

J7322-M1

Table S1. Specific Velocities $v/[enzyme]_0$ for the Decarboxylation of Pyruvate with Catalysis by ZMPDC and by SCPDC as a Function of Pyruvate Concentration

[pyruvate], mM	SCPDC			[pyruvate], mM	ZMPDC		
	specific velocity, s^{-1}				specific velocity, s^{-1}		
0.20	7.37	6.22	7.33	0.10	53.4	60.9	59.3
0.40	20.4	20.8	20.3	0.20	97.6	97.7	101
0.60	38.3	38.0	38.2	0.40	159	163	158
0.80	53.4	51.3	51.7	0.60	209	212	198
1.00	74.7	71.0	76.6	0.80	236	224	229
1.40	106	106	108	1.00	277	263	260
2.00	143	137	137	2.00	330	329	342
3.00	190	195	191	3.00	380	384	369
4.00	223	216	224	4.00	399	396	391
6.00	240	239	239	7.00	406	427	408
8.00	266	260	264	10.0	432	420	440
10.0	270	273	267	20.0	403	427	414
15.0	274	268	269	30.0	423	429	402
20.0	285	287	277				
30.0	282	279	280				

Table S2. Initial rate for decarboxylation of pyruvate v_{12} and $^{13}\text{C}_1$ -labeled pyruvate v_{13} with catalysis by ZMPDC as a function of pyruvate concentration at pH 6.2, 0.1 M citrate buffer, temperature = 30.0 °C.

[pyruvate], mM	v_{12} , 10^4 dA/sec	v_{13} , 10^4 dA/sec
0.40 [ZMPDC = 0.116 U/ml]	21.146, 21.695, 22.194, 21.289, 21.275	20.978, 21.371, 21.318, 21.344, 21.246
0.80 [ZMPDC = 0.058 U/ml]	15.153, 15.152, 15.291, 15.170, 15.560	15.099, 14.822, 15.138, 14.937, 15.100
4.00 [ZMPDC = 0.058 U/ml]	25.403, 26.746, 26.254, 26.282, 26.000, 26.151, 25.969	25.612, 25.464, 26.183, 25.400, 25.539, 25.809, 26.232
10.0 [ZMPDC = 0.058 U/ml]	27.311, 28.157, 27.969, 27.697, 27.554, 28.417, 28.107, 28.821	28.643, 27.119, 27.756, 27.112, 27.022, 27.731, 27.423, 26.822
15.0 [ZMPDC = 0.058 U/ml]	26.482, 27.010, 26.276, 26.363, 26.482, 26.575	25.968, 25.693, 25.705, 26.297, 25.929, 26.586

Table S3.^a Rate constants, standard-state free energies of activation, free energies of reactant and transition states relative to the apoenzyme assembly, and free energies of reactant and transition state stabilization for the enzymic and non-enzymic decarboxylation of pyruvate at 298 K and pH 6.2.

Observed rate constants	Standard-state rate constants, s ⁻¹	ΔG^\ddagger , kJ/mol	Transition state or intermediate	G _{rel} , kJ/mol
Non-enzymic reactions				
Deprotonation of ThDP 4.3x10 ⁶ M ⁻¹ s ⁻¹	6.8x10 ⁻²	79.6	Deprotonation	79.6
Protonation of anion 1.7x10 ¹⁰ s ⁻¹	1.7x10 ¹⁰	14.6	Anion	65.0
Specific-base catalyzed addition of ThDP to pyruvate 1.3 M ⁻² s ⁻¹	2.1x10 ⁻¹¹	133.9	Addition	133.9
Specific-base catalyzed reversion of adduct 1.3 M ⁻¹ s ⁻¹	2.1x10 ⁻⁸	116.8	α -Lactylthiamin	17.1
Decarboxylation of α -Lactylthiamin 5.0x10 ⁻⁵ s ⁻¹	5.0x10 ⁻⁵	97.5	Decarboxylation	114.6
Product release ^b 6.0x10 ⁻⁸ s ⁻¹	6.0x10 ⁻⁸	114.2	Product release	131.3
Enzymic reaction, SCPDC				
Binding of ThDP K _d = 23 μ M			E:ThDP assembly	-9.4
Reversible binding of pyruvate into the regulatory site K _d = 8 mM			SE	-4.2
Activation of SE to SE* 0.49 s ⁻¹	0.49	74.7	Activation	70.5
Deactivation from SE* to SE 0.033 s ⁻¹	0.033	81.4	Activated enzyme, SE*	-10.9
Overall binding of pyruvate into the catalytic site 8.2x10 ⁴ M ⁻¹ s ⁻¹	82	62.0	Substrate binding	51.1
Overall release of pyruvate from the catalytic site 120 s ⁻¹	120	61.1	E: α -Lactyl-ThDP assembly	-10.0

Decarboxylation 640 s ⁻¹	640	57.0	Decarboxylation	47.0
Product release	640	57.0	Product release	47.0
Enzymic reaction, ZMPDC				
Binding of ThDP ¹³ 0.353 μM			E:ThDP	-19.7
Overall binding of pyruvate into the catalytic site 8.0x10 ⁵ M ⁻¹ s ⁻¹	800	56.4	Substrate binding	36.7
Overall release of pyruvate from the catalytic site 300 s ⁻¹	300	58.8	E:α-Lactyl-ThDP assembly	-22.1
Decarboxylation 1200 s ⁻¹	1200	55.4	Decarboxylation	33.3
Product release 750 s ⁻¹	750	56.6	Product release	34.5

^a The data for non-enzymic reactions and enzymic reaction of SCPDC were taken from Table 1 in reference 4. ^b Taking $1.2 \pm 0.1 \times 10^{-4} \text{ s}^{-1}$ as first order rate constant for product release at pH 9.5 from the work of Kluger, R.; Lam, J. R.; Kim, C-S.; *Bioorganic Chemistry* **1993**, *21*, 275-283, in which benzaldehyde release from its adduct with thiamin was studied, and adjusting the pH to 6.2 (specific base catalysis). Acetaldehyde release will occur at a different rate from benzaldehyde release, so this estimate is only a rough approximation.