Supplementary Information

Structural and kinetic studies on native intermediates and an intermediate analogue in benzoylformate decarboxylase reveal a least-motion mechanism with an unprecedented short-lived pre-decarboxylation intermediate

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Supp. Fig. 1

Initial $F_o - F_c$ omit map (top row, contoured at 3σ) and simulated-annealing $2F_o - F_c$ omit map (bottom row, contoured at 1σ) of the PMThDP adduct bound to BFDC from *P. putida*.

The electron density maps were calculated from model phases of a model prior to inclusion of the PMThDP atoms. For calculation of the simulated-annealing omit map, a slow cooling protocol (1000 K start temperature, 25 K cooling per cycle, 0.004 ps time steps, 6 steps per cycle) with torsion angle dynamics was applied using CNS (1).

- 1.) Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination, *Acta Crystallographica Section D-Biological Crystallography* 54, 905-921.

Supp. Fig. 2

Circular dichroism spectra obtained after addition of increasing amounts of substrate analog methylbenzoylphosphonate to 2 mg/mL BFDC in 50 mM potassium phosphate buffer, pH 6.5 supplemented with 2.5 mM MgSO_4 and 200 μM ThDP at 20 $^{\circ}\text{C}$.

Supp. Fig. 3

Kinetics of PMThDP formation after addition of 2.5 mM MBP to 7.5 mg/mL in 50 mM potassium phosphate buffer, pH 6.5 with 2.5 mM MgSO_4 at 30 °C as analyzed by ^1H NMR spectroscopy after acid quench isolation of reaction intermediates.

(A) Sections of selected ^1H NMR spectra showing the 2'- CH_3 and 4- CH_3 signals of quench-isolated ThDP and PMThDP after different reaction times.

(B) The kinetic analysis reveals a pseudo-first-order rate constant of $k_{\text{obs}} = 3.1 (\pm 0.7) \text{ s}^{-1}$ being in fair agreement with the stopped-flow results (forward rate constant of PMThDP formation $k_{+2} = 2.33 \pm 0.17 \text{ s}^{-1}$).

Supp. Fig.
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Stereo view of the X-ray structure of PMThDP trapped in the active site of BFDC with selected amino acid side chains shown. The electron density is contoured at 1.0σ in a $2F_o - F_c$ map. For clarity, the electron density of the intermediate is depicted in red and that of the protein in blue. The electron density map was calculated from model phases of a model after inclusion of the MBP moiety of the PMThDP adduct.