Supporting Information

Efficient glycine synthesis from CO_2-derived oxalate via an *in vitro* multi-enzyme cascade

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Calculation of glycine synthesis rate

The synthesis rates were calculated based on the following equation:

$$\text{Synthesis rate } (\text{mg L}^{-1}\,\text{h}^{-1}) = \frac{\text{C (mM/L)} \times \text{M } (g/mol)}{\text{t (h)}}$$

where:

C is the concentration of glycine determined by HPLC (mmol L^{-1}).

M is the molar mass of glycine (75.07 g mol^{-1}).

t is the reaction time (h).

For the 1 mL system:

Glycine concentration (C): 10 mM

Reaction time (t): 7 h

$$\text{Synthesis rate} = \frac{10\ \text{mM} \times 75.07\ \text{g/mol}}{7\ \text{h}} = 107.2\ \text{mg L}^{-1}\,\text{h}^{-1}$$

For the 10 mL system:

Glycine concentration (C): 9.57 mM

Reaction time (t): 4 h

$$\text{Synthesis rate} = \frac{9.57\ \text{mM} \times 75.07\ \text{g/mol}}{4\ \text{h}} = 179.6\ \text{mg L}^{-1}\,\text{h}^{-1}$$

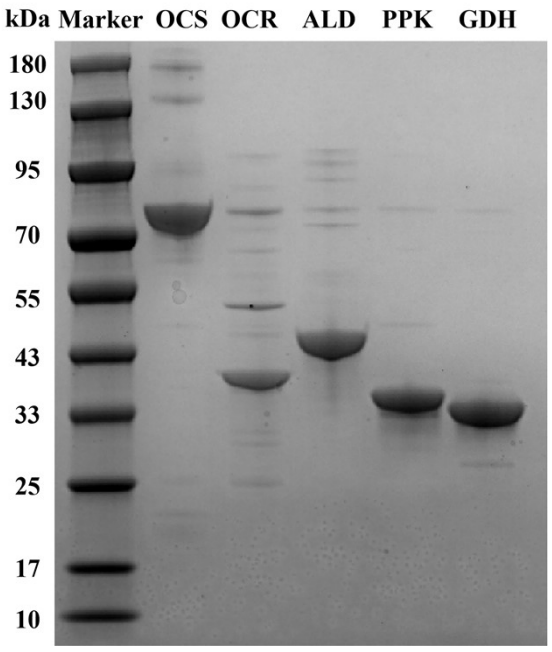

Fig. S1 SDS-PAGE analysis of the recombinant enzymes.

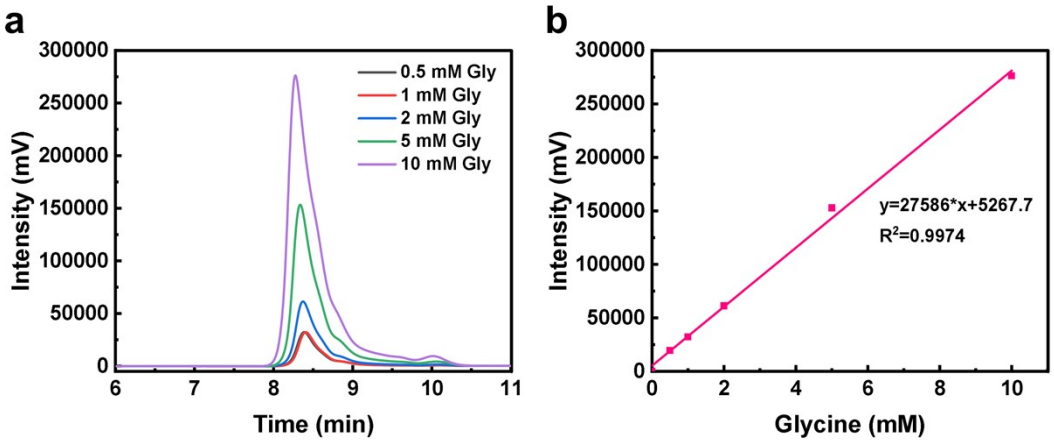

Fig. S2 Analysis of glycine by HPLC. (a) Representative HPLC chromatogram showing the detection peak of glycine. (b) Standard calibration curve for glycine quantification. This curve is constructed by plotting the relationship between peak height and glycine concentration.

Table S1. Information of enzymes used in this work.

Enzyme	EC Number	Source	Reaction	ΔG'° (kJ/mol)	K'_{eq}	Sp. act (U/mg)
Oxalyl-CoA synthetase (OCS)	6.2.1.8	*Lathyrus sativus L.*	Oxalate + ATP + CoA ⇌ Oxalyl-CoA + AMP + PPi	-28.9	1.2×10^5	0.39
Oxalyl-CoA reductase (OCR)	1.2.1.17	*Methylobacterium extorquens AM1*	Oxaly-CoA + NADPH + H^+ ⇌$NADP^+$ + Glyoxylate + CoA	19.9	3.2×10^{-4}	35.32 (reverse direction)
Alanine dehydrogenase (ALD)	1.4.1.10	*Mycobacterium tuberculosis*	Glyoxylate + NADH + NH_3 + H^+ ⇌ NAD^+ + Glycine + H_2O	-47.2	1.9×10^8	88.7
Polyphosphate kinase (PPK)	2.7.4.1	*Cytophaga hutchinsonii*	AMP + Poly P_n ⇌ ATP + Poly P_{n-2}	38.9	1.5×10^{-7}	28.64 (AMP→ADP) 15.98 (ADP→ATP)[1]
Glucose 1-dehydrogenase (GDH)	1.1.1.47	*Bacillus megaterium IAM 1030*	D-glucose + $NAD(P)^+$ ⇌ D-glucono-1,5-lactone + NAD(P)H + H^+	-2.8 (NAD^+) -4.8 ($NADP^+$)	3 (NAD^+) 7 ($NADP^+$)	1.54 (NAD^+) 0.77 ($NADP^+$)

Standard Gibbs free energy change of each step. All reactions were under conditions of pH 7.0 and an ionic strength of 0.1 M.

