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Supporting Information

Dynamic, Electrostatic Model for the Generation and Control of High-Energy Radical Intermediates by a Coenzyme B_{12}-Dependent Enzyme

Zhi-Gang Chen,[a] Monika A Ziętek,[b] Henry J. Russell,[b] Shirley Tait,[b] Sam Hay,[b] Alex R. Jones,*[c] and Nigel S. Scrutton*[b]

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Experimental Section

Materials.

Ccoenzyme B12 (> 98 % Sigma), methylcobalalmin (Sigma), 2-aminoethanol (ethanolamine, > 99 % Sigma), yeast alcohol dehydrogenase (YADH from *Saccharomyces cerevisiae*, ≥ 300 units mg^{-1} protein, Sigma) and β-nicotinamide adenine dinucleotide (β-NADH, Sigma) were used as purchased without further purification. The plasmid, pET-SEAL, encoding the small (32.0 kDa) and large (49.1 kDa) subunits of wild-type (WT) EAL from *Salmonella enterica* (kindly donated by Prof. George Reed) was overexpressed in *E. coli* and purified as described previously.[1] Site-directed mutagenesis was performed using Phusion kit (New England Biolabs), using the following primers: 5'-GCCTGTACTTTGACACCGGGCAAGGG-3' (for E287D); CAA (for E287Q); GCA (for E287A). All EAL E287 variants were overexpressed and purified as for the WT protein (SDS PAGE, Figure S2).

Coenzyme binding

An excess of each coenzyme (coenzyme B_{12} / methylcobalamin) was added to separate samples of the EAL wild-type and E287 variants and incubated on ice for 10-15 minutes. Unbound cofactor was removed by passing down a CentriPure P25 gel filtration column (emp BIOTECH). UV-visible spectra were then acquired between 200-800 nm.

Steady-state turnover.

The activity of each EAL variant was measured using a coupled assay similar to that described by Kaplan and Stadtman.[2] Briefly, the consumption of NADH (150 μM) was monitored by spectrophotometry at 340 nm as the product of the EAL catalysed reaction, acetaldehyde, was turned over by YADH (100 units ml^{-1}). Final EAL concentrations were: WT, 5 nM and E287D/Q/A, 1-1.5 μM. Data were acquired using an Agilent Cary 60 UV-Vis Spectrophotometer in a final volume of 1 ml under both aerobic and anaerobic conditions in HEPES (100 mM), pH 7.5 at 25 °C. Anaerobic data were acquired in a Belle Technology glove box. Steady-state kinetic parameters were calculated by fitting the initial rate as a function of substrate (2-aminoethanol) concentration to the Michaelis-Menten equation (Figure S4a-e and Equation (1)).

Stopped-flow spectrometry.

Single wavelength measurements were made at 525 nm under aerobic and anaerobic conditions in an Applied Photophysics SX.18 MV-R stopped-flow spectrophotometer. Data were acquired in HEPES (100 mM), pH 7.5 at 25 °C after rapid mixing of EAL variants (E287D/Q, 30-40 μM final concentration) with equal volumes of saturating concentrations of 2-aminoethanol (60 mM final concentration). Anaerobic data were acquired in a Belle Technology glove box. Data were fit to a single exponential function where possible to extract the observed pre-steady-state rate coefficient, k_{obs}.

Substrate titration into E287A.

2-aminoethanol (final concentration of 110 mM) was added to E287A (~ 10 μM) and a spectrum acquired between 300-700 nm in an Agilent Cary 60 UV-Vis Spectrophotometer at 60 s intervals until spectral changes ceased. Data were acquired under both aerobic and anaerobic conditions in a 100 μl cuvette, HEPES (200 mM), pH 7.5 at 25 °C. Anaerobic data were acquired in a Belle Technology glove box.

