

SUPPORTING INFORMATION

2-Aminoacrylate stress damages diverse PLP-dependent enzymes *in vivo*

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Table S1

Figure S1

Figure S2

Figure S3

Literature Cited.

1. McKinney, J., et al., *Tightly regulated gene expression system in Salmonella enterica serovar Typhimurium*. J. Bacteriol., 2002. **184**(21): p. 6056-9.
2. Way, J.C., et al., *New Tn10 derivatives for transposon mutagenesis and for construction of lacZ operon fusions by transposition*. Gene, 1984. **32**(3): p. 369-379.

TABLE S1. Bacterial strains, plasmids, and primers

Strain	Genotype
DM10000	<i>Salmonella enterica</i> LT2 (Wildtype)
DM13509 ¹	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL</i>
DM17050	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d)²</i>
DM17051	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d) / pDM1602</i>
DM17053	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d) / pDM1507</i>
DM17181	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL pDM1507</i>
DM17182	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL pDM1504</i>
DM17183	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d) / pDM1504</i>
DM17184	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL / pDM1505</i>
DM17185	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d) / pDM1505</i>
DM17186	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL /pDM1506</i>
DM17187	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d) / pDM1506</i>
DM17292	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL / pDM1579</i>
DM17293	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d) / pDM1579</i>
DM17294	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL / pDM1577</i>
DM17295	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d) / pDM1577</i>
DM17296	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL / pDM1578</i>
DM17297	<i>aadA::araC-P_{BAD}-T7pol⁺ xyL rpsL ridA1::Tn10(d) / pDM1578</i>
Plasmid	Description
pDM1578	pET20b- <i>ilvA</i>
pDM1602	pET20b- <i>alr</i> (wildtype)
pDM1504	pET20b- <i>alr57</i> (R209E)
pDM1505	pET20b- <i>alr56</i> (R209A)
pDM1506	pET20b- <i>alr55</i> (R209D)
pDM1507	pET20b- <i>alr54</i> (R209S)
pDM1577	pET28b- <i>ilvE</i>

Table S1 (cont)

Primer	Sequence
LT2_ <i>alr</i> _R209D	5'-agtctcactttgactgggcgGATccgggcatcattttgatgg-3'
LT2_ <i>alr</i> _R209A	5'-agtctcactttgactgggcgGCGccgggcatcattttgatgg-3'
LT2_ <i>alr</i> _R209E	5'-agtctcactttgactgggcgGAAccgggcatcattttgatgg-3'
LT2_ <i>alr</i> _R209S	5'-agtctcactttgactgggcgAGCccgggcatcattttgatgg-3'

¹A His⁺ derivative of the SB300AI *S. enterica* strain harboring the chromosomally borne gene for T7 polymerase under control of the arabinose-inducible P_{BAD} promoter (*aadA::araC-P_{BAD}-T7pol⁺*) [1].

²Tn10(d) refers to the transposition-defective mini-Tn10 (Tn10Δ16Δ17) [2].

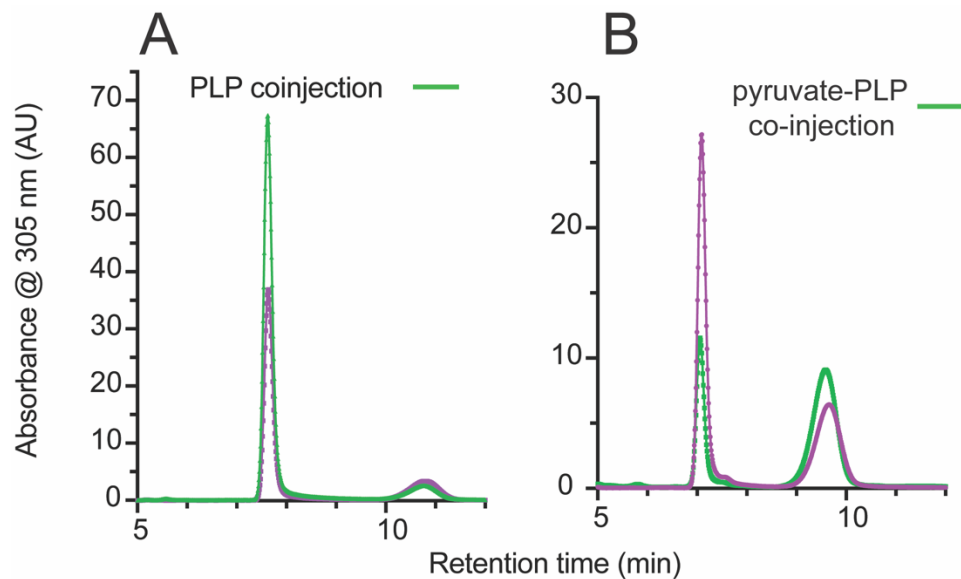


Figure S1. Assignment of peaks to PLP and pyruvate-PLP. Alr was purified from a *ridA* background, the cofactors were released from 0.4 mg of enzyme with base, and separated on HPLC as described (purple). Green chromatogram tracing shows the released cofactors with added PLP(A) or pyruvate-PLP (B). In each case the cofactors released from Alr took up half of the total volume and concentrated cofactor (PLP or PLP/pyruvate adduct) generated the other half of the volume.

