Supplemental Information

for

Structure and Catalytic Mechanism of the Nicotinate (Vitamin B_3) Degradative Enzyme Maleamate Amidohydrolase from *Bordetella Bronchiseptica RB50*[†,‡]

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List of Supplementary Figures:

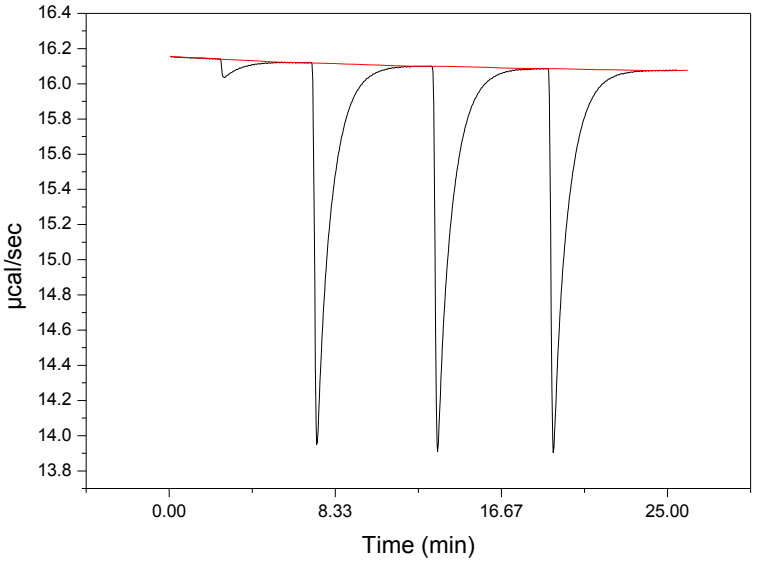


Figure S1. Isothermal calorimetric measurement of the ΔH_{app} for maleamate hydrolysis. Each peak represents the thermal power required to keep the cell at a constant temperature (25 ºC) during the course of each reaction (injection). Complete conversion of substrate to product occurs for each injection, in which the cell contains NicF (2.25 µM) and the syringe contains maleamate (2.5 mM), each made in the identical solution (bicine, 100 mM, pH 7.5). Numerical integration of each injection provides the total heat for maleamate hydrolysis during that injection (ΔH_{app}). The consistent shape and size of each peak confirms no inhibition by product formation and that the K_{eq} of this reaction greatly favors maleate formation under these conditions. Identical values of ΔH_{app} [–9.17 (±0.02) kcal/mol] were obtained from each injection.

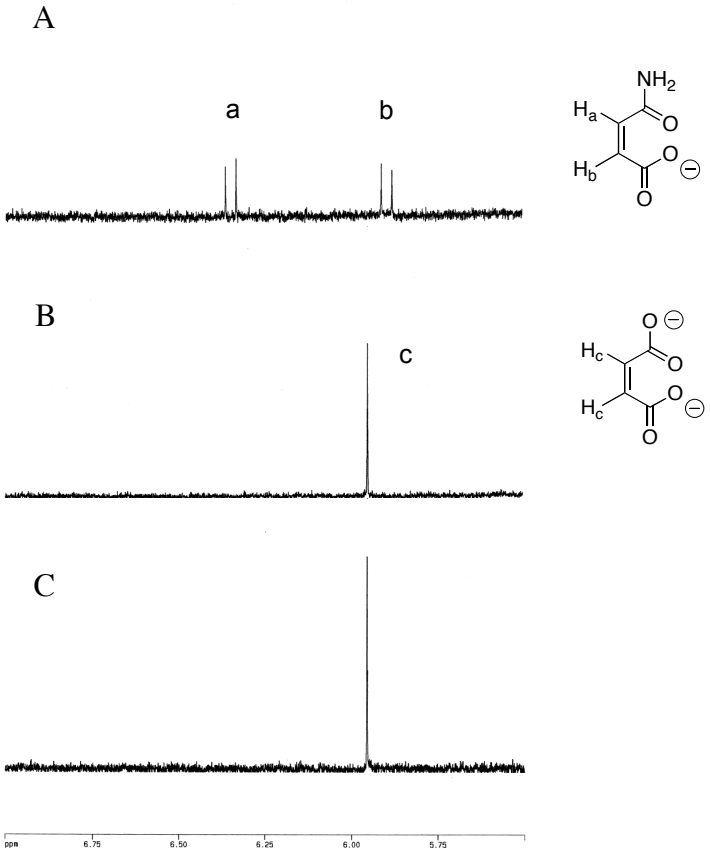
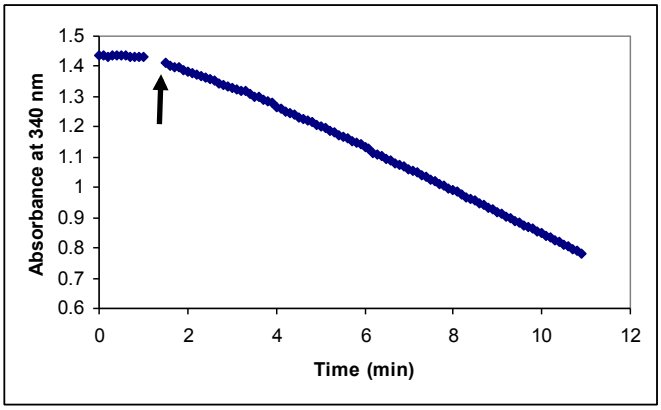


