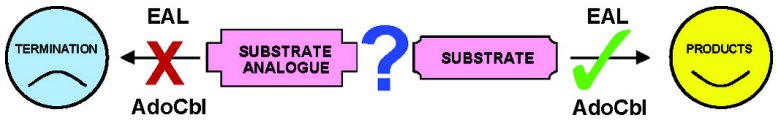
Article

Divergent Mechanisms of Suicide Inactivation for Ethanolamine Ammonia-Lyase

Gregory M. Sandala, David M. Smith, and Leo Radom

J. Am. Chem. Soc., **2005**, 127 (24), 8856-8864• DOI: 10.1021/ja051527k • Publication Date (Web): 27 May 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 05/27/2005

Divergent Mechanisms of Suicide Inactivation for Ethanolamine Ammonia-Lyase

Gregory M. Sandala,[†,‡] David M. Smith,*[,§] and Leo Radom*[,†,‡]

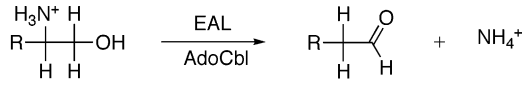
Contribution from the School of Chemistry, University of Sydney, Sydney, NSW 2006, Australia, Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia, and Rudjer Boskovic Institute, 10002 Zagreb, Croatia

Received March 9, 2005; E-mail: david.smith@irb.hr; radom@chem.usyd.edu.au

Abstract: Ab initio molecular orbital calculations have been used to study the mechanism of suicide inactivation of ethanolamine ammonia-lyase induced by three different substrate analogues. Analysis of the normal catalytic mechanism with 2-aminoethanol (ethanolamine) as substrate predicts that both the hydrogen-abstraction and hydrogen-reabstraction steps involving the B_{12}-cofactor are likely to be exothermic. On the other hand, the proposed inactivation mechanism for the first substrate analogue, glycolaldehyde, leads to a highly stabilized radical that results in a very endothermic (by ca. 90 kJ mol^{-1}) hydrogen-reabstraction step, which is thought to halt the normal function of the enzyme. Curiously, the energy requirements for a catalytically imposed mechanism in the case of the second substrate analogue, 2-hydroxyethylhydrazine (HEH), parallel those for the catalytic substrate, despite the fact that HEH is found to inactivate EAL experimentally. However, further analysis reveals the presence of a lower energy pathway for HEH that leads to the formation of the highly stabilized hydrazinium radical cation. In a manner similar to when glycolaldehyde is the substrate analogue, this results in an endothermicity for the hydrogen-reabstraction step that is prohibitively large. In contrast to these related inactivation mechanisms, the third substrate analogue, 2-aminoacetaldehyde, apparently accomplishes the inactivation of EAL in an entirely different manner. A pathway for the experimentally observed formation of acetic acid and ammonium cation has been identified and appears catalytic in the sense that 5′-deoxyadenosyl radical is regenerated. However, mechanisms to account for the subsequent formation of 4′,5′-anhydroadenosine and degradation of the corrinoid ring of the cofactor have not been elucidated.

Introduction

Ethanolamine ammonia-lyase (EAL) is an adenosylcobalamin (AdoCbl) dependent enzyme that catalyzes the deamination of vicinal amino alcohols (**1**) to produce the corresponding aldehydes (**2**):[1]



1 **2**

a: R = H
b: R = CH_3

The generally accepted mechanism for this reaction involves five steps (Scheme 1).[2] The first step (**A**), believed to be initiated upon substrate binding,[3] is the homolytic cleavage of the Co−C bond of AdoCbl, yielding the cob(II)alamin and 5′-deoxyadenosyl (Ado-$CH_2^{\bullet}$) radicals. Subsequently, step **B** involves hydrogen abstraction by Ado-$CH_2^{\bullet}$ from the substrate (**1**) to form 5′-deoxyadenosine (Ado-CH_3) and a substrate-derived radical (**3**). Rearrangement of **3** to the product-related radical (**4**) then occurs in step **C**, which is followed by hydrogen-atom reabstraction by **4** from Ado-CH_3 (step **D**). This last step produces the aminated product (**5**) as well as regenerating the 5′-deoxyadenosyl radical (Ado-$CH_2^{\bullet}$), which can then recombine with the cob(II)alamin radical to re-form AdoCbl and dissociate from EAL. The final step (**E**) involves the enzyme-catalyzed deamination of the aminated product to yield the product aldehyde (**2**) plus ammonium cation.

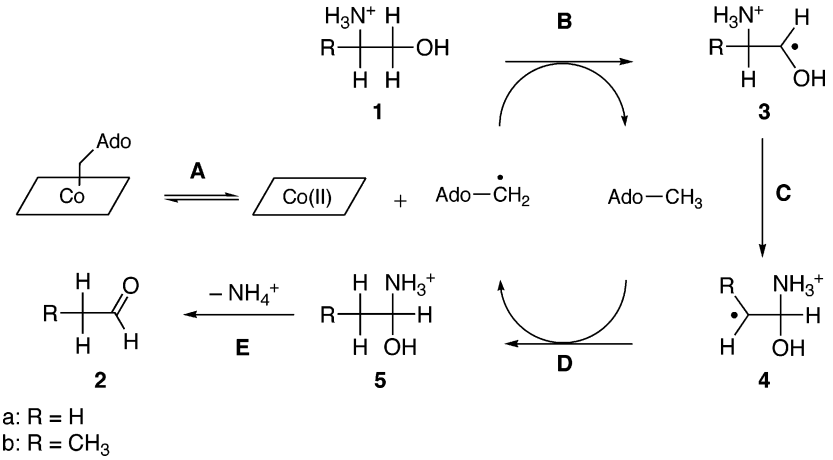
Important experimental results that have contributed to the understanding of the reactions catalyzed by EAL include electron paramagnetic resonance (EPR) spectroscopic studies that have established the reversibility of Ado-CH_3 formation and identified cob(II)alamin and the substrate-derived radical **3** as intermediates of the reaction (steps **A** and **B** of Scheme 1).[4] Further evidence to support the viability of the pathway depicted in Scheme 1 comes from the laboratories of Reed and

[†] University of Sydney.
[‡] Australian National University.
[§] Rudjer Boskovic Institute.

(1) (a) Babior B. M. *J. Biol. Chem.* **1969**, *244*, 449−456. (b) Babior, B. M. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982. (c) Bradbeer, C. *J. Biol. Chem.* **1965**, *240*, 4669−4674.
(2) See, for example: Bandarian, V.; Reed, G. H. In *Chemistry and Biochemistry of B_{12}*; Banerjee, R., Ed.; Wiley-Interscience: New York, 1999; pp 811−833.
(3) Law, P. Y.; Brown, D. G.; Lien, E. L.; Babior, B. M.; Wood, J. M. *Biochemistry* **1971**, *10*, 3428−3435.
(4) (a) Babior, B. M.; Carty, T. J.; Abeles, R. H. *J. Biol. Chem.* **1974**, *249*, 1689−1695. (b) Babior, B. M.; Moss, T. H.; Orme-Johnson, W. H.; Beinert, H. *J. Biol. Chem.* **1974**, *249*, 4537−4544. (c) Bandarian, V.; Reed, G. H. *Biochemistry* **2002**, *41*, 8580−8588.