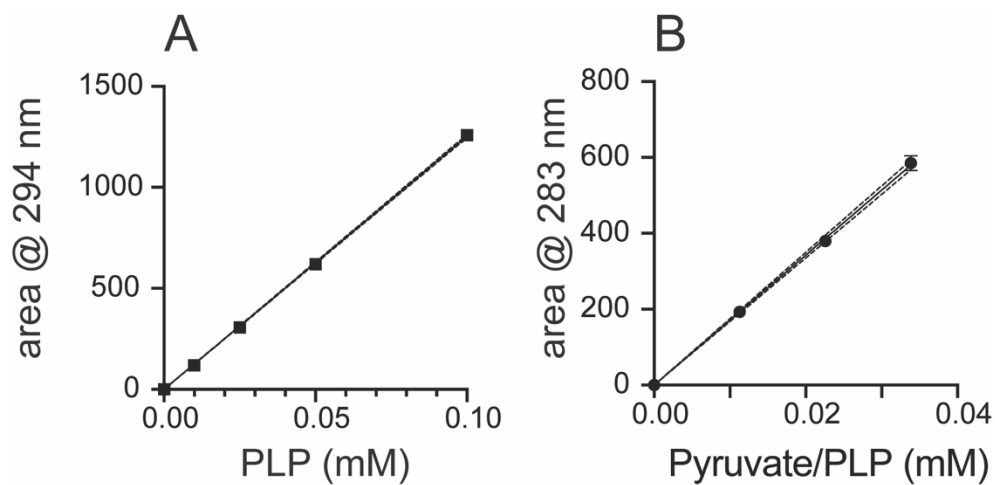


Figure S2. Standard curve for PLP and pyruvate-PLP. The standard curves for (A) PLP and (b) pyruvate/PLP are plotted. The y axis is the area of each peak at a known concentration and the x axis are the corresponding concentrations. The area of PLP and PLP/pyruvate are monitored at their individual absorbance maximum, which for PLP is 294nm, and for PLP/pyruvate, 283nm. (The R square value for both is greater than 0.99).

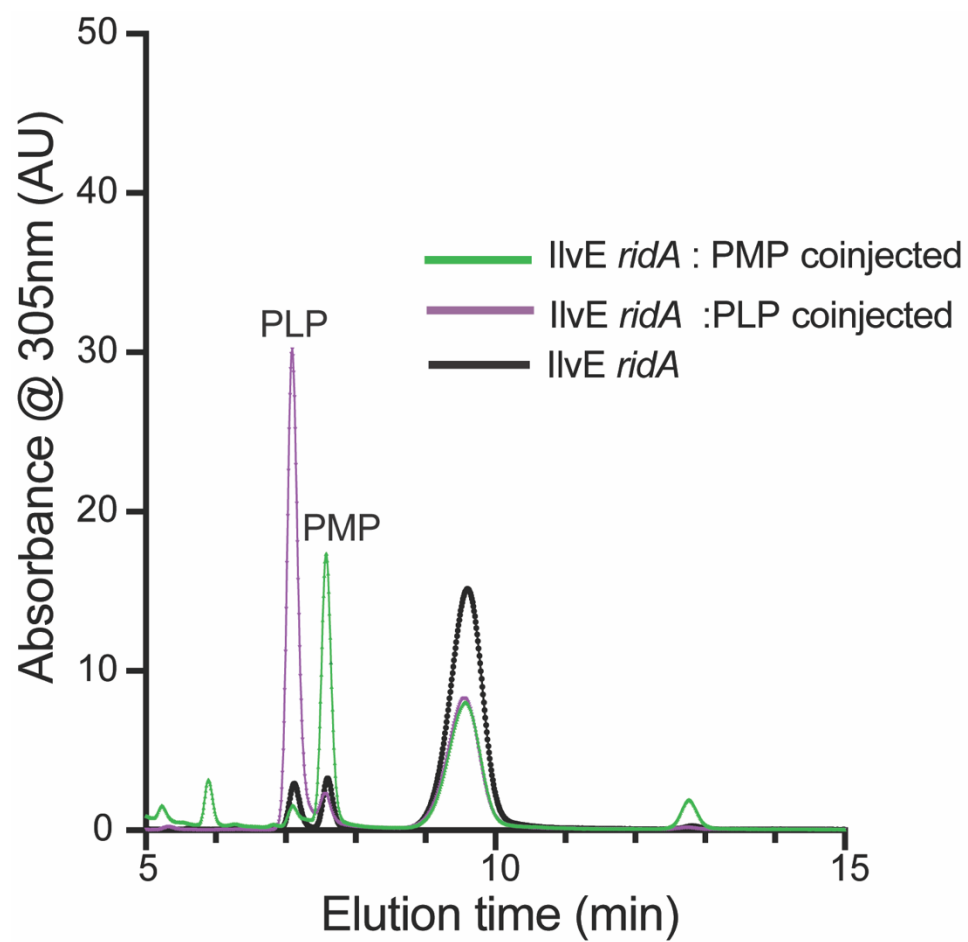


Figure S3. Peak assignment for PMP. The chromatogram of the cofactors released from IlvE purified from a *ridA* strain (black). The same sample including PLP (purple) or PMP (green).

