Supporting Information

X-ray crystallography-based structural elucidation of enzyme-bound intermediates along the 1-deoxy-D-xylulose 5-phosphate synthase reaction coordinate

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Figure S1. Oxygenase activity of DXPS. Oxygenase activity of *Dr*DXPS (A) was confirmed by monitoring enzyme-and pyruvate-dependent consumption of O₂ (B). In the presence of saturating pyruvate (500 μ M) and *Dr*DXPS (5 μ M) at 25 °C, O₂ depletion is observed (v = 20.7 ± 0.9 μ M/min), indicating that O₂ contributes to pre-decarboxylation intermediate instability on *Dr*DXPS.

E1-PDH	1	SERFPNDVDPIETRDWLQAIESVIREEGVERAQYLIDQLLAEARKGGVNVAAGTGISNYINTIPVEEQPE	70
<i>Dr</i> DXPS	1	MNELPGTSDTPLLDQIHGPKDLK	23
<i>Ec</i> DXPS	1	MSFDIAKYPTLALVDSTQELR	21
E1-PDH	71	YPGNLELERRIRSAIRWNAIMTVLRASKKDLELGGHMASFQSSATIYDVCFNHFFRARNEQDGGDLVYFQ	140
<i>Dr</i> DXPS	24	R-LSREQLPALTEELRGEIVRVCSRGGLHLASSLGAVDI-ITALHYVLDSPRDRILFDV	80
<i>Ec</i> DXPS	22	L-LPKESLPKLCDELRRYLLDSVSRSSGHFASGLGTVEL-TVALHYVYNTPFDQLIWDV	78
E1-PDH	141	GHISPGVYARAFLEGRLTQEQLDNFRQEVHGNGLSSYPHPKLMPEFWQFPTVSMGLGPIGAIYQAKFLKY	210
<i>Dr</i> DXPS	81	GHQAYAHKILTGRRDQMADIKKEGGISGFTKVSESE-HDAITVGHASTSLANALGMALARDA	141
<i>Ec</i> DXPS	79	GHQAYPHKILTGRRDKIGTIRQKGGLHPFPWRGESE-YDVLSVGHSSTSISAGIGIAVAAEK	139
E1-PDH	211	LEHRGLKDTSKQTVYAFLGDGEMDEPESKGAITIATREKLDNLVFVINCNLQRLDGPVTGNGKIINEL	278
<i>Dr</i> DXPS	142	QGKDFHVAAVIGDGSLTGGMALAALNTIGDMGR-KMLIVLNDNEMSISENVGAMNKFMRGLQV	203
<i>Ec</i> DXPS	140	EGKNRRTVCVIGDGAITAGMAFEAMNHAGDIRP-DMLVVLNDNEMSISENVGALNNHLAQLLS	201
E1-PDH	279	EGIFEGAGWNVIKVMWGSRWDELLRKDTSGKLIQLMNETVDGDYQTFKSKDGAYVREHFF	338
<i>Dr</i> DXPS	204	QKWFQEGEGAGKKAVEAVSK-PLADFMSRAKNSTRHFFDPASVNPFAAMGVRYV	256
<i>Ec</i> DXPS	202	GKLYSSLREGGKKVFSGVPPIKELLKRTEEHIKGMVVPGTLFEELGFNYI	251