10.1021/ja051527k CCC: $30.25

Scheme 1. Generally Accepted Mechanism for the Rearrangement Catalyzed by EAL



Warncke.[5] Using isotope labels in combination with pulsed electron nuclear double resonance (ENDOR) spectroscopy and X-band two-pulse electron spin−echo envelope modulation (ESEEM) spectroscopy, these elegant studies have revealed close proximity (∼3.2 Å) between the C1 of the substrate-derived radical and the C5′ of the 5′-deoxyadenosyl moiety. These findings have strongly implicated the 5′-deoxyadenosyl radical as the sole species responsible for mediating the hydrogen-transfer steps for EAL. Significantly, these results argue against earlier suggestions that a protein-bound radical mechanism is operational in the hydrogen-transfer steps of EAL, which was originally proposed in order to account for the very large kinetic isotope effects observed with EAL.[6] However, while supporting the basic concept of the mechanism shown in Scheme 1, these results do not provide direct information as to whether **3**, once formed, does indeed proceed via **4** and **5** in the subsequent generation of product.

Actually, a possible alternative to the pathway via **4** and **5** for the EAL-catalyzed conversion of vicinal amino alcohols to their equivalent aldehydes involves elimination of ammonium cation from the substrate-derived radical (**3**), rather than from the closed-shell product (**5**). For this "direct elimination" mechanism to be operative, hydrogen-atom reabstraction from Ado-CH_3 by the resulting allyloxy radical (not shown) is required in order to regenerate Ado-CH_2•. While conceptually feasible, there is growing evidence against this proposition. For example, the identification of the product-related radical derived from 2-aminoethanol (**4a** in Scheme 1) by EPR and ESEEM spectroscopy[7] provides clear support for the migration pathway in favor of the direct elimination pathway. Separate studies have characterized the cryotrapped product-related radical state using various EPR spectroscopic techniques, though at that time the migration and direct elimination pathways could not be conclusively differentiated from one another.[8]

In addition to these experimental studies, detailed theoretical work by Semialjac and Schwarz[9b,c] has demonstrated that the direct elimination pathway represents a higher energy, and thus less likely, reaction pathway. In contrast, the migration pathway (Scheme 1) was found to be viable under certain conditions. More specifically, these model theoretical studies[9] showed that a combination of essentially full protonation of the migrating amino group plus interaction of a Brönsted base with the spectator hydroxyl group at C1 provides a barrier that is consistent with experiment. Similar synergistic interactions have also been successfully applied to the migration step of diol dehydratase (DDH),[10] an analogous coenzyme B_{12}-dependent enzyme that catalyzes the conversion of vicinal diols into their respective aldehydes.[11] The theoretical studies[9a] also showed that hydrogen reabstraction from acetaldehyde radical (required in the direct elimination pathway) has a significantly higher energy requirement than hydrogen reabstraction from **4a** (required in the migration pathway), again making the former less likely.

Thus, although the precise nature of the pathway linking **3a** and **2a** remains somewhat open, (a) the theoretical finding[9b,c] that the migration pathway is more favorable from energy considerations than elimination of ammonia to form acetaldehyde radical, (b) the theoretical finding[9a] that hydrogen reabstraction by acetaldehyde radical from 5′-deoxyadenosine (Ado-CH_3) is energetically much more demanding than reabstraction by **4a**, and (c) the experimental evidence[7] for the product radical **4a**, all favor rearrangement over elimination.

An additional source of information for the reactions catalyzed by the coenzyme B_{12}-dependent enzymes is provided by experiments performed with analogues of catalytic substrates. This is the focus of the present study. Suitably chosen substrate analogues have been found to disrupt the catalytic cycle of the enzyme and have thus provided insight into enzyme functionality. For example, early studies revealed that addition of glycolaldehyde resulted in the inactivation of diol dehydratase (DDH).[12] Recently, again using glycolaldehyde as the substrate analogue, DDH and EAL were both found to become inactivated, and they displayed similar EPR spectra from the

(5) (a) LoBrutto, R.; Bandarian, V.; Magnusson O. T.; Chen, X.; Schramm, V. L.; Reed, G. H. *Biochemistry* **2001**, *40*, 9−14. (b) Warncke, K.; Utada, A. S. *J. Am. Chem. Soc.* **2001**, *123*, 8564−8572.
(6) Cleland, W. W. *CRC Crit. Rev. Biochem.* **1982**, *13*, 385−428.
(7) Warncke, K.; Canfield, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 5930−5931.
(8) (a) Ke, S.-C.; Warncke, K. *J. Am. Chem. Soc.* **1999**, *121*, 9922−9927. (b) Warncke, K.; Schmidt, J. C.; Ke, S.-C. *J. Am. Chem. Soc.* **1999**, *121*, 10522−10528.
(9) (a) Wetmore, S. D.; Smith, D. M.; Bennett, J. T.; Radom, L. *J. Am. Chem. Soc.* **2002**, *124*, 14054−14065. (b) Semialjac, M.; Schwarz, H. *J. Am. Chem. Soc.* **2002**, *124*, 8974−8983. (c) Semialjac, M.; Schwarz, H. *J. Org. Chem.* **2003**, *68*, 6967−6983.
(10) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 1664−1675.
(11) For recent reviews, see for example: (a) Frey, P. A. *Chem. Rec.* **2001**, *1*, 277−289. (b) Toraya, T. *Chem. Rev.* **2003**, *103*, 2095−2127.
(12) Wagner, O. W.; Lee, H. A.; Frey, P. A.; Abeles, R. H. *J. Biol. Chem.* **1966**, *241*, 1751−1762.

inactivation process.[13] From these results, an inactivation mechanism was proposed.[13] For EAL, these inactivation studies potentially provide valuable information since the crystal structure has yet to be obtained.

From a theoretical perspective, the computational studies mentioned above[9] have primarily focused on the rearrangement step in the EAL-catalyzed reactions. In addition to this work, Semialjac and Schwarz have carried out a detailed examination of the hydrogen abstraction from **1a** (and related compounds; step B) using the 1,5-dideoxyribose-5-yl radical as a model for Ado-$CH_2^{\bullet}$ and a series of amino acid mimics.[14] However, there has been no theoretical characterization to date of either the barriers of the "reverse" hydrogen transfer (step **D**) or the phenomenon of substrate-analogue-induced suicide inactivation of EAL. Accordingly, we have used high-level ab initio calculations in the present contribution to examine the hydrogen-abstraction steps of ethanolamine ammonia-lyase and how they relate to substrate-analogue-induced suicide inactivation of this enzyme. Such studies, we hope, will further illuminate the specificity and functionality of EAL. As in previous work, we use small model systems to gain insight into the salient features of these events.[15,16] Also, following our recent work on the suicide inactivation of the coenzyme B_{12}-dependent enzyme, diol dehydratase (DDH),[17] we examine this phenomenon within the context of a catalytic mechanism, in this case when 2-aminoethanol is the substrate. On the basis of these results, we then examine the fate of the substrate analogues glycolaldehyde (section 2), 2-hydroxyethylhydrazine (section 3), and 2-aminoacetaldehyde (section 4) and examine how each is able to effect the suicide inactivation of EAL.

