SUPPORTING INFORMATION

Enzyme Architecture: Breaking Down the Catalytic Cage that Activates Orotidine 5'-Monophosphate Decarboxylase for Catalysis.

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RESULTS

The kinetic parameters from Figure 2A were determined using the Michaelis-Menten equation, with the assumption that [OMP] >> [E•OMP], where [OMP] and [E•OMP] are the concentration of free OMP and the Michaelis complex, respectively. Table S1 shows the values of [E•OMP] calculated for Figure 2A using the kinetic parameters obtained from the fit of the kinetic data to the Michaelis-Menten equation, and the initial enzyme concentration $[E]_o$ and substrate concentration $[OMP]_o$. Table S1 shows for all cases that [E•OMP] is < 10 % of $[OMP]_o$. The uncertainty in the derived kinetic parameter (k_{cat}/K_m) introduced by the accumulation of E•OMP is along the lines of that introduced into steady-state kinetic treatments by the small decrease in initial velocity observed over the first *ca* 10% reaction of substrate.

Table S1. Comparison of initial substrate concentrations $[S]_0$ with the concentration of substrate present after correction for the formation of Michaelis complex: $[S] = ([S]_0 - [ES])$.

Mutant OMPDC	$[E]_o$ mM	$[OMP]_o$ mM	$(v/[E]_o)/10^{-4}$ s^{-1}	$(v/(v_{max})$	[E•OMP] (mM)[a]	$[S]_0 - [E•OMP]$ mM
S154A/R235A $(v_{max}/[E]) = k_{cat} = 0.54 \times 10^{-4}$ s^{-1} $K_m = 0.0020$ M	0.20	0.50	0.109	0.20	0.040	0.46
	0.20	1.0	0.17	0.31	0.062	0.94
	0.20	2.0	0.22	0.41	0.081	1.92
	0.20	3.0	0.29	0.54	0.11	2.89
S154A/Q215A/Y217F $(v_{max}/[E]) = k_{cat} = 4.2 \times 10^{-4}$ s^{-1} $K_m = 0.0015$ M	0.10	0.50	1.07	0.25	0.025	0.475
	0.10	1.0	1.94	0.46	0.046	0.95
	0.10	2.0	2.76	0.66	0.066	1.93
	0.10	3.0	3.39	0.81	0.081	2.92

[a] $[E•OMP] = [(v/(v_{max})][E]_o$, where v_{max} is the observed activity when $[E•OMP] = [E]_o$.

Figure S1 shows the linear plot of v/[E] against [FOMP] for *Ec*OMPDC-catalyzed decarboxylation of FOMP, with slope of k_{cat}/K_m = 2.7 x 10^6 M^{-1} s^{-1}. Figures S2A–D shows time-courses for the change in absorbance at 282 nm for decarboxylation of FOMP catalyzed by the S154A/Y217F (Figure S2A), S154A/R235A (Figure S2B), S154A/Q215A/Y217F (Figure S2C) and the S154A/Q215A/R235A (Figure S2D) mutants of *Sc*OMPDC. The solid lines show the fit of the experimental data for each Figure to the exponential decay for the pseudo first-order enzyme-catalyzed reaction of FOMP, to give the observed first order rate constant k_{obsd} (s^{-1}) for OMPDC-catalyzed decarboxylation. In each case the data are fit by a simple exponential decay, which shows that the reactions are first order in [FOMP] and that the starting concentration [FOMP] = 0.06–0.15 mM << K_m for the OMPDC-catalyzed decarboxylation reaction. Each of the experiments from Figures S2 were repeated at least two times, and the values of k_{obsd}/[E] for a given mutant *Sc*OMPDC-catalyzed decarboxylation were reproducible to better than ±10%.