E1-PDH	339	GKYPETAALVADWTDEQIWALNRGGHDPKKIYAAFKKAQETKGKATVILAHTIKGYGMGDAAEGKNIAHQ	408
<i>Dr</i> DXPS	257	GPVDGHNVQELVWLLERLVDLD-GPTILHIVTTKGKGLSYAE	297
<i>Ec</i> DXPS	252	GPVDGHDVLGLITTLKNMRDLK-GPQFLHIMTKKGRGYEPAE	292
E1-PDH <i>Dr</i> DXPS <i>Ec</i> DXPS	409 298 293	VKKMNMDGVRHIRDRFNVPVSDADIEKLPYITFPEGSEEHTYLHAQRQKLHGYLPSRQPNFTEKLELPSL ADPIYWHGPAKFDPATGE	478 315 310
E1-PDH <i>Dr</i> DXPS <i>Ec</i> DXPS	479 316 311	QDFGALLEEQSKEISTTIAFVRALNVMLKNKSIKDRLVPIIADEARTFGMEGLFRQIGIYSPNGQQYTPQ YVPSSAYSWSAAFGEAVTEWAKTDPRTFVVTPAMREGSGLVEF	548 358 355
E1-PDH	549	DREQVAYYKEDEKGQILQEGINELGAGCSWLAAATSYSTNNLPMIPFYIYYSMFGFQRIGDLCWAAGDQQ	618
<i>Dr</i> DXPS	359	SRVHPHRYLDVGIAEEVAVTTAAGMALQGMRPVVAIYSTF-LQRAYDQVLHDVAIE	413
<i>Ec</i> DXPS	356	SRKFPDRYFDVAIAEQHAVTFAAGLAIGGYKPIVAIYSTF-LQRAYDQVLHDVAIQ	410
E1-PDH	619	ARGFLIGGTSGRTTLNGEGLQHEDGHSHIQSLTIPNCISYDPAYAYEVAVIMHDGLERMYGEKQENVYYY	688
<i>Dr</i> DXPS	414	HLNVTFCIDRAGIV-GADGATHNGVFDLSFLRSIPGVRIGLPKDAAELRGMLKYAQTHDGPFAIR	477
<i>EC</i> DXPS	411	KLPVLFAIDRAGIV-GADGQTHQGAFDLSYLRCIPEMVIMTPSDENECRQMLYTGYHYNDGPSAVR	475
E1-PDH	689	IT-TINENYHMPAMPEGAEEGIRKGIYKLETIEGSKGKVQLLGSGSILRHVREAAEILAKDYGVGSDVYS	757
<i>Dr</i> DXPS	478	YPRGNTAQVPAGTWPDLKWGEWERLKGGDDVVILAGGKALDYALKAAEDLPGVGVVN	534
<i>Ec</i> DXPS	476	YPRGNAVGVELTPLEKLPIGKGIVKCRGEKLAILNFGTLMPEAAKVAESLNATLVD	531
E1-PDH	758	VTSFTELARDGQDCERWNMLHPLETPRVPYIAQVMNDAPAVASTDYMKLFAEQVRTYVPADDYR	821
<i>Dr</i> DXPS	535	ARFVKPLDEEMLREVGGRARALITVEDNTVVGGFGGAVLEALNSMNLHPTVR	586
<i>Ec</i> DXPS	532	MRFVKPLDEALILEMAASHEALVTVEENAIMGGAGSGVNEVLMAHRKPVSVL	583
E1-PDH <i>Dr</i> DXPS <i>EC</i> DXPS	822 587 584	VLGTDG-FGRSDSRENLRHHFEVDASYVVVAALGELAKRGEIDKKVVADAIAKFNIDADKVNPRLA VLGIPDEFQEHATAESVHARAGIDAPAIRTVLAELGVDVPIEV	