Theoretical Methodology

Standard ab initio[18] and density functional theory[19] calculations have been used for this study. Geometries and scaled vibrational frequencies have been obtained at the MPW1K/6-31+G(d,p) level of theory.[20] Relative energies were obtained with the high-level composite method G3(MP2)-RAD, which is an improved version[21a] of that originally proposed.[21b] Briefly, this implementation of the G3(MP2)-RAD methodology approximates the URCCSD(T)/G3MP2large level of theory by performing a series of single-point energy calculations on the MPW1K/6-31+G(d,p) geometries, a level of theory found recently to give good performance for abstraction reaction barriers.[22] The single-point energy calculations include URCCSD(T)/6-31G(d), RMP2/6-31G(d), and RMP2/ G3MP2large. Finally, a higher level correction, which has been optimized to accurately reproduce experimental thermochemical data, is included in the final energy expression.[21a] All energies at this level of theory refer to isolated molecules in the gas phase at 0 K.

Because the mechanism catalyzed by EAL involves the separation of charged species, specifically in the deamination step (step **E** in Scheme 1), some calculations were carried out using a polarizable continuum model (PCM) with a dielectric constant of 4.0 to approximate the weakly polar nature of the active site.[23] In the gas phase, any separation of charge will correspond to an unfavorable process and thus the use of a continuum solvation model may better describe the deamination step. We note at the outset that this correction significantly affects the deamination step alone, with only a small effect on the other steps of the reaction. The PCM solvation free energies were obtained using the integral equation formalism (IEF−PCM) as implemented in Gaussian 03[24] from calculations at the MPW1K/6-311+G(3df,2p)//MPW1K/6-31+G(d,p) level of theory.

For the hydrogen-abstraction steps of the EAL-catalyzed reactions, ethanol was chosen as a model for 5′-deoxyadenosine (Ado-CH_3). This choice is based on the results of a previous study that demonstrated it to be an adequate model for the hydrogen-abstraction steps of the coenzyme B_{12}-dependent enzymes,[25] in addition to providing a balance between computational cost and accuracy. We note that the transition structures obtained for the hydrogen-abstraction steps are not necessarily those of lowest energy for our model system. The lower energy structures in such cases are an artifact of strong intermolecular hydrogen bonding involving our ethanol model. To remedy this, we consistently choose conformations that avoid such interactions for each substrate.

Relative energies were obtained for each reaction scheme by maintaining a constant stoichiometry throughout the scheme. That is, the sum of the energies of the isolated species prior to any reaction is taken as the zero level and subsequent relative energies along the reaction coordinate are determined by maintaining the constant overall stoichiometry.

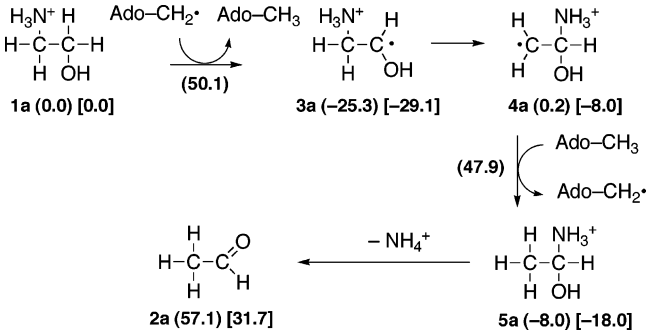
All calculations were performed with the MOLPRO 2002.6 program[26] and Revisions B.03 and B.05 of the Gaussian 03 program.[24] G3(MP2)-RAD total energies as well as Gaussian archive entries for the optimized species are provided in Tables S1 and S2 of the Supporting Information.

Results and Discussion

1. Mechanism for the Catalytic Substrate 2-Aminoethanol. The ability of EAL to catalyze the rearrangement and deamination of a natural substrate is exemplified by its reaction with 2-aminoethanol (**1a**, ethanolamine; Scheme 2).[1] We have chosen the fully protonated form of **1a** on the basis of pK_a arguments[27] and previous computational studies.[9,28] From Scheme 2, it can be seen that initial hydrogen abstraction at C1 of 2-aminoethanol (**1a**) by 5′-deoxyadenosyl radical needs to overcome a barrier

(13) Abend, A.; Bandarian, V.; Reed, G. H.; Frey, P. A. *Biochemistry* **2000**, *39*, 6250−6257.
(14) Semialjac, M.; Schwarz, H. *Chem.−Eur. J.* **2004**, *10*, 2781−2788.
(15) (a) Wetmore, S. D.; Smith, D. M.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 8678−8689. (b) Wetmore, S. D.; Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 7963−7972. (c) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 1664−1675. (d) Wetmore, S. D.; Smith, D. M.; Radom, L. *J. Am. Chem. Soc.* **2000**, *122*, 10208−10209. (e) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 9388−9399. (f) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 5700−5704. (g) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 1383−1384. (h) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 1037−1044.
(16) For a recent review, see: Smith, D. M.; Wetmore, S. D.; Radom, L. In *Theoretical Biochemistry−Processes and Properties of Biological Systems*; Eriksson, L. A., Ed.; Elsevier: Amsterdam, 2001; pp 183−214.
(17) Sandala, G. M.; Smith, D. M.; Coote, M. L.; Radom, L. *J. Am. Chem. Soc.* **2004**, *126*, 12206−12207.
(18) (a) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986. (b) Jensen, F. *Introduction to Computational Chemistry*; Wiley: New York, 1999.
(19) Koch, W.; Holthausen, M. C. *A Chemist's Guide to Density Functional Theory*; Wiley-VCH: Weinheim, Germany, 2000.
(20) (a) Lynch, B. J.; Fast, P. L.; Harris, M.; Truhlar, D. G. *J. Phys. Chem. A* **2000**, *104*, 4811−4815. (b) Lynch, B. J.; Truhlar, D. G. *J. Phys. Chem. A* **2001**, *105*, 2936−2941.
(21) (a) Henry, D. J.; Sullivan, M. B.; Radom, L. *J. Chem. Phys.* **2003**, *118*, 4849−4860. (b) Henry, D. J.; Parkinson, C. J.; Mayer, P. M.; Radom, L. *J. Phys. Chem. A* **2001**, *105*, 6750−6756.
(22) Coote, M. L. *J. Phys. Chem. A* **2004**, *108*, 3865−3872.
(23) Indeed, many theoretical investigations of enzyme-catalyzed reactions have successfully approximated the active site with a dielectric of 4.0. See, for example: Himo, F.; Siegbahn, P. E. M. *Chem. Rev.* **2003**, *103*, 2421−2456, and references therein.
(24) Frisch, M. J.; et al. *Gaussian 03*; Gaussian, Inc.: Pittsburgh, PA, 2003.
(25) Wetmore, S. D.; Smith, D. M.; Radom, L. *ChemBioChem* **2001**, *12*, 919−922.
(26) MOLPRO 2002.6 is a package of ab initio programs written by Werner, H.-J.; et al.
(27) Means, G. E.; Congdon, W. I.; Bender, M. I. *Biochemistry* **1972**, *11*, 3564−3571.
(28) Although we have generally used protonated forms of the substrates and substrate analogues in all the reaction schemes examined throughout this paper, we have omitted the "protonated" designation within the text for simplicity. However, the nature of the various species is given fully within the schemes.

