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Understanding the Mechanism of Action of B_{12}-Dependent Ethanolamine Ammonia-Lyase: Synergistic Interactions at Play

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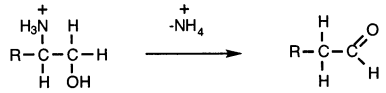
Abstract: Ab initio molecular orbital calculations are used to examine the mechanism of action of B_{12}-dependent ethanolamine ammonia-lyase involving the conversion of 2-aminoethanol to acetaldehyde plus ammonia. We attempt to elucidate the mechanism by which the enzyme facilitates this reaction through interactions between active-site residues and the substrate. Our calculations suggest a preferred pathway involving a 1,2-shift in the associated radical and also suggest that interactions between the enzyme and the migrating group of the substrate that afford an almost fully protonated migrating group will lead to the most efficient catalysis. However, this criterion on its own is insufficient to fully understand the rearrangement. Additional synergistic interactions between the spectator hydroxyl group in the substrate and active-site residues on the enzyme are required to lower the barrier height to a value consistent with experimental observations.

Introduction

Ethanolamine ammonia-lyase (EAL)[1−4] is a coenzyme-B_{12}-dependent enzyme that catalyzes the conversion of (protonated)[5] 2-aminoethanol (**1**) to ethanal (acetaldehyde, **2**) plus ammonium (equation 1):[6]



(1)

1 (R = H)
1a (R = CH_3)

2 (R = H)
2a (R = CH_3)

This enzyme is also known to catalyze the conversion of both enantiomers of (protonated) 2-aminopropanol (**1a**) to propionaldehyde (**2a**) plus ammonium.

The first step in the proposed pathway for the reactions catalyzed by ethanolamine ammonia-lyase is the homolytic cleavage of the Co−C bond in coenzyme-B_{12} to form a cobalt-centered radical plus a 5′-deoxyadenosyl radical (Ado·). The 5′-deoxyadenosyl radical then abstracts a hydrogen atom from protonated 2-aminoethanol (Scheme 1), forming the protonated 2-amino-1-hydroxyethyl radical (**3,** Scheme 1) plus 5′-deoxyadenosine (Ado-H). A protein radical has also been proposed to participate in the generation of **3**,[7] which may possibly explain the unusually large isotope effects measured for ethanolamine ammonia-lyase. Regardless of the exact mechanism for its generation, the substrate-derived radical (**3**) has two major possibilities for further reaction (Scheme 1). The first involves a 1,2-shift of the NH_3^+ group to give a product-related radical (**4**). This species then abstracts a hydrogen atom from 5′-deoxyadenosine to give protonated 1-aminoethanol (**5**), which may eliminate ammonium via heterolytic means to afford the product aldehyde (**2**). The alternative fate for the substrate-derived radical (**3**) is a direct elimination of ammonium to give the allyloxy radical (**6**), which may then abstract a hydrogen