Figure S2. ^{1}H NMR (400 MHz) spectra from 5.5 ppm to 7.0 ppm of (A) standard of maleamate (1 mM), (B) standard of maleate (1 mM), and (C) product of the reaction in which maleamate was incubated with purified gene product from *Bb*1774. Results confirm the functional assignment of this protein as NicF.

A



B

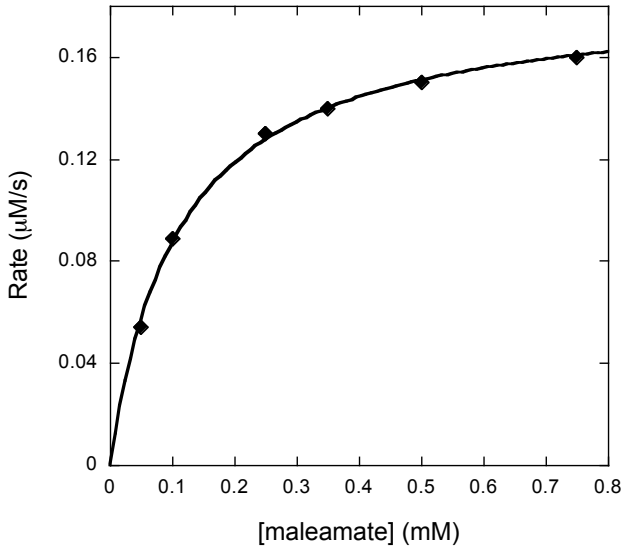


Figure S3. (A) Change in UV absorbance at 340 nm in the glutamate dehydrogenase-coupled assay for ammonia production as a function of time. The arrow indicates the time in which maleamate was added and mixed into the cuvette. Linear fit to the data provides the rate of ammonia production. Solution contained glutamate dehydrogenase (20 units), α-ketoglutarate (10 mM), EDTA (1 mM), NADPH (250 mM), and NicF (10 nM) in phosphate buffer (100 mM, pH 7.4). (B) Nonlinear fit of the initial rate of ammonia production as a function of maleamate concentration provides a k_{cat} = 18.4 (± 0.3) s^{-1} and K_M = 107 (±7) μM.

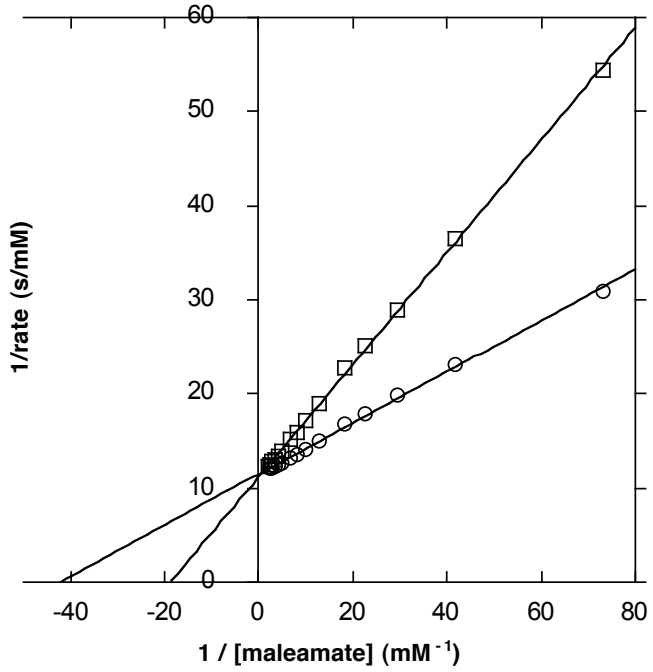


Figure S4. Double reciprocal plot of initial rate data obtained by multi-injection isothermal titration calorimetry of NicF activity (at 25 ºC) with varying concentrations of maleamate and fixed concentrations of maleate (0 mM – circles; 4.0 mM – squares). Satisfactory linear regression analyses ($R^2 = 0.998$) assuming competitive inhibition provides a $K_I = 4.3$ (±0.3) mM.