Scheme 2. Proposed Mechanism for the EAL-Catalyzed Reaction of (Protonated) 2-Aminoethanol (Gas-Phase G3(MP2)-RAD Relative Energies in Parentheses and PCM-Corrected Values in Square Brackets, kJ mol^{-1})

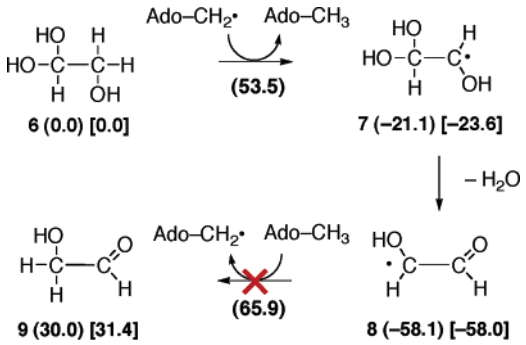


of 50.1 kJ mol^{-1} to generate 5′-deoxyadenosine and the substrate-derived 2-amino-1-hydroxyethyl radical (**3a**). This abstraction step is predicted to be exothermic by 25.3 kJ mol^{-1}. If initial hydrogen-atom abstraction were to occur from C2 of **1a** instead of C1, the corresponding endothermicity would be 12.8 kJ mol^{-1}, and thus this process is less likely to occur (data not shown). After formation of **3a**, rearrangement then occurs to give the product-related 2-amino-2-hydroxyethyl radical (**4a**). A detailed discussion of the salient features of this migration can be found elsewhere.[9] This step is calculated to be endothermic by 25.5 kJ mol^{-1}. We note that the endothermicity of this step is associated with the enhanced stabilization of **3a** resulting from the hydroxyl substituent at the radical center.[29] For the product-related radical **4a**, there is instead a destabilization caused by the β-hydroxyl group.[30] As noted in the Introduction, the alternative possibility of direct elimination from **3a** to give the acetaldehyde radical (instead of rearrangement of **3a** to give **4a**) is considered less likely. After the rearrangement to the product-related radical **4a**, hydrogen-atom reabstraction from Ado-CH_3 by **4a** is calculated to require 47.7 kJ mol^{-1}, with an associated exothermicity of 8.2 kJ mol^{-1}.

Note that the second barrier for hydrogen-atom reabstraction is slightly lower in energy than the initial hydrogen-atom abstraction barrier (47.7 kJ mol^{-1} vs 50.1 kJ mol^{-1}). To examine the effects of imposing a "push−pull-type" mechanism on these barriers, we have used NH_4^+ to protonate the migrating amino group and NH_3 to partially deprotonate the spectator hydroxyl group. Such a model has been successfully applied to the amine migration step for EAL.[9a,c] For the initial hydrogen-atom abstraction, we find that there is essentially no effect on the abstraction barrier when a push−pull mechanism using NH_4^+/NH_3 is employed, the barrier remaining at 50.1 kJ mol^{-1}. This value is in qualitative agreement with those of 58.6 and 61.5 kJ mol^{-1} obtained for an analogous push−pull mechanism by Semialjac and Schwarz in their more extensive investigation of the initial H-atom abstraction.[14] However, we find that the barrier for hydrogen-atom reabstraction slightly increases (by 5.2 kJ mol^{-1}) from the isolated model, bringing the barrier to 53.1 kJ mol^{-1}. Thus use of an improved model for the hydrogen-abstraction steps of the EAL-catalyzed reaction leads to a slightly increased reabstraction barrier relative to the initial abstraction.

(29) Henry, D. J.; Parkinson, C. J.; Mayer, P. M., Radom, L. *J. Phys. Chem. A* **2001**, *105*, 6750−6756.
(30) Henry, D. J.; Beckwith, A. L. J.; Radom, L. *Aust. J. Chem.* **2003**, *56*, 429−436.

Scheme 3. Proposed Mechanism for the EAL-Catalyzed Reaction of Ethane-1,1,2-triol (Gas-Phase Relative Energies in Parentheses and PCM-Corrected Values in Square Brackets, kJ mol^{-1})



In any case, following hydrogen-atom reabstraction from Ado-CH_3 by the product-related radical **4a**, enzyme-catalyzed deamination to form the product acetaldehyde (**2a**) plus ammonium cation occurs and is calculated to be endothermic by 65.1 kJ mol^{-1}. Because deamination involves charge separation, it is energetically unfavorable in the gas phase. Thus, we have also characterized this step using a polarizable continuum model (see Theoretical Methodology) and find that this reduces the endothermicity of the deamination step to 49.7 kJ mol^{-1}.

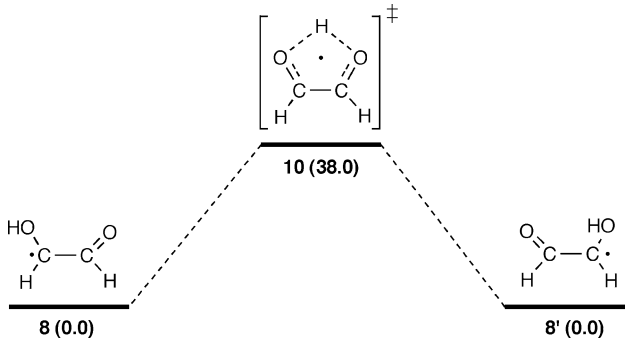
In summary, for the EAL-catalyzed transformation of 2-aminoethanol to acetaldehyde plus ammonium cation, we observe exothermic reactions for each of the hydrogen-abstraction steps. At the same time, the presence of a Brönsted acid (NH_4^+) (partially) protonating the migrating amino group and a Brönsted base (NH_3) (partially) deprotonating the spectator hydroxyl group influences the calculated barrier for the hydrogen reabstraction step. In addition, the migration of the amino group is an overall endothermic process, a result that can be attributed to reduced stabilizing effects at the radical center for the product-related radical. And finally, we calculate the deamination step to be endothermic, even in the presence of a model polar environment.

Having examined the catalytic features of this mechanism, we now turn our attention to reactions of analogues of the catalytic substrate, namely, glycolaldehyde (section 2), 2-hydroxyethylhydrazine (section 3), and 2-aminoacetaldehyde (section 4), to examine their contributions to the phenomenon of suicide inactivation. As noted earlier, these substances have been directly implicated in the suicide inactivation of EAL. It is therefore of interest to examine how and why these substances promote the termination of further EAL-catalyzed reactions.

2. Mechanism for the Substrate Analogue Glycolaldehyde. Recent EPR spectroscopic studies have revealed that glycolaldehyde rapidly causes the inactivation of both DDH and EAL.[13] In an attempt to elucidate the mechanism of the inactivation process for EAL with glycolaldehyde, we have calculated the barriers and enthalpy changes for the hydrogen-atom abstraction steps when the hydrate of glycolaldehyde, namely, ethane-1,1,2-triol (**6**), is the substrate (Scheme 3). Such a scheme parallels the approach that we recently used for diol dehydratase.[17]

The barrier for initial hydrogen abstraction from C2 of **6** by Ado-CH_2• is calculated to be 53.5 kJ mol^{-1}. The substrate-derived 1,2,2-trihydroxyethyl radical (**7**) is formed in this process, which is exothermic by 21.1 kJ mol^{-1}. We note in passing that hydrogen abstraction from C1 of **6** leads to a radical that lies 3.7 kJ mol^{-1} higher in energy than **7**. In any case, the

Scheme 4. Schematic Profile of the Degenerate Rearrangement of Glycolaldehyde Radical (**8**) (Gas-Phase G3(MP2)-RAD Relative Energies in Parentheses, kJ mol^{-1})



barrier and exothermicity of the hydrogen abstraction reaction from C2 of **6** are close to those found when **1a** is the substrate (50.1 and 24.4 kJ mol^{-1}, respectively; see Scheme 2). Apparently the presence of two hydroxyl groups on C1 in lieu of an amino moiety does not influence this step significantly.