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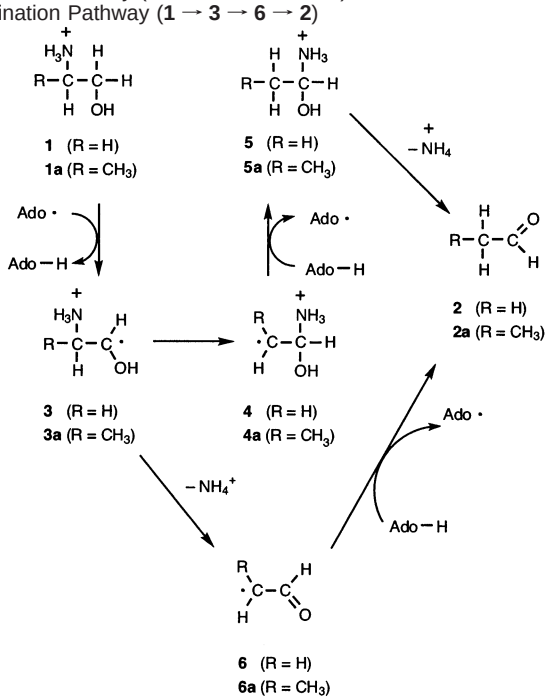
(1) (a) Babior, B. M. *BioFactors* **1988**, *1*, 21−26. (b) Babior, B. M. In *B_{12}*; Dolphin, D., Ed.; John Wiley & Sons: New York, 1982; pp 263−287.
(2) (a) Trommel, J. S.; Warncke, K.; Marzilli, L. G. *J. Am. Chem. Soc.* **2001**, *123*, 3358−3366. (b) Warncke, K.; Utada, A. S. *J. Am. Chem. Soc.* **2001**, *123*, 8564−8572. (c) Ke, S.-C.; Torrent, M.; Museav, D. G.; Morokuma, K.; Warncke, K. *Biochemistry* **1999**, *38*, 12681−12689. (d) Warncke, K.; Schmidt, J. C.; Ke, S.-C. *J. Am. Chem. Soc.* **1999**, *121*, 1, 10522−10528. (e) Ke, S.-C.; Warncke, K. *J. Am. Chem. Soc.* **1999**, *121*, 9922−9927.
(3) Bandarian, V.; Reed, G. H. In *Chemistry and Biochemistry of B_{12}*; Banerjee, R., Ed.; John Wiley & Sons: New York, 1999; pp 811−833.
(4) (a) LoBrutto, R.; Bandarian, V.; Magnusson, O. T.; Xiangyang, C.; Schramm, V. L.; Reed, G. H. *Biochemistry* **2001**, *40*, 9−14. (b) Bandarian, V.; Reed, G. H. *Biochemistry* **2000**, *39*, 12069−12075. (c) Bandarian, V.; Poyner, R. R.; Reed, G. H. *Biochemistry* **1999**, *38*, 12403−12407. (d) Bandarian, V.; Reed, G. H. *Biochemistry* **1999**, *38*, 12394−12402. (e) Abend, A.; Bandarian, V.; Nitsche, R.; Stupperich, E.; Rétey, J.; Reed, G. H. *Arch. Biochem. Biophys* **1999**, *1*, 138−141.
(5) (a) On the basis of the pK_a of ethanolamine of 9.5,[5b] we expect that the amino group of the substrate will be protonated and we therefore adopt such a model in our calculations as indicated. (b) Means, G. E.; Congdon, W. I.; Bender, M. I. *Biochemistry* **1972**, *11*, 3564−3571.
(6) (a) Frey, P. *Chem. Rev.* **1990**, *90*, 1343−1357. (b) Rétey, J. In *Chemistry and Biochemistry of B_{12}*; Banerjee, R., Ed.; John Wiley & Sons: New York, 1999; pp 271−287. (b) Golding, B. T.; Anderson, R. J.; Ashwell, S.; Edwards, C. H.; Garnett, I.; Kroll, F.; Buckel, W. In *Vitamin B_{12} and B_{12} Proteins*; Krautler, B., Arigoni, D., Golding, B. T., Eds.; Wiley-VCH: Weinheim, 1998; pp 201−216. (c) Golding, B. T.; Buckel, W. In *Comprehensive Biological Catalysis*; Sinnott, M. L., Ed.; Academic Press: London, 1997; pp 239−259. (d) Golding, B. T. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, Chapter 15.
(7) O'Brien, R. J.; Fox, J. A.; Kopczynski, M. G.; Babior, B. M. *J. Biol. Chem.* **1985**, *260*, 16131−16136.

10.1021/ja027579g CCC: $22.00

Scheme 1. Proposed Mechanisms for the Ethanolamine-Ammonia-Lyase-Catalyzed Reaction Involving the (Protonated) 2-Amino-1-hydroxyethyl Radical (**3**) and Including the Migration Pathway (**1** → **3** → **4** → **5** → **2**) and the Direct Elimination Pathway (**1** → **3** → **6** → **2**)



atom from 5′-deoxyadenosine to form the product directly (without the intervening recombination of ammonium).