Figures

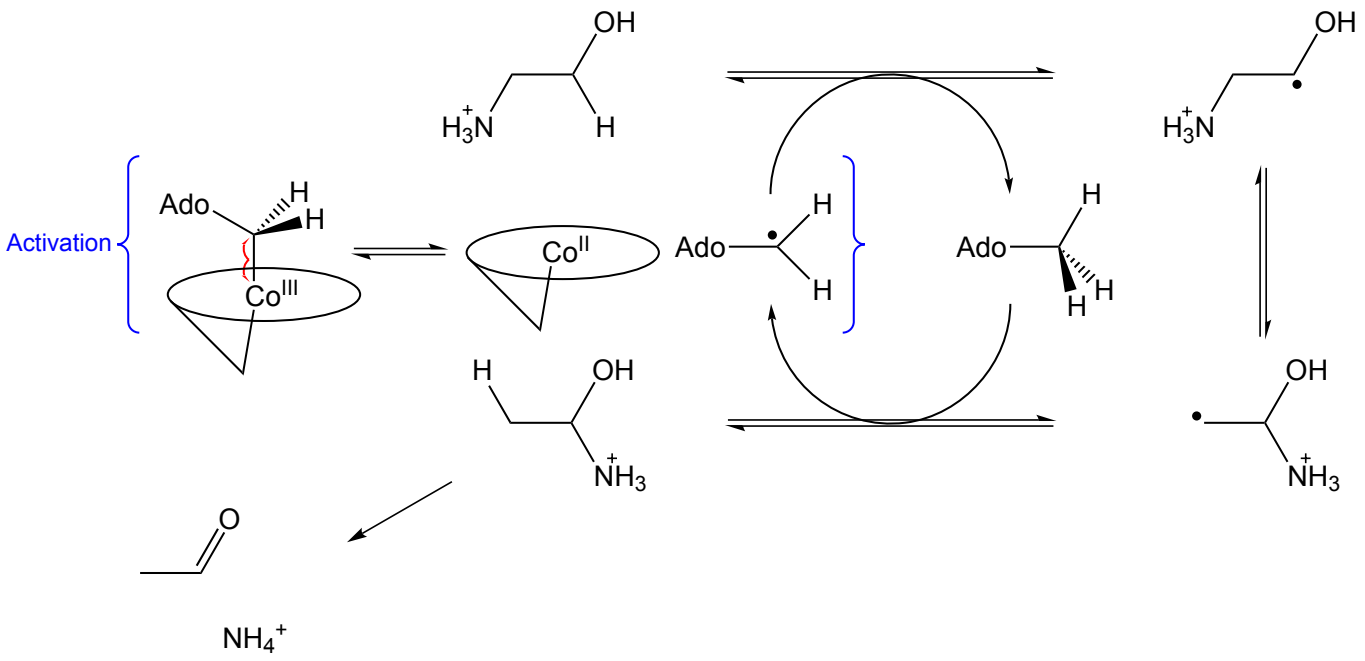


Figure S1. Proposed cycle of the coenzyme B_{12}-dependent ethanolamine ammonia lyase catalysed conversion of 2-aminoethanol to ethanal and ammonia. Refer to main article for description.

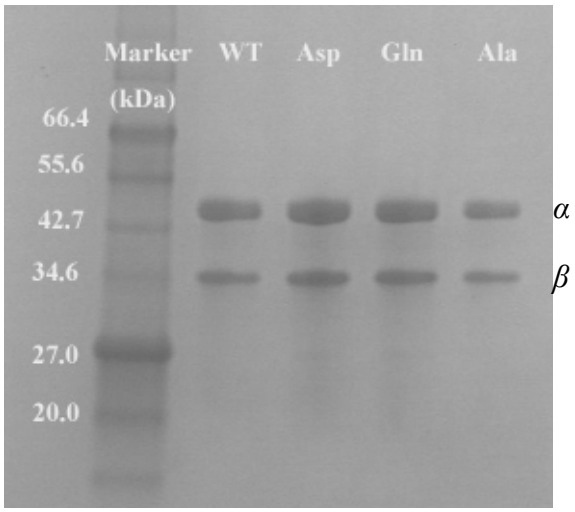


Figure S2. SDS-PAGE of WT, E287D (Asp), E287Q (Gln), and E287A (Ala) variants of EAL. The bands for the small (*β*, 32.0 kDa) and large (*α*, 49.1 kDa) subunits are indicated.