Figure S2. Sequence alignment of *EcDXPS* (2018), *DrDXPS* (201X), and E1-PDH (2G25). PROMALS3D (1) was used for the alignment. Secondary structure is indicated below the corresponding sequence where helix and beta sheet are denoted by a cylinder or arrow, respectively.



Figure S3. Michaelis-Menten kinetic analysis of *Dr***DXPS.** Crystallization of *Dr*DXPS was conducted under anoxic conditions; thus, DXP-forming activity was confirmed under anoxic conditions. Michaelis-Menten analysis was conducted by varying pyruvate concentration (A) or varying D-GAP concentration (B). Similar to the kinetic parameters for other DXPS enzymes, $K_m^{\text{pyruvate}} = 54 \pm 3 \,\mu\text{M}$, $K_m^{\text{D-GAP}} = 11 \pm 2 \,\mu\text{M}$, and $k_{\text{cat}} = 45 \pm 2 \,\min^{-1}$ for *Dr*DXPS. Error represents standard error, n = 3 for K_m and n = 6 for k_{cat} .



Figure S4. Morrison curves for (A) MAP and (B) BAP inhibition of DXP formation on *Dr*DXPS under anoxic conditions



Figure S5. IC₅₀ curves for (A-B) MAP and (C-D) BAP inhibition of DXP formation by *Dr*DXPS under anoxic conditions. Concentrations in μ M were used for logarithm calculation. IC₅₀ curves in panels (A) and (C) were conducted in the presence of $2 \times K_m^{pyruvate}$, whereas curves in panel (B) and (D) were conducted in the presence of $10 \times K_m^{pyruvate}$.



Figure S6. Determination of MOI of (A) MAP and (B) BAP against *Dr*DXPS. A positive slope indicates increasing IC_{50} with increasing [pyruvate] suggesting MAP and BAP are competitive with respect to pyruvate as expected. Error represents standard deviation, n = 3



Figure S7. Accumulation of LThDP on *Dr***DXPS.** The CD signal increases with the concentration of pyruvate (12.5, 25, 37.5, 50, 75, 100, 150, 200, 300, 400, and 500 μ M), indicating the formation of the stable LThDP intermediate on *Dr*DXPS (50 μ M).



Figure S8. Composite omit electron density maps of ThDP-intermediate states captured in this study. (A) Composite omit map contoured to 1.0σ in blue mesh for PLThDP; (B) Composite omit map contoured to 1.0σ in blue mesh for the enamine. The structures are colored in the same color scheme as Fig. 2. Tyr395, Arg423 and Arg480, which have been implicated in D-GAP binding (2), are labeled.



Figure S9. Composite omit electron density maps of PLThDP-bound E1-PDH (PDB ID: 2G25). (3) Composite omit map contoured to 1.0σ in blue mesh for PLThDP. The composite omit map was calculated using Phenix (4) with the dataset deposited on PDB. The structures are colored in the same color scheme as Fig. 3B. Key active site residues are labeled.



Figure S10. The active site of PLThDP-bound *Dr*DXPS with modeled D-GAP. The active site of *Dr*DXPS is large enough to bind PLThDP and D-GAP without clashes. The coordinates of D-GAP were reproduced from previous docking analysis (5). His51, His304, and His434, three predicted PLThDP-binding residues, are shown as sticks. Tyr395, Arg423 and Arg480, which have been implicated in D-GAP binding (2), are also shown as sticks.



Figure S11. SDS-PAGE analysis of *Dr***DXPS cocrystallized with pyruvate.** Lane 1, molecular weight markers (in kDa); Lanes 2, purified sample; Lane 3, crystallization drops; Lane 4, *Dr*DXPS crystals. *Dr*DXPS crystals were washed in 500 nL of well solution before harvested for SAS-PAGE analysis. *Dr*DXPS crystals show no sign of proteolysis.



Figure S12. The composite omit electron density maps for the spoon motif in the structure of DXPS in the presence of (A) PLThDP and (B) the enamine. Composite omit maps are contoured to 1.0σ in blue meshes for the spoon motif. The continuous electron density validates the assigned conformations for the spoon motif in both structures. The structures are colored in the same color scheme as Fig. 5A.



Figure S13. Active site rearrangements in *Dr*DXPS. The structure of *Dr*DXPS in the presence of PLThDP is overlaid with the structure of *Dr*DXPS cocrystallized with pyruvate. The spoon motif (orange) is shifted by 17 Å between two structures, and Ala307 is shifted by 13.1 Å. Tyr395, Arg423 and Arg480, which have been implicated in D-GAP binding (2), are labeled. Cas of Gly290, Gly292, and Ala307 (if present in structures) are shown as spheres to highlight the conformational changes.

Table S1. Summary of kinetic parameters for DXP formation and inhibitory constants $(K_i^{MAP} \text{ and } K_i^{BAP})$ for *Dr*DXPS and *Ec*DXPS (6,7) under anoxic conditions. Error represents standard error, n = 3 for K_m and K_i ; n = 6 for k_{cat} .