Both possible mechanisms are consistent with available experimental evidence. In elegant ESR studies, Warncke and co-workers[2] have associated part of the observed spectrum with a product-related radical, but a distinction between **4** and **6** is not possible.[2d] The migration pathway (**1** → **3** → **4** → **5** → **2**, Scheme 1) is supported by speculation that recombination of ammonia at the adjacent carbon (i.e., fragmentation−recombination) may be enhanced by groups at the active site that prevent full separation of the relevant species.[6a] Additionally, the analogous pathway has been demonstrated experimentally for diol dehydratase.[8] On the other hand, the direct elimination route (**1** → **3** → **6** → **2**, Scheme 1) has chemical precedence from solution studies of model radical-mediated elimination reactions,[6d,9] which show that α-hydroxy radicals with a leaving group in the β-position undergo a similar fragmentation process. An important distinction between the two mechanisms arises in the step in which a hydrogen atom is abstracted from 5′-deoxyadenosine (Ado-H). The position from which this hydrogen needs to be abstracted is unactivated and therefore one would expect that a radical with a higher reactivity is required to accomplish the abstraction more readily. In the direct elimination pathway, the relevant radical (**6**) is stabilized by conjugative interaction with the carbonyl double bond and hence would not be particularly reactive. The analogous radical in the migration pathway (**4**), however, does not receive any special stabilization and could consequently be considered more likely to abstract the unactivated hydrogen atom from 5′-deoxyadenosine (Ado-H).

In the current work, we use high-level ab initio calculations to study the reaction catalyzed by ethanolamine ammonia-lyase. As in previous work,[10,11] we use ab initio calculations on small model systems to accomplish this analysis. We initially focus on the relative reactivity of radicals **4** and **6**. On the basis of these results, we conclude that the migration pathway (**1** → **3** → **4** → **5** → **2**) is more likely than the direct elimination (**1** → **3** → **6** → **2**). The remainder of our efforts then focus on understanding the possible roles for the enzyme within the confines of the radical rearrangement mechanism.

After the current work was substantially complete, we became aware of a parallel computational study of the mechanism of ethanolamine ammonia-lyase by Semialjac and Schwarz.[12] The emphasis of this latter work is a comparison between the rearrangement and direct elimination pathways rather than on the detailed role of the enzyme in the rearrangement. The work also concludes that the migration pathway (**1** → **3** → **4** → **5** → **2**) is more likely than the direct elimination route (**1** → **3** → **6** → **2**), though either is deemed to be possible. Thus, the reader is encouraged to consult this second study as a complementary reference to the results presented herein.

Computational Details

Standard ab initio[13] and density functional theory[14] calculations were carried out using the GAUSSIAN 98,[15] MOLPRO-98,[16a] and MOLPRO-2000[16b] programs. In accordance with our other recent studies of the

(8) (a) Rétey, J.; Robinson, J. A. In *Stereospecificity in Organic Chemistry and Enzymology;* Ebel, H. F., Ed.; Verlag Chemie: Weinheim, 1982; pp 185−207. (b) Arigoni, D. In *Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} Intrinsic Factor*; Zagalak, B., Friedrich, W., Eds.; W de Gruyter: Berlin, 1979; pp 389−410. (c) Rétey, J.; Umani-Ronchi, A.; Seibl, J.; Arigioni, D. *Experientia* **1966**, *22*, 502. (d) Rétey, J.; Umani-Ronchi, A.; Arigoni, D. *Experientia* **1966**, *22*, 72−73.

(9) (a) Buley, A. L.; Norman, R. O. C.; Pritchett, R. J. *J. Chem. Soc. B* **1966**, 849−852. (b) Walling, C.; Johnson, R. A. *J. Am. Chem. Soc.* **1975**, *97*, 2405−2407.

(10) (a) Wetmore, S. D.; Smith, D. M.; Radom, L. *ChemBioChem* **2001**, *2*, 919−922. (b) Wetmore, S. D.; Smith, D. M.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 8678−8689. (c) Wetmore, S. D.; Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 7963−7972. (d) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 1664−1675. (e) Wetmore, S. D.; Smith, D. M.; Radom, L. *J. Am. Chem. Soc.* **2000**, *122*, 10208−10209. (f) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 9388−9399. (g) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 5700−5704. (h) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 1383−1384. (i) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 1037−1044.