While the next step in the catalytic mechanism involves a migration of the amino group (see Scheme 2), the analogous migration of a hydroxyl group for the 1,2,2-trihydroxyethyl radical **7** would merely generate an identical structure. An alternative mechanism involves the dehydration of **7** to generate the glycolaldehyde radical (**8**; Scheme 3). This is calculated to be exothermic by 37.0 kJ mol^{-1}. Further processing of the resulting **8** requires reabstraction of a hydrogen atom from Ado-CH_3 to regenerate Ado-$CH_2^{\bullet}$ and form glycolaldehyde (**9**). However, the calculated barrier for such an event is 124.0 kJ mol^{-1}, with an associated endothermicity of 88.1 kJ mol^{-1}! These results demonstrate the exorbitant energy cost of destroying the delocalized character of **8**, an expense that EAL cannot afford. Consequently, EAL is not able to regenerate Ado-$CH_2^{\bullet}$, and the EAL/cob(II)alamin complex may thus remain stable, under anaerobic conditions, for a period of several days.[13] The catalytic cycle is thus terminated.

At this point, we wish to digress slightly from the mechanism of suicide inactivation of EAL by glycolaldehyde in order to address the original interpretation[13] of the EPR spectra in terms of *cis*-ethanesemidione radical (**10**), and how this provides insight into the nature of the active site of EAL (a valuable exercise since the crystal structure has yet to be obtained for EAL). The anaerobic inactivated complex of the reaction between DDH or EAL, coenzyme B_{12}, and glycolaldehyde was found to include cob(II)alamin radical and an organic radical.[13] The organic radical species responsible for the inactivation process was identified as **10**. However, we recently found this symmetric form (**10**) to be a transition structure for the degenerate rearrangement of equivalent glycolaldehyde radical structures, lying 38.0 kJ mol^{-1} higher in energy than the glycolaldehyde radical **8** itself (Scheme 4).[17]

We suggested that the species observed in the EPR spectra probably corresponds to a complex between the glycolaldehyde radical **8** and an active site amino acid residue.[17] For DDH, whose crystal structure has been determined, an aspartic acid residue (Asp335) is a suitable candidate for this purpose.[31] Indeed, using the formate anion as a model for Asp335 of DDH, we found that the barrier for interconversion of equivalent glycolaldehyde radical structures is reduced from 38.0 to just 14.1 kJ mol^{-1} through such complexation.[17] Given that the incubation experiments were performed at room temperature prior to quenching the system to temperatures amenable to EPR (77 K), it is quite likely that an equilibrium is established which gives rise to the observed spectra for DDH. Because the EPR spectra observed in the glycolaldehyde-induced inactivation of EAL are virtually identical to those observed in the glycolaldehyde-induced inactivation of DDH, it is therefore reasonable to speculate that an equivalent amino acid residue (e.g., an Asp or Glu) exists within the active site of EAL.

This conjecture is in fact supported by earlier experimental work on EAL.[32] By treating the enzyme with a carboxyl-blocking reagent in the absence of substrate, it was found that after subsequent addition of substrate the residual activity of EAL was decreased by more than 80%. However, if the substrate was incubated with EAL prior to carboxyl-blocking reagent addition, EAL activity was not significantly diminished. These results suggest that an active-site carboxyl group is vital to the mechanism catalyzed by EAL. We note that the active site of the analogous coenzyme-B_{12}-dependent enzyme DDH includes His143, Glu170, Asp335, and Gln296.[31] For this enzyme, it has been postulated that the acid/base pair of His143 and Glu170 facilitate the migration step of the hydroxyl group.[10] In connection with the suicide inactivation of DDH, we postulated that Asp335 will facilitate partial deprotonation of glycolaldehyde radical, giving rise to the observed EPR spectra.[17] Accordingly, given that DDH and EAL operate on similar substrates, it may well be that EAL also contains such acid/base pairs. If this is indeed the case, a suitable acid/base pair would facilitate the migration step and then, in an analogous fashion to Asp335 of DDH, another (carboxylate) residue would be capable of (partially) deprotonating the glycolaldehyde radical. Overall, this proposal implies that the active sites of the coenzyme B_{12}-dependent enzymes DDH and EAL include conserved amino acid moieties that catalyze very similar reactions.

Having completed our digression, we conclude this section by summarizing our explanation for the suicide inactivation of EAL by glycolaldehyde and its hydrate. Our calculations suggest that a series of exothermic steps are exploited by EAL in order for **6** to reach **8**. The mechanistic essence of suicide inactivation of EAL lies with the overwhelming energy cost required to destroy the stabilization inherent in **8**, as exemplified by the very large endothermicity of the hydrogen reabstraction step (88.1 kJ mol^{-1}). Thus, it is the inability of the stabilized glycolaldehyde radical to reabstract the unactivated hydrogen atom from 5′-deoxyadenosine that results in this enzymatic stalemate.

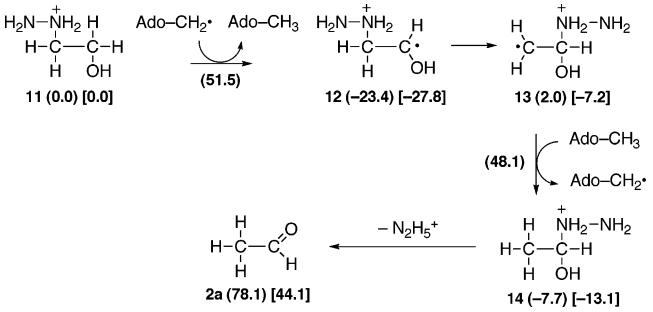
3. Mechanism for the Substrate Analogue 2-Hydroxyethylhydrazine. Reed and co-workers have recently established that 2-hydroxyethylhydrazine (**11**), another analogue of 2-aminoethanol, also causes the rapid, irreversible inactivation of EAL.[33] Incubation of EAL, coenzyme B_{12} (AdoCbl), and **11** leads to the homolysis of the Co−C bond of AdoCbl, and the products of this reaction were identified as acetaldehyde and the hydrazinium radical cation. Following similar arguments to

(31) Shibata, N.; Nakanishi, Y.; Fukuoka, M.; Yamanishi, M.; Yasuoka, N.; Toraya, T. *J. Biol. Chem.* **2003**, *278*, 22717−22725.

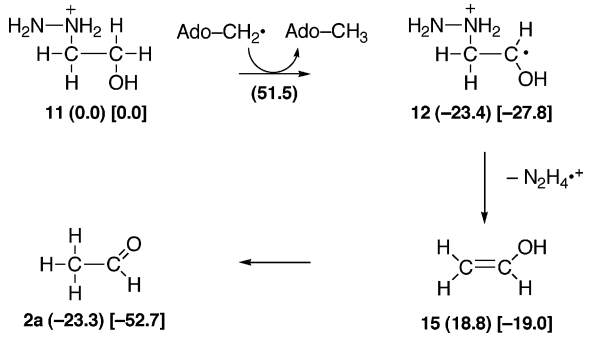
(32) Kopczynski, M. G.; Babior, B. M. *J. Biol. Chem.* **1984**, *259*, 7652−7654.

