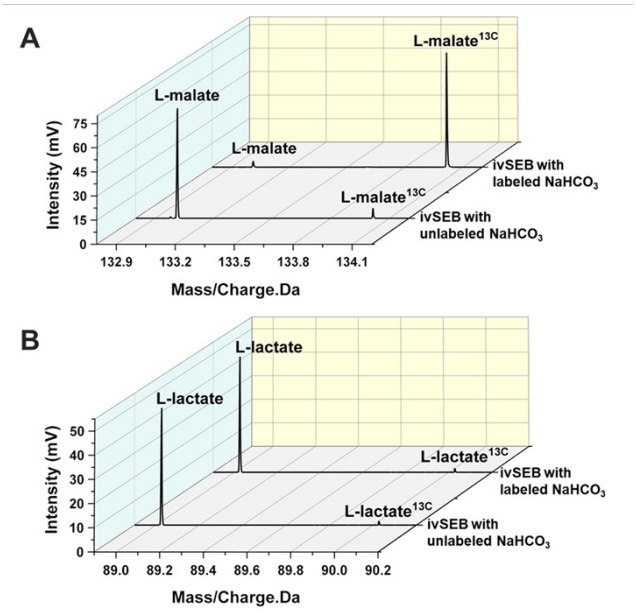

Fig. S6 High performance liquid chromatography-mass spectrometry (LC-MS) test confirmed ^{13}C-labeled products. (A) The LC-MS profiles of L-malate production from D-glucose with and without ^{13}C-labeled. (B) The LC-MS profiles of L-lactate production from D-glucose with and without ^{13}C-labeled. The reaction was performed in 100 mM HEPES-NaOH buffer (pH 7.0) at 50 °C containing 5 mM D-glucose, 100 mM $NaHCO_3$, 50 mM NH_4Cl, 2 mM DTT, 5 mM $MgCl_2$, 0.5 mM Mn^{2+}, 2.0 mM NAD^+, 1.0 U/mL GDH, 1.0 U/mL DHAD, 1.0 U/mL KDGA, 1.0 U/mL ALDH, and 1.0 U/mL MDH.