(11) For a recent review, see: Smith, D. M.; Wetmore, S. D.; Radom, L. In *Theoretical Biochemistry - Processes and Properties of Biological Systems*; Ericksson, L. A., Ed.; Elsevier: Amsterdam, 2001; pp 183−214.

(12) Semialjac, M.; Schwarz, H. *J. Am. Chem. Soc.* **2002**, *124*, 8974−8983.

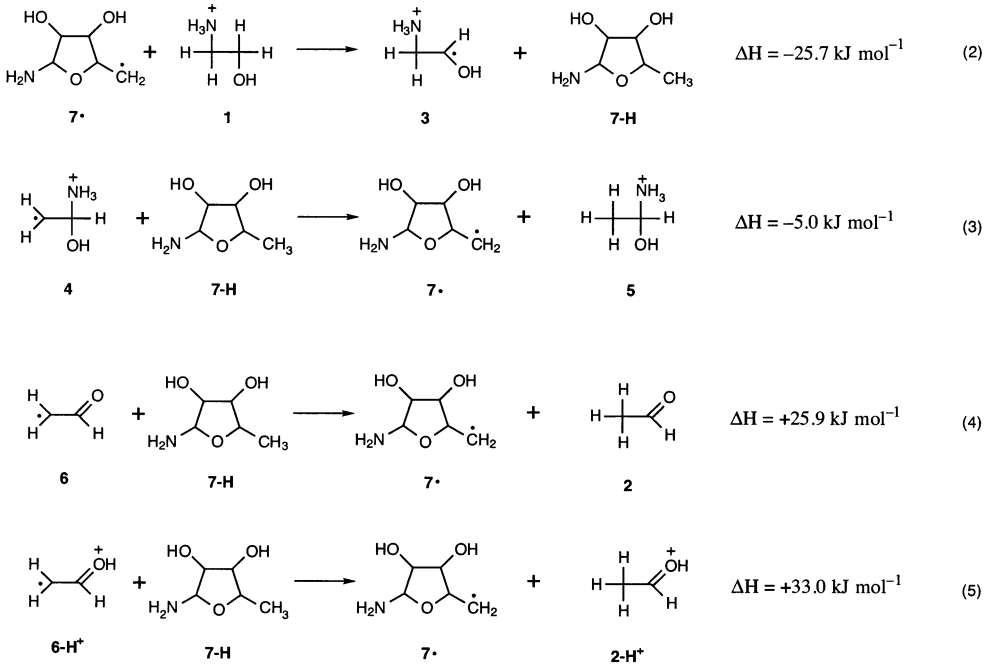
(13) (a) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986. (b) Jensen, F. *Introduction to Computational Chemistry*; Wiley: New York, 1999.

(14) Koch, W.; Holthausen, M. C. *A Chemist's Guide to Density Functional Theory*; Wiley-VCH: Weinheim, 2000.

(15) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; J. A. Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *GAUSSIAN 98*, Revision A.7; Gaussian Inc.: Pittsburgh, PA, 1998.

(16) (a) MOLPRO 98 is a package of ab initio programs written by Werner, H.-J.; Knowes, P. J. with contributions from Amos, R. D.; Berning, A.; Cooper, D. L.; Deegan, M. J. O.; Dobbyn, A. J.; Eckert, F.; Elbert, S. T.; Hampel, C.; Lindh, R.; Lloyd, A. W.; Meyer, W.; Nickless, A.; Peterson, K.; Pitzer, R.; Stone, A. J.; Taylor, P. R.; Mura, M. E.; Pulay, P.; Schütz, M.; Stoll, H.; Thorsteinsson, T. (b) MOLPRO 2000 is a package of ab initio programs written by Werner, H.-J. and Knowles, P. J with contributions from Amos, R. D.; Bernhardsson, A.; Berning, A.; Celani, P.; Cooper, D. L.; Deegan, M. J. O.; Dobbyn, A. J.; Eckert, F.; Hampel, C.; Hetzer, G.; Korona, T.; Lindh, R.; Lloyd, A. W.; McNicholas, S. J.; Manby, F. R.; Meyer, W.; Mura, M. E.; Nicklass, A.; Palmieri, P.; Pitzer, R.; Rauhut, G.; Schütz, M.; Stoll, H.; Stone, A. J.; Tarroni, R.; Thorsteinsson, T.