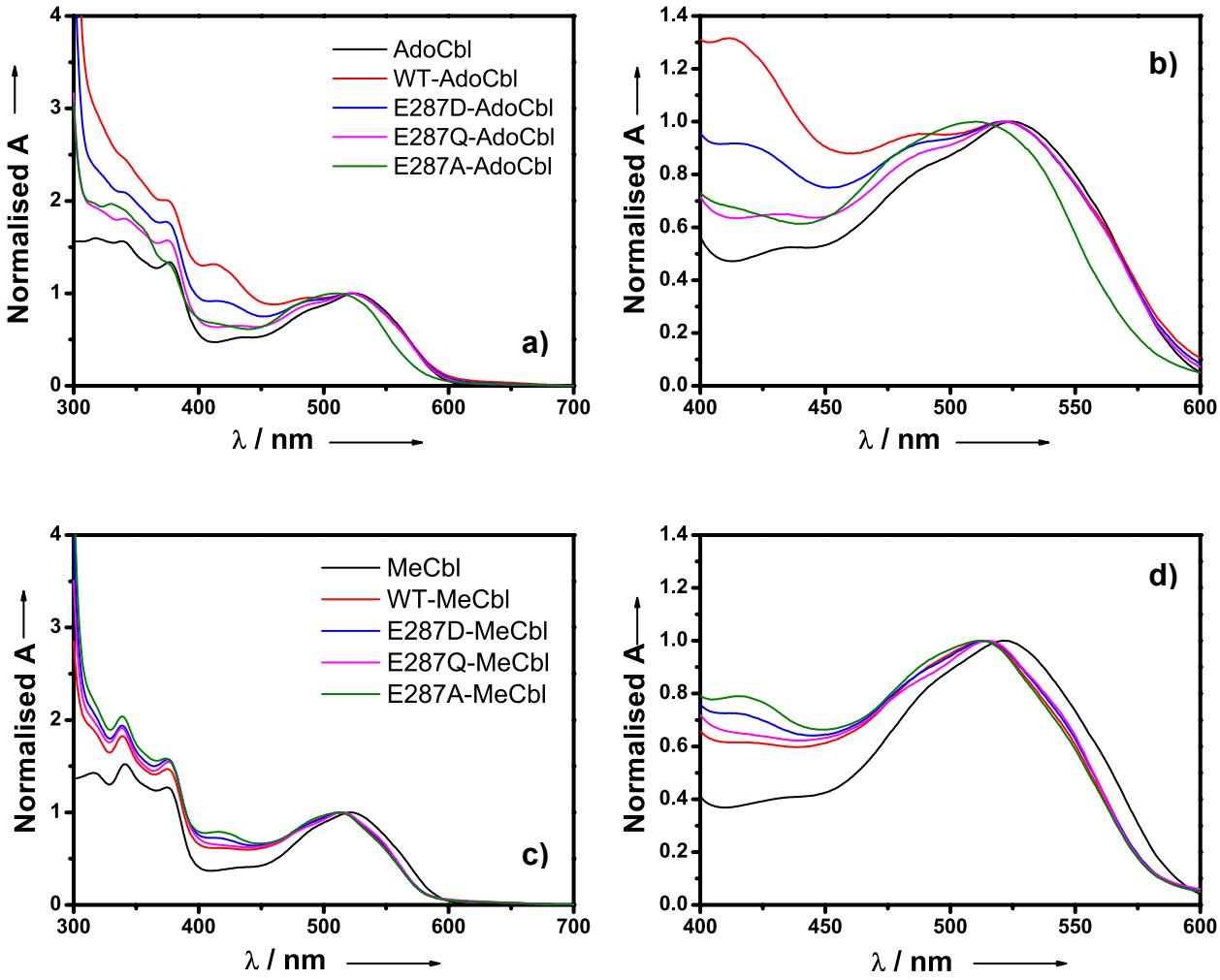