(33) (a) Bandarian, V.; Reed, G. H. *Biochemistry* **1999**, *38*, 12394−12402. (b) Bandarian, V.; Poyner, R. R.; Reed, G. H. *Biochemistry* **1999**, *38*, 12403−12407.

Scheme 5. Proposed Catalytic EAL Reaction of (Protonated) 2-Hydroxyethylhydrazine (Gas-Phase G3(MP2)-RAD Relative Energies in Parentheses and PCM-Corrected Values in Square Brackets, kJ mol^{-1})



Scheme 6. Proposed Mechanism for Suicide Inactivation of EAL by (Protonated) 2-Hydroxyethylhydrazine (Gas-Phase G3(MP2)-RAD Relative Energies in Parentheses and PCM-Corrected Values in Square Brackets, kJ mol^{-1})



those used when glycolaldehyde (or its hydrate) is the substrate, the inactivation process was attributed to the inability of the hydrazinium radical cation to recapture a hydrogen atom from Ado-CH_3. The result is the irreversible cleavage of the cofactor AdoCbl and tight, irreversible binding of the cob(II)alamin radical to EAL. Given our results for glycolaldehyde, such a mechanism indeed seems plausible. Nevertheless, to explore this point further, we have explicitly calculated the reaction profiles for the conversion of 2-hydroxyethylhydrazine to acetaldehyde plus the hydrazinium radical cation. However, before we examine this inactivation pathway, we first explore the consequences of imposing a *catalytic* mechanism for 2-hydroxyethylhydrazine turnover.

The catalytically imposed EAL reaction of 2-hydroxyethylhydrazine (**11**) is depicted in Scheme 5.[28] The barrier for initial hydrogen-atom abstraction at C1 of **11** by Ado-$CH_2^\bullet$ is calculated to be 51.5 kJ mol^{-1}. This reaction yields the substrate-derived radical **12**, with an exothermicity of 23.4 kJ mol^{-1}. We note that if hydrogen abstraction initially occurred at C2, the corresponding derived radical (not shown) lies 38.6 kJ mol^{-1} higher in energy than **12**. Consequently, the overall hydrogen-abstraction step would be endothermic (by 16.2 kJ mol^{-1}) and not consistent with the catalytic mechanism of 2-aminoethanol presented in Scheme 2. Therefore, initial hydrogen abstraction by Ado-$CH_2^\bullet$ is likely to occur from C1 of **11** to generate the substrate-derived radical **12** in an exothermic reaction. By comparison, the barrier and exothermicity when 2-aminoethanol is the substrate are calculated to be 50.1 and 25.3 kJ mol^{-1}, respectively. Thus, the change from an amino to a hydrazine group does not substantially influence this step.

Following the generation of the substrate-analogue-derived radical **12**, migration of the hydrazine moiety leads to the product-related radical **13**. This step is calculated to be endothermic by 25.4 kJ mol^{-1}. Again, this step does not differ significantly in its energy requirements from the corresponding step when **1a** is the substrate (endothermic migration of 25.5 kJ mol^{-1}).[34] After the migration step, the barrier for hydrogen-atom reabstraction from Ado-CH_3 by the product-related radical **13** is calculated to be 46.1 kJ mol^{-1} and the reaction is exothermic by 9.7 kJ mol^{-1}. Once more, we note the similarity of this step to the analogous barrier and exothermicity for 2-aminoethanol (47.7 and 8.2 kJ mol^{-1}, respectively). Finally, to complete the "catalytic" cycle, dehydrazination must occur, giving acetaldehyde and the hydrazinium cation. In the gas phase, this step is calculated to be endothermic by 85.8 kJ mol^{-1}, but this result is reduced to 57.2 kJ mol^{-1} when a polarizable continuum model (PCM) with a dielectric constant of 4.0 is employed to simulate the protein environment.

The energy requirements for each step of the catalytically imposed EAL reaction of **11** (Scheme 5) seemingly parallel those for when 2-aminoethanol is the substrate (Scheme 2). On this basis alone, it is thus quite surprising that EAL cannot facilitate the transformation of **11** to acetaldehyde plus the hydrazinium cation. Nevertheless, despite these striking energetic similarities, **11** is found to inactivate EAL, yielding acetaldehyde and the hydrazinium radical cation as products. Why is this the case? To try to answer this question, we now examine a reaction pathway by which these events might occur.

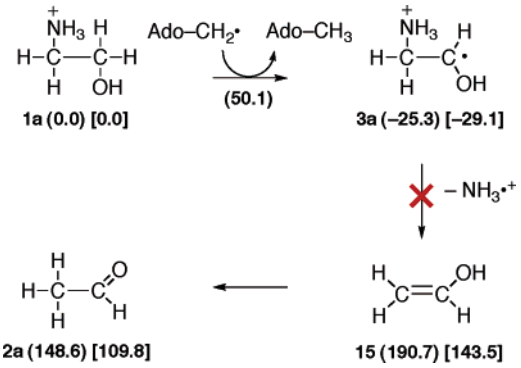
Scheme 6 illustrates a reaction profile that would lead to the suicide inactivation of EAL by **11**, with acetaldehyde and the hydrazinium radical cation as products. The initial step for this transformation is identical to that in Scheme 5, with a barrier and exothermicity of 51.5 and 23.4 kJ mol^{-1}, respectively. The alternative pathway shown in Scheme 6 involves the loss of the hydrazinium radical cation from the substrate-derived radical **12**, generating the observed hydrazinium radical cation and the enol form of acetaldehyde. In the gas phase, the loss of the hydrazinium radical cation from **12** is calculated to be endothermic by 42.2 kJ mol^{-1}.[35] However, using a PCM model the endothermicity of this step is reduced to just 8.8 kJ mol^{-1}. Thus, the dissociation of the hydrazinium radical cation from the substrate-derived radical **12** appears quite feasible, possibly during a failed migration process. Finally, conversion of the enol form of acetaldehyde to its keto counterpart is calculated to be exothermic by 42.1 kJ mol^{-1} in the gas phase and 33.7 kJ mol^{-1} if the effects of solvation are included.

The fact that the hydrazinium radical cation is experimentally observed implies that hydrogen-atom reabstraction by NH_2-$NH_2^{\bullet+}$ from Ado-CH_3 does not occur to a significant extent. To explore this further, we have calculated the enthalpy change

(34) We note that the alternative possibility of elimination of protonated hydrazine ($N_2H_5^+$) from **12** to yield the acetaldehyde radical is quite unfavorable, being endothermic by 75.0 kJ mol^{-1} (43.4 kJ mol^{-1} with PCM).

(35) Both Schemes 5 and 6 show 2-hydroxyethylhydrazine (**11**) to be protonated at the nonterminal nitrogen. The alternative isomer, protonated at the terminal nitrogen, is calculated to be only 1.1 kJ mol^{-1} higher in energy than **11**. The energy profiles associated with the "catalytic" mechanism involving this isomer (data not shown) are found to be quite similar to the values shown in Scheme 5. However, the loss of $NHNH_3^{\bullet+}$ from this alternative isomer is predicted to be endothermic by 149.5 kJ mol^{-1}, in contrast to the 42.2 kJ mol^{-1} shown in Scheme 6 for the loss of NH_2-$NH_2^{\bullet+}$ from **12**.