Scheme 2. Isodesmic Reactions Modeling the Hydrogen-Transfer Steps of the Migration and Direct Elimination Pathways Including Enthalpies (kJ mol^{-1}) Obtained with G3(MP2)-RAD(p)



reactions catalyzed by a variety of B_{12}-dependent enzymes,[10a−c] the geometries and zero-point vibrational energies for neutral and positively charged species were obtained using UB3-LYP/6-31G(d,p). Relative energies (at 0 K) were obtained using a previously defined G3(MP2)-RAD(p) procedure.[10c] This technique is a modification of the G3(MP2) method[17] in which a restricted-open-shell coupled-cluster calculation (URCCSD(T)/6-31G(d)) replaces the UQCISD(T)/6-31G(d) computation, and the basis set extension is evaluated with restricted-open-shell perturbation theory (RMP2) rather than with the unrestricted formalism (UMP2). The geometries and frequencies are obtained with UB3-LYP/6-31G(d,p) rather than with UHF/6-31G(d) or UMP2/6-31G(d).

Since it has been found that the reliability of G2- and G3-type theories for anionic systems is improved by direct inclusion of diffuse functions, rather than relying on the additivity approximation,[18] the G3(MP2)(+)-RAD(p) method is used here for the negatively charged systems. In this modification, the 6-31G(d) basis set in the G3(MP2)-RAD(p) formalism is replaced by the slightly larger 6-31+G(d) basis set. We find that including diffuse functions can also have a significant effect on the geometry and frequency calculations for the negatively charged systems investigated in the present work, and thus the UB3-LYP/6-31+G(d,p) procedure is used to obtain these properties for the anionic species.

Results and Discussion

A. The Difference in Radical Reactivities. As outlined in the introductory remarks, the relative stabilities of the various radicals involved in the proposed mechanisms may be important in discriminating between possible pathways. Although it is possible to measure such stabilities via bond dissociation energies (BDEs) or radical stabilization energies (RSEs),[19] a more transparent measure in the current application is available through the construction of appropriate isodesmic reactions, such as those shown in Scheme 2. In this Scheme, as in previous work,[10c] we have used 2-amino-5-methyltetrahydrofuran-3,4-diol (**7-H**) as a model for 5′-deoxyadenosine (Ado-H). The enthalpies of the reactions in Scheme 2 should provide an indication as to the thermodynamics governing possible hydrogen-transfer steps involved in the EAL rearrangement.

The results in Scheme 2 show that the proposed hydrogen transfer from the substrate (**1**) to the 5′-deoxyadenosyl radical (Ado•) (i.e., reaction 2) is exothermic by 25.7 kJ mol^{-1}. This result is expected because of the stabilizing influence of the α-hydroxy substituent in the substrate-derived radical (**3**). As this step is present in both the migration and direct elimination mechanisms, the exothermicity is encouraging for both but does not help discriminate between the two pathways.

An important distinction between the migration and direct elimination pathways comes in the step involving hydrogen reabstraction from the unactivated methyl group of 5′-deoxyadenosine. In the migration mechanism, the (protonated) 2-hydroxy-2-aminoethyl radical (**4**) accomplishes this task. Scheme 2 shows that this hydrogen transfer (reaction 3) is exothermic, albeit by only 5.0 kJ mol^{-1}. In the direct elimination mechanism, there are two alternative radicals that could accept the hydrogen atom from the coenzyme. The first, which results from the direct elimination of NH_4^+ from the substrate-derived radical, is the allyloxy radical (**6**). Scheme 2 shows that the transfer of hydrogen to this radical (reaction 4) is endothermic by 25.9 kJ mol^{-1}. The second alternative, which results from the loss of NH_3 from the substrate-derived radical, is the vinyl alcohol radical cation (**6-H**$^+$). The hydrogen transfer involving this species (reaction 5) is even more endothermic (33.0 kJ mol^{-1}) than that involving **6**.