Enzyme	$K_{\rm m}^{\rm D-GAP}$ (μM)	$K_{\rm m}^{\rm pyruvate}(\mu{ m M})$	$k_{\rm cat}({\rm min}^{-1})$	$K_{i}^{MAP}(\mu M)$	$K_{i}^{BAP}(\mu M)$
Dr DXPS	11 ± 2	54 ± 3	45 ± 2	2.8 ± 0.2	5.7 ± 0.5
<i>Ec</i> DXPS	6 ± 2^{a}	20.77 ± 0.01^{a}	92 ± 5^{a}	3.24 ± 0.02^{b}	5.6 ± 0.8^{b}

^aData from DeColli, A. A., Nemeria, N. S., Majumdar, A., Gerfen, G. J., Jordan, F., and Freel Meyers, C. L. (2018) Oxidative decarboxylation of pyruvate by 1-deoxy-D-xyulose 5-phosphate synthase, a central metabolic enzyme in bacteria. *J. Biol. Chem.* **293**, 10857-10869

^bData from Smith, J. M., Vierling, R. J., and Freel Meyers, C. L. (2012) Selective inhibition of *E. coli* 1deoxy-D-xylulose-5-phosphate synthase by acetylphosphonates. *MedChemComm* **3**, 65-67

	DrDXPS	DrDXPS
	with MAP bound	with enamine bound
PDB ID	60UV	60UW
Beamline	APS 24-ID-C	APS 24-ID-C
Space group	P2 ₁ 2 ₁ 2 ₁	C2
Cell dimensions (A)	a = 78.50, b = 125.28, c = 151.70	a = 129.75, b = 85.54, $c = 60.57, B = 114, 77^{\circ}$
Wavelength (Å)	0 9791	c = 00.57, p = 114.77 0 9791
Resolution $(\mathbf{A})^{\dagger}$	100-1.94(2.01-1.94)	50 -2 40 (2 49-2 40)
# unique reflections	110177	23154
π unique reflections	98.6(92.3)	98.2(92.2)
Redundancy [†]	66(57)	(92.2)
$< I/\sigma I > \dagger$	22 A (2 A)	7.8(2.7)
$\sim 1/01 \sim$	22.4(2.4)	7.8(2.7)
K _{sym}	(0.806)	(0.832)
$CC_{1/2}$	(0.800)	(0.855)
Resolution (Å)	96.6-1.94	49.4-2.40
# unique reflections	110075	23132
R_{work} (%) / R_{free} (%)	15.6/17.4	15.8/19.6
RMS bond lengths (Å)	0.003	0.003
RMS bond angles (°)	0.62	0.59
Number of		
Atoms/Molecules		
Protein atoms	8985	4093
PLThDP	2	0
enamine	0	1
Water molecules	607	114
Na ⁺	2	1
Average B-factor ($Å^2$)	44.8	54.4
Protein atoms	44.9	54.6
PLThDP	36.0	-
enamine	-	56.2
Water molecules	45.3	47.3
Na ⁺	33.5	52.7
Ramachandran plot		
Favored (%)	96.99	97.03
Allowed (%)	3.01	2.97
Outliers (%)	0.00	0.00
Rotamer outliers (%)	0.54	1.19

Table S2. Data collection and model refinement statistics for structures of *Dr*DXPS.

[†]Values in parentheses indicate the highest-resolution bin.

Table S3. Residues and cofactors modeled in each chain	(1-629) of both structures.
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	PLThDP-bound <i>Dr</i> DXPS	Enamine-bound DrDXPS
A	8-208, 217-224, 244-629 1 PLThDP, 1 Na ⁺	7-185, 247-291, 307-626 1 enamine, 1 Na ⁺
B	8-200, 244-626 1 PLThDP, 1 Na ⁺	N/A

Table S4. Comparisons of model refinement statistics among the structure of *Dr*DXPS with MAP bound and two DXPS structures published before this work.

	PLThDP-bound	DrDXPS	Proteolyzed
PDB ID	60UV	201X (3)	201S (3)
Resolution (A)	96.60-1.94	29.25-2.90	29.77-2.40
Clash score	1.66	13	19
R_{work} (%) / R_{free} (%)	15.6/17.4	20.9/27.2	19.1/23.4
Ramachandran outliers (%)	0.00	1.7	0.4
Rotamer outliers (%)	0.54	9.7	4.3

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