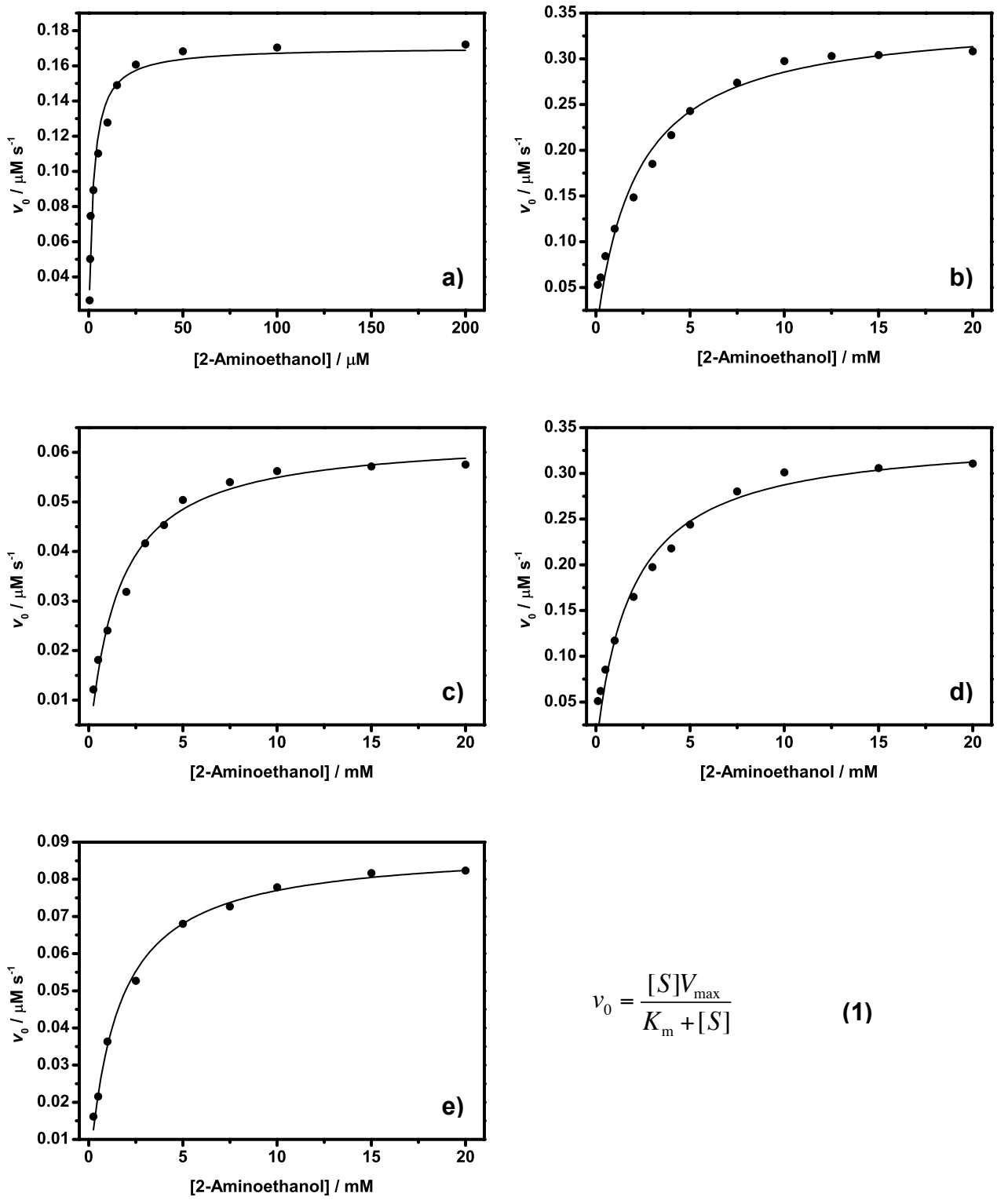