The results in Scheme 2 give rise to one measure of discrimination between the NH_3^+ migration and the direct elimination mechanisms. The key fact is that, as part of the initial activation of the substrate by the coenzyme, a relatively inert methyl group is produced in Ado-H, from which a hydrogen atom must be removed at some later point during the reaction. A reactive radical such as the protonated 2-hydroxy-

(17) Curtiss, L. A.; Redfern, P. C.; Raghavachari, K.; Rassolov, V.; Pople, J. A. *J. Chem. Phys.* **1999**, *110*, 4703−4709.
(18) Gronert, S. *J. Am. Chem. Soc.* **1993**, *115*, 10258−10266.
(19) See, for example: Henry, D. J.; Parkinson, C. J.; Mayer, P. M.; Radom, L. *J. Phys. Chem. A* **2001**, *105*, 6750−6756.

2-aminoethyl radical (**4**) would seem to be well-suited for this task. On the other hand, a stabilized radical such as **6** or **6**-H^+ may simply not be reactive enough to recapture the hydrogen atom and thus participate in the catalytic cycle.

Such a conclusion is supported by recent experimental studies on the suicide inactivation of ethanolamine ammonia-lyase by glycolaldehyde.[20] This substrate analogue inactivates the complex of EAL with coenzyme B_{12} within a few minutes. EPR studies indicate the formation of the cobalt-centered radical (B_{12r} or cob(II)alamin) and an organic radical in the same time frame. This EPR-active complex is stable for several days under anaerobic conditions and does not react further. Isotopic labeling experiments led to the assignment of the organic radical as the ethanesemidione radical, a resonance-stabilized radical that is structurally similar to the allyloxy radical (**6**). It was concluded that the suicide inactivation arises because this delocalized radical "does not possess sufficient oxidizing capacity to recapture a H-atom from 5′-deoxyadenosine".[20]

However, the above results are not definitive in ruling out the direct elimination mechanism. They simply provide a plausible argument why the mechanism involving NH_3^+ migration could well be preferred. For a thorough investigation of the elimination mechanism, as well as many other important facets of the ethanolamine-ammonia-lyase-catalyzed reactions, the reader is referred to the article of Semialjac and Schwarz.[12] For the remainder of the present work, we will focus on the migration mechanism. We aim to shed light on the possible influence of enzyme functional groups on the important radical rearrangement step.

B. The Unassisted 1,2-NH_3^+-Shift. The pK_a of 2-aminoethanol (9.5)[5b] is sufficiently large that at neutral pH, or within the active site of ethanolamine ammonia-lyase, we expect the amino group of the substrate to be protonated. In addition, the calculated proton affinities for (closed-shell) 2-aminoethanol (921.6 kJ mol^{-1}) and the (open-shell) 2-amino-1-hydroxyethyl radical (921.1 kJ mol^{-1}) are sufficiently similar that the substrate-derived radical is also expected to be protonated. Therefore, we begin our examination of the radical rearrangement step of the migration mechanism by considering the migration of NH_3^+ in the protonated 2-amino-1-hydroxyethyl radical (**3**) to form the protonated 2-amino-2-hydroxyethyl radical (**4**).

The rearrangement of **3** proceeds through an intramolecular pathway with a barrier of 65.8 kJ mol^{-1} (see Figure 1 and Table 1). The reaction is endothermic by 28.4 kJ mol^{-1}. The presence of the "spectator" hydroxyl group has a substantial effect on the reaction. Specifically, the barrier for the 1,2-NH_3^+ shift in the protonated 2-amino-1-hydroxyethyl radical (65.8 kJ mol^{-1}) is significantly less than that for the analogous shift in the unsubstituted protonated 2-aminoethyl radical (104.8 kJ mol^{-1}).[10e]