Table S2. List of sequences of the synthesized genes.

Gene	Sequence
sumo-ocs	ATGAGCGGCATGAGCGATAGCGAAGTGAACCAAGAAGCGAAGCCGGAAG TGAAACCGGAAGTGAAGCCGGAAACCCATATTAACCTGAAAGTGAGCGA TGGCAGCAGCGAAATTTTTTTTAAAATTAAAAAAACCACCCCGCTGCGCC GCCTGATGGAAGCGTTTGCGAAACGCCAAGGCAAAGAAATGGATAGCCT GCGCTTTCTGTATGATGGCATTCGCATTCAAGCGGATCAGACCCCGGAAG ATCTGGATATGGAAGATAACGATATTATTGAAGCGCATCGCGAACAGATT GGCGGCGAAATTGATGCGATGCTGCATCAGACCGGCGGCATGAGCAAAC ATCACCATCACAGTGGCCATCACCATACCGGCCATCATCACCACAGCGGC AGTCATCACCATAGCGGCAGTGCGGCCGGCGGCGAAGAAGATAAAAAAC CGGCGGGTGGCGAAGGCGGTGGCGCGCATATTAACCTGAAAGTGAAAGG CCAAGATGGCAACGAAGTGTTTTTTCGCATTAAACGCAGCACGCAGCTGA AAAAACTGATGAACGCGTATTGCGATCGTCAGAGCGTGGATATGACCGCG ATTGCGTTTCTGTTCGATGGCCGCCGCCTGCGCGCGGAACAGACCCCGGA TGAACTGGAAATGGAAGATGGCGATGAAATTGATGCGATGCTGCATCAG ACCGGCGGCGAAACCGCGACCACCCTGACCGGCCTGCTGCAGAGTGTGG CGAAAACCTTTCCGAGCCGCCGCGGCATTAGCCTGGCGGGCAAATTTGAT CTGACCCATAGCCATCTGAACGAACTGGTGGAAAGCGCGGCGGATCATCT GATTAGCGCGGGCATTAAACCGAACGATGTGGTGGCGCTGACCTTTCCGA ACACCGTGGAATATGTGATTCTGTTTCTGGCGGTGATTCGCGTGCGCGCG ACCGCGGCGCCGCTGAACGCGGCGTATACCGCGGAAGAATTTGAATTTTA TCTGAGCGATAGTGAAAGCAAACTGTTACTGACCCCGCTGGAAGGCAACA AACCGGCGCAAGATGCGGCGAGCAAACTGAGCATTCCGCTGGGCAGCGC GAGTTTAACCAAAAGCGAAGAGGAAACCAAACTGACCATTAGCCTGAAA CATCCGGAAAGCGGCCTGAAAAGCGATAGCGTGAACAGCGTGGCGAAAC TGATCAACGAACCGAGCGATGTGGCGCTGTTTCTGCATACGAGCGGCACC ACGAGCCGCCCGAAAGGCGTGCCGCTGACGCAGCATAACCTGGTGAGCA GCGTGAAAAACATTCAGAGCGTGTATCAGCTGACCGAAAGCGATAGCAC CGTTATTGTGCTGCCGCTGTTTCATGTGCATGGTCTGATTGCGGGCCTGCT GAGCAGCCTGGGCAGCGGCGCGGCCGTGGTTCTGCCGGCGGCGGGCCGC TTTAGCGCGAGCACCTTTTGGAAAGATATGATTCAGTATAACGCGACCTG GTATACCGCGGTGCCGACCATTCATCAGATTATTCTGGATCGCCATCTGA ACAACCCGGAACCGGCGTATCCGAAACTGCGCTTTATTCGCAGCTGTAGC GCGAGCCTGGCGCCGGTGATTCTGGGCCGCCTGGAAGAAAGCTTTGGCGC GCCGGTGCTGGAAGCCTACGCGATGACCGAAGCGAGCCATCTGATGAGC AGCAACCCGCTGCCGCAGAACGGCCCGCATAAAGCGGGCAGCGTGGGCA AACCGGTGGGCCAAGAAATGGCGATTCTGGATGAAAGCGGCCGCGTTCT GGAAGCGGGCGTGAACGGCGAAGTGTGCATTCGCGGCGAAAACGTGACC AAAGGCTATAAAAACAACGAAGCGGCGAACACCGCGGCGTTTCTGTTTG GCTGGTTTCATACCGGCGATATTGGCTATTTTGATAGCGATGGCTATCTGC ATCTGGTGGGCCGCATTAAAGAACTGATTAACCGCGGCGGCGAAAAAATT AGCCCGATTGAAGTGGATGCGGTGCTGCTGAGCCATCCGGATGTGGCGCA AGCGGTGGCGTTTGGCATTCCGGATCAGAAATATGGCGAAGAAATTCATT

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References

1 B. P. Nocek, A. N. Khusnutdinova, M. Ruszkowski, R. Flick, M. Burda, K. Batyrova, G. Brown, A. Mucha, A. Joachimiak, L. Berlicki and A. F. Yakunin, *ACS Catal.*, 2018, **8**, 10746-10760.