SUPPORTING INFORMATION

Glyoxylate carboligase: a unique thiamin diphosphate-dependent enzyme that can cycle between the 4'-aminopyrimidinium and the 1',4'-iminopyrimidine tautomeric forms in the absence of the conserved glutamate.

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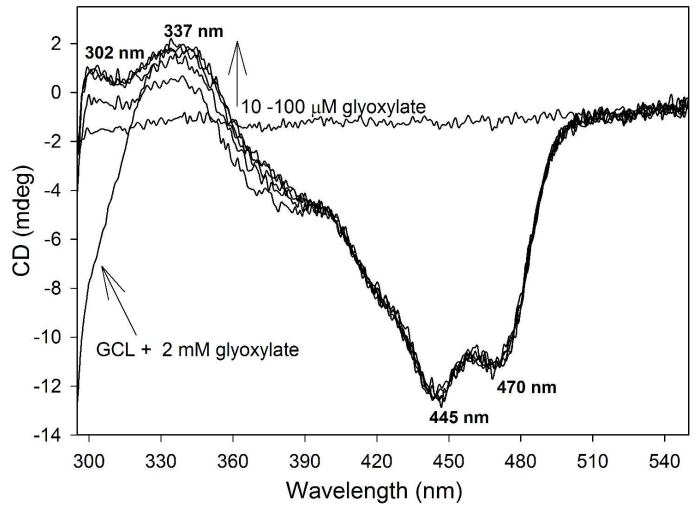
Running title: Stabilization of a rare thiamin tautomer on glyoxylate carboligase

Figure S1. Reaction of GCL with glyoxylate monitored by CD. (Top) Near-UV CD spectra of GCL titrated by glyoxylate. The GCL (1.5 mg/mL, concentration of active centers = 23 μM) in 0.1 M KH_2PO_4 (pH 7.6) containing 0.5 mM ThDP, 2.5 mM $MgCl_2$, 1.0 mM DTT, 10 μM FAD and 1% glycerol was titrated with glyoxylate (0.010–2 mM). Spectra were recorded at 5 ^oC. (Bottom) Time course of 1',4'-iminoglycolylThDP formation on GCL. The GCL (46 μM concentration of active centers) in the buffer as in Top in one syringe was mixed at 6 ^oC with an equal volume of 20 μM glyoxylate in the second syringe. The data points were collected for 20 s and were fitted to a double exponential equation [$CD_{302-304}$ (t) = $CD_1e^{-k_1t}$ + CD_2 e^{-k_2t} + c].

Figure S2. Time-dependent formation of (*S*)-acetolactate by I393A GCL. The I393A GCL (2.5 mg/ml, concentration of active centers = 38.6 μM) in 0.1 M KH_2PO_4 (pH 7.6) containing 5 mM $MgCl_2$ and 0.5 mM ThDP was titrated by pyruvate (0.020 - 4.0 mM) at 4 ^oC. On addition of 4 mM pyruvate, temperature was raised to 25 ^oC and time–dependent formation of (S)-acetolactate was recorded. Inset: CD spectrum of (*S*)-acetolactate after protein was removed from the reaction mixture using Ultracel-30K Centrifugal Filter Unit (Millipore).

Figure S3. Time-course of acetolactate-ThDP complex formation on V51D/I393A GCL. The V51D/I393A GCL (7.0 mg/mL, concentration of active centers=108 μM) in 0.1 M KH_2PO_4 (pH 7.6) containing 0.5 mM ThDP, 2.5 mM $MgCl_2$, 1.0 mM DTT, 10 μM FAD and 1% glycerol in one syringe was mixed with 1.2 mM pyruvate at 6 ^oC in the second syringe. The reaction was monitored for 50 s and data points were fitted to a double exponential as in Figure S1.

Figure S1.


