Supporting Information

A Conserved Tyr-Glu Catalytic Dyad in Evolutionarily-Linked Enzymes: Carbapenam Synthetase and β-Lactam Synthetase†

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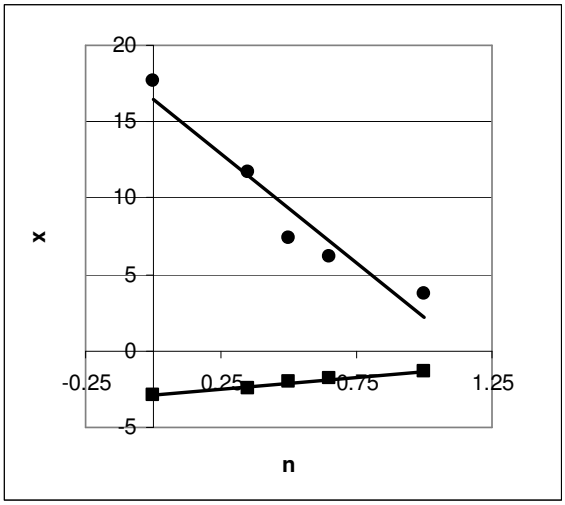
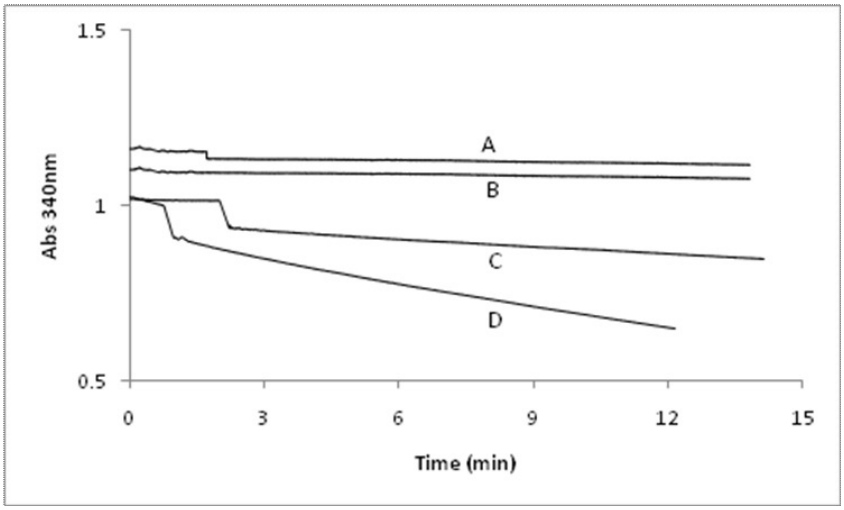
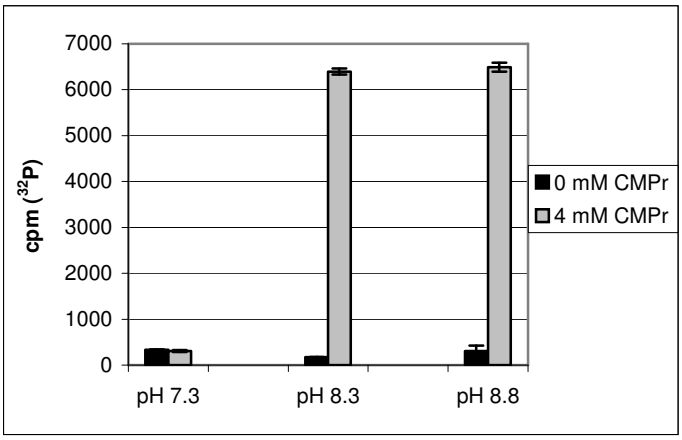
SUPPORTING FIGURES



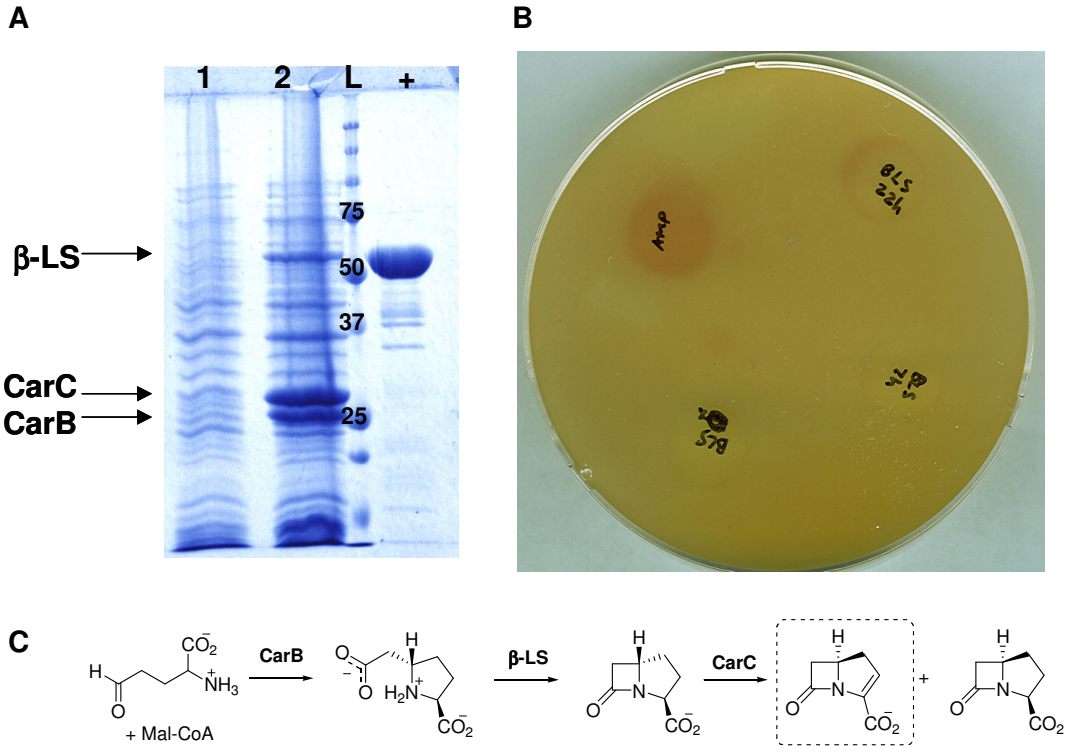
S. Figure 1. Linear proton inventory plot fits of β-LS mutant, Y348F, second-order rate constant versus mole fraction of D_2O (**n**). Linear fits were achieved as a function of k_{cat}/K_m at varying amounts of D_2O ($k_{\mathbf{n}}$) versus **n** and is represented as **x**. A ln $k_{\mathbf{n}}$ versus **n** plot (■) shows many-proton reactant-state fractionation factors (φ^R) and gave the best fit ($R^2 = 0.981$). A less favorable fit to a $k_{\mathbf{n}}^{-1}$ versus **n** plot (•) predicts a single site reactant-state fractionation factor ($R^2 = 0.928$).