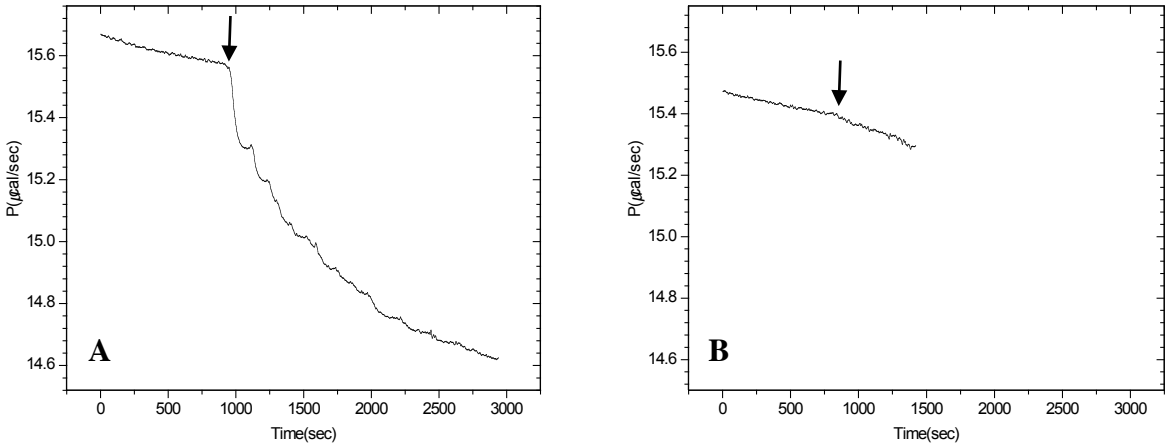
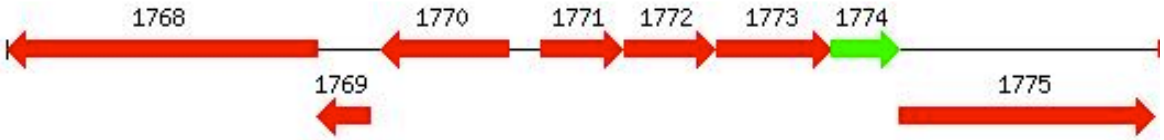


Figure S5. Inactivation of NicF activity by iodoacetamide measured by isothermal titration calorimetry. (A) Reaction measured in the absence of iodoacetamide showing the change in observed power upon injection of maleamate. (B) Reaction measured using identical conditions shown in panel A, except NicF had been pre-incubated with iodoacetamide (10:1, iodoacetamide:NicF). Arrows indicate time of first maleamate injection. Results are consistent with a critical role for a cysteine side chain thiol in the mechanism of NicF-catalyzed maleamate hydrolysis.



peg	current annotation in 'The SEED'	putative functional assignment
1768	putative diheme cytochrome c-553	nicB
1769	isoquinoline 1-oxidoreductase α	nicA
1770	salicylate monooxygenase	nicC (Moore, T., Story, R., Bauerle, M., and Snider, M. J., unpublished)
1771	maleate cis-trans isomerase	nicE
1772	probable alpha/beta hydrolase	nicD
1773	leucyl aminopeptidase	nicX (Henke, M. and Snider, M. J. unpublished)
1774	N-carbamoylsarcosine amidase	nicF (this paper)
1775	isoquinoline 1-oxidoreductase ß subunit	nicB

Figure S6. Genome analysis in *Bordetella bronchiseptica RB50* from 'The SEED' database (*1*), of a putative *nic* gene cluster.

REFERENCES

1. Overbeek, R., Disz, T., and Stevens, R. (2004) The SEED: A peer-to-peer environment for genome annotation. Communications of the ACM 47, 46-51. http://theseed.uchicago.edu/FIG/index.cgi