337 nm
302 nm
10 -100 μM glyoxylate
GCL + 2 mM glyoxylate
470 nm
445 nm
CD (mdeg)
Wavelength (nm)

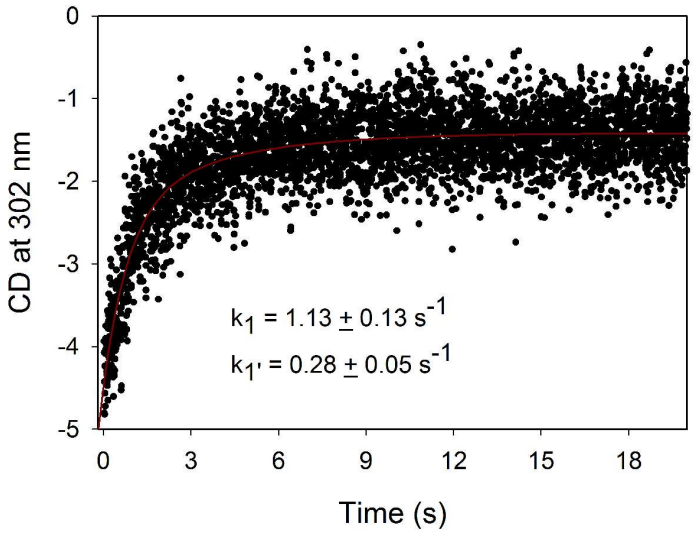

CD at 302 nm
k_1 = 1.13 ± 0.13 s^{-1}
$k_{1'}$ = 0.28 ± 0.05 s^{-1}
Time (s)

Figure S2.

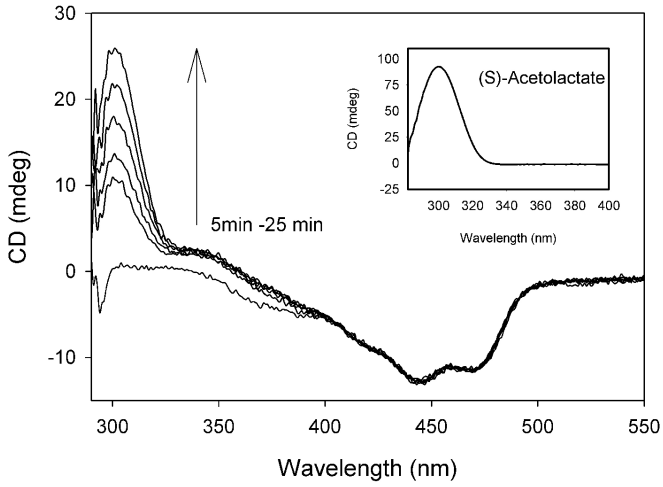
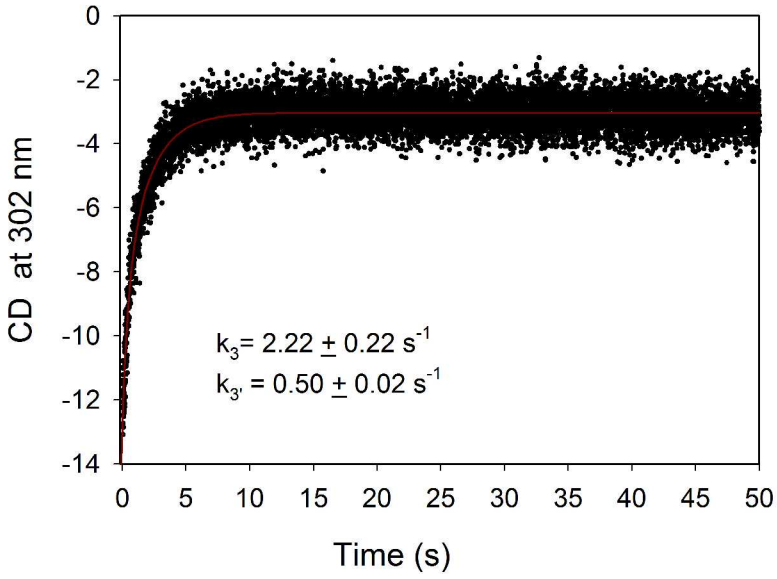

30
20
10
0
-10
CD (mdeg)
5min -25 min
300
350
400
450
500
550
Wavelength (nm)
100
75
50
25
0
-25
CD (mdeg)
(S)-Acetolactate
300 320 340 360 380 400
Wavelength (nm)

Figure S3.


0
-2
-4
-6
-8
-10
-12
-14
CD at 302 nm
0
5
10
15
20
25
30
35
40
45
50
Time (s)
k3= 2.22 ± 0.22 s-1
k3' = 0.50 ± 0.02 s-1