Scheme 7. Potential Mechanism of Ammonia Radical Cation Loss in the EAL-Catalyzed Reaction of (Protonated) 2-Aminoethanol (Gas-Phase G3(MP2)-RAD Relative Energies in Parentheses and PCM-Corrected Values in Square Brackets, kJ mol^{-1})



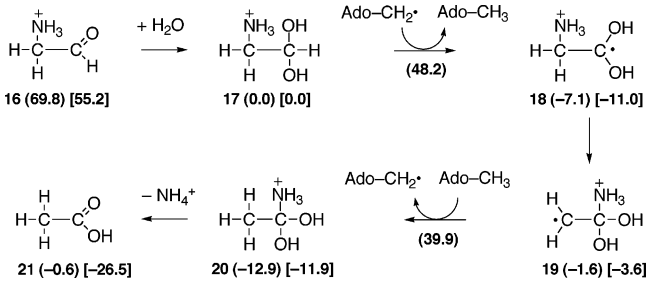
resulting from the hydrogen abstraction by the hydrazinium radical cation from Ado-CH_3 and find that this process is endothermic by 101.4 kJ mol^{-1}. This result is similar to that obtained for the hydrogen abstraction by the glycolaldehyde radical from Ado-CH_3 in the glycolaldehyde-induced suicide inactivation of EAL. In this latter case, the endothermicity is calculated to be 88.1 kJ mol^{-1} (section 2). Therefore, like the glycolaldehyde radical, the hydrazinium radical cation is too stabilized to initiate hydrogen-atom transfer from the unactivated methyl group of 5′-deoxyadenosine.

At this point it is instructive to ponder why the ammonia radical cation is not generated when 2-aminoethanol is the substrate. We examine this in Scheme 7.

Beginning with **1a**, the hydrogen abstraction by Ado-$CH_2^{\bullet}$ from C1 of **1a** is calculated to have a barrier of 50.1 kJ mol^{-1}, with an overall exothermicity of 25.3 kJ mol^{-1}, generating the substrate-derived radical **3a** (Scheme 7). Note that this step is identical to that in Scheme 2. At this stage, instead of the catalytically accessible migration of the amino group, the ammonia radical cation may dissociate from 2-aminoethyl radical (**3a**) to form the enol of acetaldehyde, which is mechanistically analogous to the corresponding step in the 2-hydroxyethylhydrazine-based suicide inactivation of EAL. While plausible in principle, it is immediately seen that the propensity for $NH_3^{\bullet+}$ to dissociate from **3a** is prohibited by the *very* large endothermicity (216.0 kJ mol^{-1}) calculated for this process. Even when a PCM model is employed, this step remains extremely endothermic (172.6 kJ mol^{-1}). The greater stability of $NH_2NH_2^{\bullet+}$ compared with $NH_3^{\bullet+}$ is due to a symmetrical stabilization of the radical-cation center by the adjacent nitrogen lone pair. Indeed, this phenomenon is reflected in the large differences between the calculated N−H bond dissociations energies for the hydrazinium (318.7 kJ mol^{-1}) and ammonium (511.6 kJ mol^{-1}) cations.

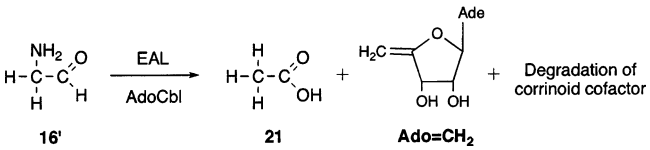
To conclude the discussion of the suicide inactivation of EAL by **11**, we note from Scheme 5 that a catalytic type mechanism is indeed energetically feasible, as evidenced by energy requirements comparable to those for the catalytic substrate 2-aminoethanol (Scheme 2). However, we propose that EAL does not commit itself to this pathway. Instead, during catalysis of 2-hydroxyethylhydrazine an overall lower energy exit channel, leading to acetaldehyde and the hydrazinium radical cation, is found. This contrasts with the scenario when glycolaldehyde is the substrate analogue (section 2), where only one pathway is

Scheme 8. Proposed Mechanism for the EAL-Catalyzed Reaction of (Protonated) 2-Aminoacetaldehyde (Gas-Phase G3(MP2)-RAD Relative Energies in Parentheses and PCM-Corrected Energies in Square Brackets, kJ mol^{-1})



possible. The very large endothermicity for the hydrogen-atom reabstraction by the hydrazinium radical cation from Ado-CH_3 epitomizes the enhanced stabilization of this radical. Thus, the 5′-deoxyadenosyl radical cannot be regenerated to recombine with the cob(II)alamin radical, which will remain tightly bound to EAL. The net result is the formation of acetaldehyde, the hydrazinium radical cation, and an enzyme that, in the absence of any reactivation, is inert.

4. Mechanism for the Substrate Analogue 2-Aminoacetaldehyde. It has been reported that the substrate analogue 2-aminoacetaldehyde (**16′**) can facilitate cleavage of the Co−C bond of adenosylcobalamin upon incubation with EAL.[36] These experiments characterized both acetic acid (**21**) and 4′,5′-anhydroadenosine (Ado=CH_2) as major products from the reaction, and also observed degradation of the corrinoid ring of the cofactor. Importantly, EPR experiments provided no evidence for species with unpaired electrons, and spectral analysis concluded that the corrinoid compound was not hydroxycob(III)alamin.[36]



These results are quite intriguing and suggest a complex reaction mechanism to account for the observed major products. In an attempt to provide some insight into the nature of this inactivation process, we have carried out appropriate calculations. We follow a similar approach to that used in sections 2 and 3 and develop the inactivation process of 2-aminoacetaldehyde within the context of the functional catalytic mechanism for 2-aminoethanol, originally presented in Scheme 2.

We begin the exploration of this suicide inactivation phenomenon between 2-aminoacetaldehyde (**16**),[28] AdoCbl, and EAL with a proposed mechanism for the transformation of **16** to **21** (Scheme 8). The process begins with the hydration of **16** to form 2-aminoethane-1,1-diol (**17**). This step is calculated to be exothermic by 69.8 kJ mol^{-1}. Adhering to the spirit of coenzyme B_{12}-dependent enzymes, hydrogen-atom abstraction from **17** by the Ado-$CH_2^{\bullet}$ is then postulated to occur, and this is also calculated to be exothermic (by 7.1 kJ mol^{-1}), generating the 2-amino-1,1-dihydroxyethyl substrate-derived radical (**18**). The barrier for this abstraction step is calculated to be 48.2 kJ

(36) Krouwer, J. S.; Schultz, R. M.; Babior, B. M. *J. Biol. Chem.* **1978**, *253*, 1041−1047.

mol^{-1}. Migration of the amino group during the transformation of the substrate-derived radical **18** to the product-related radical **19** is calculated to be slightly endothermic (by 5.5 kJ mol^{-1}). Again, as with 2-aminoethanol (Scheme 2) and 2-hydroxyethylhydrazine (Scheme 4), the migration step is endothermic due to decreased stabilization provided to the unsubstituted radical site in the product-related radical.[30] The barrier for hydrogen-atom reabstraction from Ado-CH_3 by the product-related radical **19** is calculated to be 41.5 kJ mol^{-1}, with the process being exothermic by 11.3 kJ mol^{-1}. Finally, deamination of 1-aminoethane-1,1-diol (**20**) generates the observed acetic acid product (**21**) in a process that is calculated to be endothermic by 12.3 kJ mol^{-1} in the gas phase and exothermic by 14.6 kJ mol^{-1} using a polarizable continuum model.