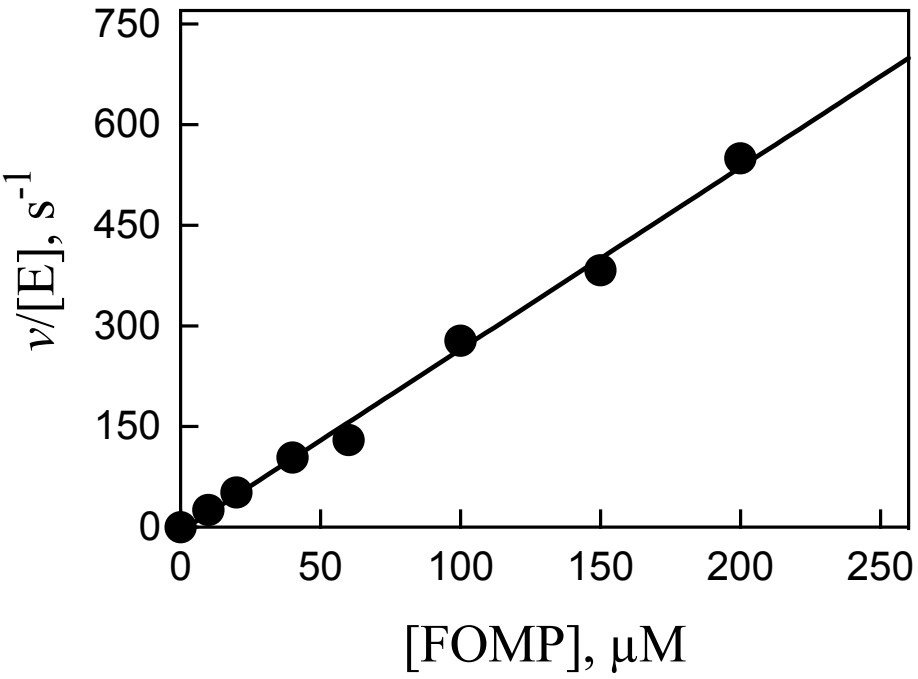

Figure S1. Dependence of $v/[E]$ (s^{-1}) for decarboxylation of FOMP catalyzed by wild-type *Ec*OMPDC on the concentration of FOMP for reactions at 25 °C, pH 7.1 (10 mM MOPS) and at $I = 0.105$ (NaCl).

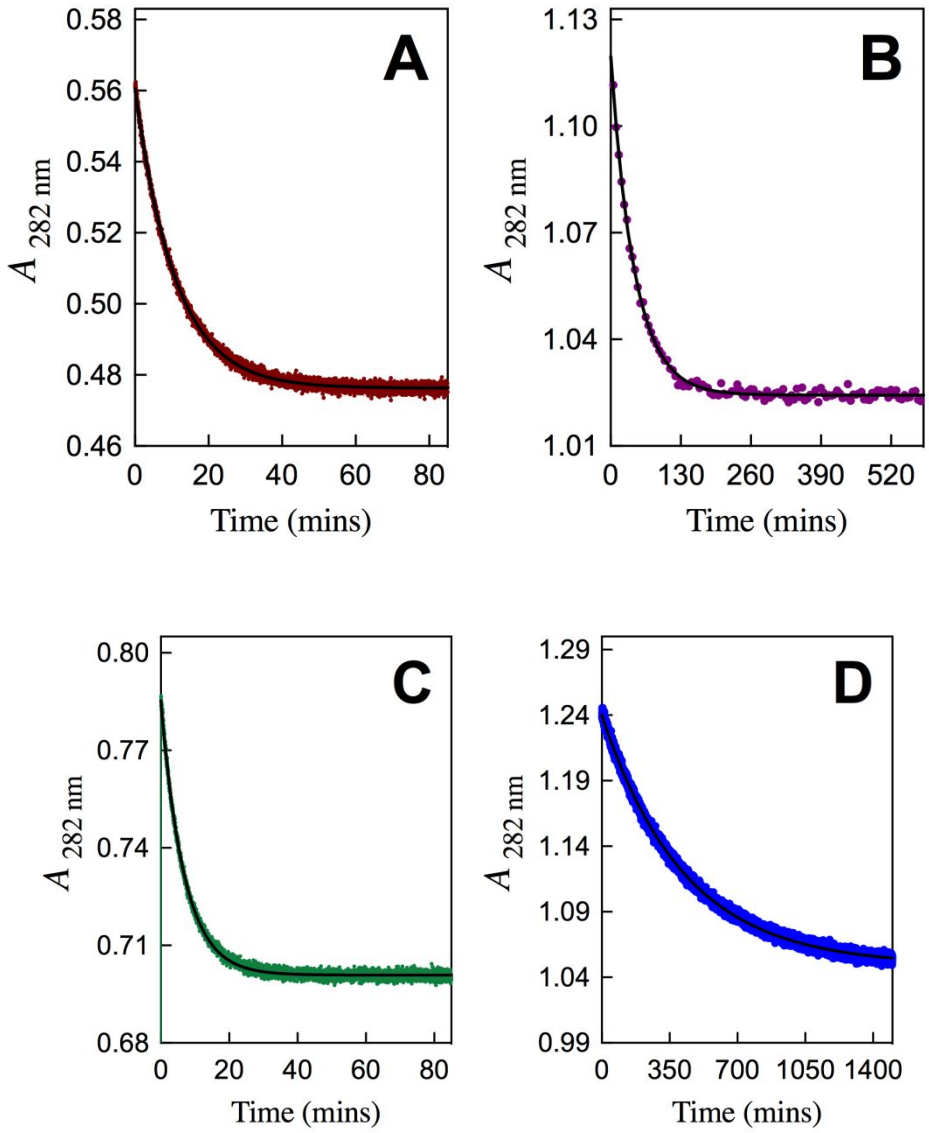

Figure S2. Full time-course for the reaction of FOMP catalyzed by mutants of *Sc*OMPDC for reactions at 25 °C, pH 7.1 (10 mM MOPS) and at I = 0.105 (NaCl). Figure S1A: decarboxylation of 60 μM FOMP catalyzed by 3.0 μM of the S154A/Y217F mutant of *Sc*OMPDC. Figure S1B: decarboxylation of 60 μM FOMP catalyzed by 0.02 mM of the S154A/R235A mutant of *Sc*OMPDC. Figure S1C: decarboxylation of 60 μM FOMP catalyzed by 0.01 mM of the S154A/Q215A/Y217F mutant of *Sc*OMPDC. Figure S1D: decarboxylation of 150 μM FOMP catalyzed by 6 μM of the S154A/Q215A/R235A mutant of *Sc*OMPDC.