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    | *Salmonella enterica* LT2 (Wildtype)          |
| DM13509¹   | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL       |
| DM17050    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\)^2 |
| DM17051    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\) / pDM1602 |
| DM17053    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\) / pDM1507 |
| DM17181    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL pDM1507 |
| DM17182    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL pDM1504 |
| DM17183    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\) / pDM1504 |
| DM17184    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL / pDM1505 |
| DM17185    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\) / pDM1505 |
| DM17186    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL /pDM1506 |
| DM17187    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\) / pDM1506 |
| DM17292    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL / pDM1579 |
| DM17293    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\) / pDM1579 |
| DM17294    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL / pDM1577 |
| DM17295    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\) / pDM1577 |
| DM17296    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL / pDM1578 |
| DM17297    | \(aadA::araC\)-P\_BAD-T7pol\^ xyL rpsL \(\text{ridA1::Tn10(d)}\) / pDM1578 |

| Plasmid    | Description |
|------------|-------------|
| pDM1578    | pET20b-ilvA |
| pDM1602    | pET20b-alr (wildtype) |
| pDM1504    | pET20b-alr57 (R209E) |
| pDM1505    | pET20b-alr56 (R209A) |
| pDM1506    | pET20b-alr55 (R209D) |
| pDM1507    | pET20b-alr54 (R209S) |
| pDM1577    | pET28b-ilvE |
Table S1 (cont)

| Primer       | Sequence                                                                 |
|--------------|--------------------------------------------------------------------------|
| LT2\_alr\_R209D | 5’-agtctcacttgacctggcgGATccggtcatcttttgatgg-3’ |
| LT2\_alr\_R209A | 5’-agtctcacttgacctggcgGCGccgggcatcttttgatgg-3’ |
| LT2\_alr\_R209E | 5’-agtctcacttgacctggcgGAAccgggcatcttttgatgg-3’ |
| LT2\_alr\_R209S | 5’-agtctcacttgacctggcgAGCccgggcatcttttgatgg-3’ |

1A His+ derivative of the SB300Al S. enterica strain harboring the chromosomally borne gene for T7 polymerase under control of the arabinose-inducible P\textsubscript{BAD} promoter (aadA::araC-P\textsubscript{BAD}-T7\textsuperscript{pol+}) [1].

2\textsuperscript{Tn10(d)} refers to the transposition-defective mini-Tn10 (Tn10\Delta16\Delta17) [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 cure 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).