The mechanism proposed in Scheme 8 is energetically reminiscent of that outlined in Scheme 2 for the catalytic substrate, 2-aminoethanol. For both mechanisms, each hydrogen-atom abstraction is an exothermic process and the migration of the amino group is slightly endothermic. The similarities between these mechanisms therefore suggest that the proposal in Scheme 8 is indeed viable for EAL. On the basis of these results alone, the conversion of **16** by EAL ought to be catalytic, since the 5′-deoxyadenosyl radical is regenerated, yet this is not found to be the case experimentally. Additionally, while the mechanism depicted within Scheme 8 is consistent with the observation of acetic acid when 2-aminoacetaldehyde is the substrate, it does not account for the formation of Ado=CH_2 or the degradation of the corrinoid portion of the cofactor. Clearly, we are confronted with a more complicated picture.

One possible species that could bring about the oxidation of Ado-$CH_2^{\bullet}$ is a protein-bound radical. Indeed, such a proposal is not without precedent (though for different motives).[6] Even so, as stated in the Introduction, recent studies using the catalytic substrates 2-aminopropanol and 2-aminoethanol have implicated Ado-$CH_2^{\bullet}$ as the sole radical mediator in the hydrogen-abstraction steps for EAL.[5] And while substrate analogues may induce aberrant side reactions within the active site of EAL and create protein-bound radicals, there is no evidence for such behavior at present.

In this light, it is worthwhile to reflect on the various products derived from the catalytic and inactivation mechanisms presented in this article to appreciate how the products derived from aminoacetaldehyde differ (see Schemes 2, 3, 6, and 8). These variations may provide clues as to the nature of the subsequent oxidation of the 5′-deoxyadenosyl radical and degradation of the cofactor. One possibility is that the presence of an unbound carboxylic acid group, e.g. acetic acid, as the product derived from aminoacetaldehyde, might somehow play a role in the inactivation process.[37] Such speculation becomes even more tempting when one considers the synthetic model work of Schrauzer and co-workers, who, using 5′-deoxyadenosylcobaloximes as model systems for 5′-deoxyadenosylcobalamins, found that under basic conditions the species 4′,5′-anhydroadenosine and cob(I)alamin are produced.[38]

If indeed the presence of an unbound carboxylic group within the confines of the active site of EAL can elicit inactivation, it is conceivable that *any* unbound enzyme-based carboxylic acid group could potentially perform a similar task. Clearly, this is an undesirable result in terms of the accepted mechanism for the EAL-catalyzed reactions. To avoid this type of inactivation, it may be that EAL and perhaps all adenosylcobalamin-dependent enzymes inhibit this detrimental side reaction by occupying the active-site carboxylates with the responsibility of binding the substrate, in yet another manifestation of negative catalysis.[39] The results of experiments designed to examine these considerations will be of interest.

In summary, our investigation of the suicide inactivation of EAL by **16** suggests that EAL can bring about the conversion of **16** to acetic acid and ammonium cation. This mechanism is catalytic in the sense that Ado-$CH_2^{\bullet}$ is regenerated. However, details of the subsequent oxidation of Ado-$CH_2^{\bullet}$ and corrinoid ring degradation remain a mystery.

Concluding Remarks

In the present study we have examined the substrate-analogue-induced suicide inactivation of ethanolamine-ammonia lyase (EAL). Details of a catalytic mechanism for EAL are presented with the native substrate 2-aminoethanol in Scheme 2. We find that each hydrogen-abstraction step is an exothermic process. In addition, use of a push−pull mechanism with an acid at the migrating amino group and a base at the spectator hydroxyl group for the hydrogen-abstraction steps increases the barrier for hydrogen reabstraction but has little effect on the initial hydrogen-abstraction step.

The catalysis by EAL of the reaction of glycolaldehyde (or its hydrate) ultimately leads to the glycolaldehyde radical. The endothermicity of the hydrogen reabstraction step by the glycolaldehyde radical is calculated to be 88.1 kJ mol^{-1}. It is the inability of the glycolaldehyde radical to reabstract a hydrogen atom from the unactivated methyl group of 5′-deoxyadenosine that results in the suicide inactivation of EAL.

Interestingly, an artificially imposed catalytic mechanism for 2-hydroxyethylhydrazine reveals that the relative energies for each step are very similar to those calculated for the catalytically active 2-aminoethanol. Despite this energetically accessible route, however, EAL apparently chooses a lower energy pathway that leads to the generation of the hydrazinium radical cation and acetaldehyde. It is this alternative that results in the suicide inactivation of EAL. In this mechanism, in a manner similar to the glycolaldehyde-induced inactivation of EAL, formation of the highly stabilized hydrazinium radical cation terminates the catalytic cycle because it cannot perform the necessary hydrogen reabstraction step from 5′-deoxyadenosine.

The suicide inactivation process of EAL when 2-aminoacetaldehyde is the substrate follows a route that is vastly different from that of either glycolaldehyde or 2-hydroxyethylhydrazine. We calculate that the turnover of 2-aminoacetaldehyde to form acetic acid plus ammonium cation is catalytic *in its own right*. That is to say, Ado-$CH_2^{\bullet}$ is regenerated and is thus capable of recombining with the cob(II)alamin radical and freely dissociating from EAL. However, these events do not transpire experimentally. Instead, Ado-$CH_2^{\bullet}$ is oxidized to 4′,5′-anhydroadenosine, and the corrinoid ring is degraded. Details of these

(37) We note that neither the catalytic nor inactivation products from the related coenzyme B_{12}-dependent enzyme diol dehydratase contain carboxylic acid functional groups. It would be intriguing to see if there were similar consequences to the EAL situation, i.e., production of 4′,5′-anhydroadenosine and degradation of the cofactor, if an inactivator were used with DDH that included or produced a carboxylic acid.

(38) (a) Schrauzer, G. N.; Sibert, J. W. *J. Am. Chem. Soc.* **1970**, *92*, 1022−1030. (b) Katz, R. N.; Vickrey, T. M.; Schrauzer, G. N. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 542−543.

(39) Rétey, J. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 355−361.

subsequent reactions remain a mystery, though there is a possibility that the acetic acid product derived from 2-aminoacetaldehyde may contribute to the inactivation process.

Acknowledgment. We gratefully acknowledge the award (to G.M.S.) of an Australian National University International Postgraduate Research Scholarship, the award (to L.R.) of an Australian Research Council Discovery grant, the appointment (of D.M.S.) as a Visiting Fellow in the School of Chemistry, University of Sydney, and generous allocations of supercomputer time from the Australian Partnership for Advanced Computing, the Australian National University Supercomputing Facility, and the Australian Centre for Advanced Computing and Communications.

Supporting Information Available: GAUSSIAN archive entries of the MPW1K/6-31+G(d,p) geometries (Table S1), corresponding G3(MP2)-RAD total energies (Table S2), and full references where applicable (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA051527K