S. Figure 2. The effect of hydroxylamine on the reactions catalyzed by the CPS active site mutants Y345A and E380A. Detection of AMP production in the absence (A, Y345A and B, E380A) and presence of 100 mM hydroxylamine for (C) E380A and (D) Y345A CPS mutants.



S. Figure 3. $^{32}PP_i$-ATP Exchange Assay of β-LS/ATP and (2*S*,5*S*)-Carboxymethyl Proline (CMPr). The bar graph shows assays with 0.02 μCi of [^{32}P]-pyrophosphate as counts per minute (cpm) of ^{32}P versus pH. The calculated background with all components except CMPr is shown in black and the ^{32}P-incorporation into ATP of assays containing all components (gray) at pH 7.3, 8.3, and 8.8. Standard deviation from assays performed in duplicate is shown as error bars.



S. Figure 4. Nitrocefin Assay Representing the Co-Expression of CarB and C with wild-type β-LS. Panel (A) 14% SDS Page Gel of the pET24a/*carBC_bls* ((*1*) and M.F. Freeman, unpublished results) expression at 28 °C with Lane **1** and **2** representing the uninduced and induced samples, respectively. Lane **L** shows the molecular weight ladder in units of kDa, and **+** is a standard of purified β-LS. Overproduction of β-LS (56 kDa), CarC (29 kDa), and CarB (27.5 kDa) shown in Lane **2** is identified with arrows. (B) The plates (BA_2 agar and *Bacillus lichenformis* (*2*)) were incubated at 37 °C for three hours after 250 uL of each respective supernatant was plated from aliquots of the CarB/β-LS/CarC co-expression media after 0, 7, 22h of induction with 1 mM IPTG. The image was captured after 350-µg/mL nitrocefin was applied to the agar. The sample labeled "βLS 0h" served as a negative control. Red indicates the prescence of a β-lactam and is ampicillin in the positive control, "AMP", and (5*R*)-carpapenem in the sample labeled "βLS 22h". Panel (C) shows the coupled reaction of CarB, β-LS, and CarC *in vivo*. The active antibiotic is shown in the box with dashed lines and is responsible for the red color visualized in Panel (B).

SUPPORTING TABLES

S. Table 1. CPS Mutant Protein Oligonucleotide Primers

Mutation	Oligonucleotide Primers (PCR QuikChange Method)
Y348F	5'-GCATGCTCACCGGATTTGGTTCCGACCTGC-3'
Y348A	5'-CATGCTCACCGGAGCGGGTTCCGACCTG-3'
E380D	5'-CGCGTTGGACAGGGGATTTTGCTACCCACGG-3'
E380Q	5'-CGCGTTGGACAGGGCAGTTTGCTACCCACG-3'
E380A	5'-GCGTTGGACAGGGGCGTTTGCTACCCACG-3'

The double mutants Y345F/E380D, Y345F/E380Q and Y345A/E380A were constructed in the same manner using the mutagenic primers for the various E380 mutations and the template vector of the single mutations Y345F and Y345A.

S. Table 2. β-LS Mutant Protein Oligonucleotide Primers

Mutation	Oligonucleotide Primers (PCR Overlap Extension Method)
Y348F	5'-TCCTCACCGGGTTCGGCGCGGACATC-3' 5'-GATGTCCGCGCCGAACCCGGTGAGGA-3'
Y348A	5'-CATCCTCACCGGGGCTGGCGCGGACAT-3' 5'-ATGTCCGCGCCAGCCCCGGTGAGGATG-3'
E382D	5'-GACGGGCTGAACGACATGTCCCCGGTGC-3' 5'-GCACCGGGGACATGTCGTTCAGCCCGTC-3'
E382Q	5'-GACGGGCTGAACCAGATGTCCCCGGTGC-3' 5'-GCACCGGGGACATCTGGTTCAGCCCGTC-3'

REFERENCES

(1) Li, R. F., Stapon, A., Blanchfield, J. T., and Townsend, C. A. (2000) Three unusual reactions mediate carbapenem and carbapenam biosynthesis. *Journal of the American Chemical Society 122*, 9296-9297.

(2) Sykes, R. B., and Wells, J. S. (1985) Screening for Beta-Lactam Antibiotics in Nature. *Journal of Antibiotics 38*, 119-121.

(3) Gerratana, B., Stapon, A., and Townsend, C. A. (2003) Inhibition and alternate substrate studies on the mechanism of carbapenam synthetase from Erwinia carotovora. *Biochemistry 42*, 7836-47.