Supporting Information

Probing Nitrogen Sensitive Steps in the Free Radical-Mediated Deamination of Amino Alcohols by Ethanolamine Ammonia-Lyase

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Supplementary Experimental Methods

Glutamate dehydrogenase, a-ketoglutarate, NADH, AdoCbl, and ADP were from Sigma. Ethanolamine was from Eastman Kodak, 2-aminopropanols were from Aldrich. ~1 M stock solutions of amino alcohols were prepared in Milli-Q water and adjusted to ~pH 7.5 with HCl prior to use. EAL was dialyzed vs. .01 M Hepes/NaOH pH 7.5 until no ammonia was detectable using the glutamate dehydrogenase coupled assay. Centrifugal concentrators were rinsed with Milli-Q water prior to use, according to manufacturer instructions.

EAL reactions were carried out in 5 or 10 mL of .01 M NaPO4 pH 7.5 containing .02 M amino alcohol and 1 mM AdoCbl, 0.4 mM EAL (ethanolamine reactions), or 50 mM AdoCbl ,50 mM EAL (2-aminopropanol reactions). Reactions were quenched by addition of 1 M HCl to a final concentration of .02 M. EAL was removed from reactions using Amicon Ultra 15 (30,000 MWCO) centrifugal concentrators at 4,000 g. Partial conversion reactions were run 15 to 40 min as appropriate to achieve ~50% conversion of amino alcohol. Conversion of ethanolamine was complete within 12 hr. Complete conversion of 2-aminopropanol could was not achieved in a single reaction because AdoCbl is slowly converted to cob(III)alamin during the reaction [S0]. Complete conversion of 2-aminopropanol was achieved by removing spent enzyme from the 12 hr reaction by ultrafiltration, and adding fresh enzyme and AdoCbl for a second 12 hr reaction period.

Assays for ammonia and amino alcohol were carried out using a cocktail containing 0.1 M Hepps/NaOH pH 8.0, 1 mM a-ketoglutarate, 0.2 mM ADP, 0.2 mM NADH, 5 mM AdoCbl, and 0.5 mg

mL-1 glutamate dehydrogenase. This cocktail was prepared at least 2 hr before use to allow residual ammonia in the reagents to be consumed. A typical assay would be as follows: record the absorbance at 340 nm of 995 μL of assay cocktail, then add 5 μl of quenched EAL reaction mixture, wait until the absorbance at 340 nm stabilizes and record the new value, then add 1 μl of 30 mg mL-1 EAL, wait for the absorbance at 340 nm to stabilize, and record the value again. The ammonia concentration is proportional to the initial drop in absorbance at 340 nm, and the amino alcohol concentration is proportional to the drop after EAL is added. A value of 6.22 mM-1cm-1 was used for the extinction coefficient of NADH at 340nm.

Discussion of Barrier Heights

Differences in the amounts, and identities of the steady state intermediates observed with the 3 different substrates, the DV/K IE's observed for EAL from Clostridium (Table S1), and the 15(V/K) IE's are indicative of different relative barrier heights in the reactions of the three amino alcohols. The relative barrier heights are portrayed in the following qualitative reaction coordinate diagram (Figure S1). Ethanolamine turns over much faster than either of the 2-aminopropanols so the barriers are uniformly lower for this substrate. A product related radical accumulates during steady-state turnover with ethanolamine indicating that the steps following product radical formation are major contributors to rate limitation. Hence, the barrier separating product radical and product is highest for this substrate. The sensitivity of the amount of substrate radical to 2H incorporation and a large D(V/K) measured with (R)-2-aminopropanol suggest that steps leading to initial formation of substrate radical are substantially rate limiting. Therefore, for (R)-2-aminopropanol, the barrier for formation of the substrate radical is elevated relative corresponding barrier for the other two substrates. For (S)-2-aminopropanol the 15(V/K) and the observation that the substrate radical dominates in the steady-state show that the steps subsequent to substrate radical formation are rate limiting. A large barrier for conversion of substrate radical to product radical is therefore indicated.

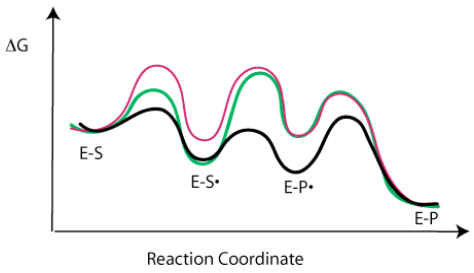

Figure S1. Qualitative reaction coordinate diagram of EAL catalyzed deamination of three amino alcohols. Red, (*R*)-2-aminopropanol; green, (*S*)-2-aminopropanol; black, ethanolamine.

S1

Table S1: D(V/K) Isotope Effects on EAL from *Clostridium*. [S1]	
Ethanolamine	9.7
(R)-2-aminopropanol	20
(S)-2-aminopropanol	5.4

References

S0. Hollaway, M. R.; White, H. A.; Joblin, K. N.; Johnson, A. W.; Lappert, M. F.; Wallis, O. C. Eur. J. Biochem. 1978, 82, 143-154.

S1. Babior, B. M., In B12 Vol. 2, Dolphin, D., Ed. John Wiley & Sons, Inc.: New York, 1982, Chapter 10.