Figure S3. Normalised UV-visible spectra of **a-b)** 5'deoxyadenosylcobalamin (AdoCbl, coenzyme B_{12}) and **c-d)** methylcobalamin (MeCbl) both free in aqueous solution and bound to the EAL variants (WT, E287D, E287Q and E287D). The spectra were normalised between 700 nm (A=0) and the peak of the αβ absorption band (A=1). The peak of the αβ absorption band in each case is blue-shifted by varying extents (b & d, Table S1 and Figure 2). There is more general variation between the EAL variants in the protein bound spectra for B_{12} (a) than for methylcobalamin (c), especially between 300-500 nm. This, and the nature of the αβ band blue shift (see Figure 2, Table S1 and discussion in main article), suggest significant interaction between E287 and the protein through direct contact with the upper axial 5'-deoxyadenosylcobalalmin.

	Peak of αβ absorption band (± 2 nm)	
	AdoCbl	MeCbl
Free	525	521
E287D	522	515
WT	520	513
E287Q	522	515
E287A	510	512

Table S1. Peak values of the αβ absorption band for 5'deoxyadenosylcobalamin (AdoCbl, coenzyme B_{12}) and methylcobalamin (MeCbl) both free in aqueous solution and bound to the EAL variants (WT, E287D, E287Q and E287D). See also Figures 2 and S3a-d. The EAL variants are arranged from top to bottom in order of increasing hydrophobicity of the 287 residue (Asp < Glu < Gln < Ala).



$$v_0 = \frac{[S]V_{max}}{K_m + [S]} \qquad \textbf{(1)}$$

Figure S4 Initial velocity (v_0) as a function of [2-aminoethanol] measured for EAL variants: **a)** WT – aerobic, **b)** E287D – aerobic, **c)** E287Q – aerobic, **d)** E287D – anaerobic, **e)** E287Q – anaerobic. All data were fit to the Michaelis-Menten equation (1), and k_{cat} calculated from V_{max} / $[E]_0$, where $[E]_0$ = EAL variant concentration.

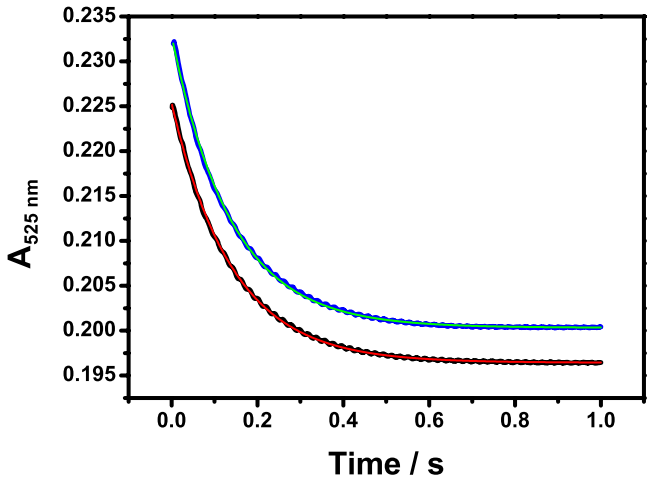


Figure S5. Pre-steady-state traces and single exponential fits representing the change in absorbance at 525 nm after rapid mixing of the E287D variant with 2-aminoethanol (2AE). Black (data) and red (fit) – 50 mM 2AE, k_{obs} = 7.10 ± 0.29; blue (data) and green (fit) – 60 mM 2 AE, k_{obs} = 7.29 ± 0.18. The rates are the same within error, suggesting saturating concentrations of substrate.

References

[1] V. Bandarian, G. H. Reed, *Biochemistry* **1999**, *38*, 12394-12402.
[2] B. H. Kaplan, E. R. Stadtman, *J. Biol. Chem.* **1968**, *243*, 1787-1793.