An estimate of the barrier for the rate-limiting step in the ethanolamine-ammonia-lyase-catalyzed reaction may be obtained from the value of $k_{cat} = 55$ s^{-1} [21] and assuming reasonable values for the entropy.[22] This suggests that the barrier for the rate-limiting step should lie between approximately 60 and 75 kJ mol^{-1} (see also refs 1b, 2d, and 12). Furthermore, experimental evidence suggests that the hydrogen-atom-transfer step leading to product formation is rate limiting.[23] On this basis, it would seem that, because the barrier for the direct migration of an amino group from the protonated 2-amino-1-hydroxyethyl radical (**3**) (65.8 kJ mol^{-1}) fits nicely within the lower end of the calculated range, no further investigation is necessary. However, during our study, we found several reasons to question such a conclusion, as discussed below.

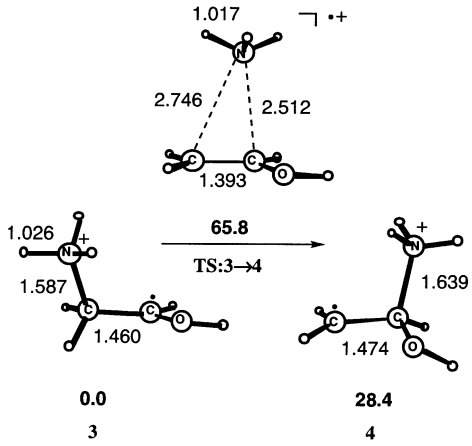


Figure 1. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for the species involved in the rearrangement of the protonated 2-amino-1-hydroxyethyl radical (**3**). Relative energies (kJ mol^{-1}) obtained with G3-(MP2)-RAD(p) (bold type).

Table 1. G3(MP2)-RAD(p) Relative Energies (kJ mol^{-1}) for the 1,2-Amino Shift in the Protonated 2-Amino-1-hydroxyethyl Radical (**3**), and the Relative Energies for the Rearrangement Mediated by Interactions between the Migrating Group and Bases (X) of Varying Strength (See Eq 6 and Figures 1−4)

species	relative energy	species	relative energy
3	0.0	**3-NH_2CH_3**	0.0
TS:3→4	65.8	**TS:3-NH_2CH_3→4-NH_2CH_3**	103.2
4	28.4	**4-NH_2CH_3**	24.7
3-HF	0.0	**8**	0.0
TS:3-HF→4-HF	78.7	**TS:8→9**	96.5
4-HF	27.2	**9**	74.6
		TS:9→10	91.4
3-OH_2	0.0	**10**	−8.0
TS:3-OH_2→4-OH_2	90.9		
4-OH_2	27.3		
3-NH_3	0.0		
TS:3-NH_3→4-NH_3	99.1		
4-NH_3	26.2		

Although the amino acid sequence has been determined for ethanolamine ammonia-lyase,[24] the X-ray crystal structure has yet to be solved. Therefore, the amino acids that may be involved in binding of the substrate at the active site of the enzyme remain unidentified. However, one probable mode for binding the positively charged amino group would be through interaction with a hydrogen-bond acceptor. Our previous work[10c] has indicated that, depending on the strength of the acceptor, such binding will lead to some degree of deprotonation of the (protonated) amino group of the substrate, and we now explore such interactions for the present situation.

C. The Effect of Interactions with the Migrating NH_3^+ Group. The effects of a putative hydrogen-bond acceptor at

(20) Abend, A.; Bandarian, V.; Reed, G. H.; Frey, P. *Biochemistry* **2000**, *39*, 6250−6257.
(21) Faust, L. P.; Babior, B. M. *Arch. Biochem. Biophys.* **1992**, *294*, 50−54.
(22) George, P.; Glusker, J. P.; Bock, C. W. *J. Am. Chem. Soc.* **1997**, *119*, 7065−7074.
(23) (a) Weisblat, D. A.; Babior, B. M. *J. Biol. Chem.* **1971**, *246*, 6064−6071. (b) Babior, B. M. *J. Biol. Chem.* **1969**, *244*, 449−456.
(24) Faust, L. P.; Connor, J. A.; Roof, D. M.; Hoch, J. A.; Babior, B. M. *J. Biol. Chem.* **1990**, *265*, 12462−12466.

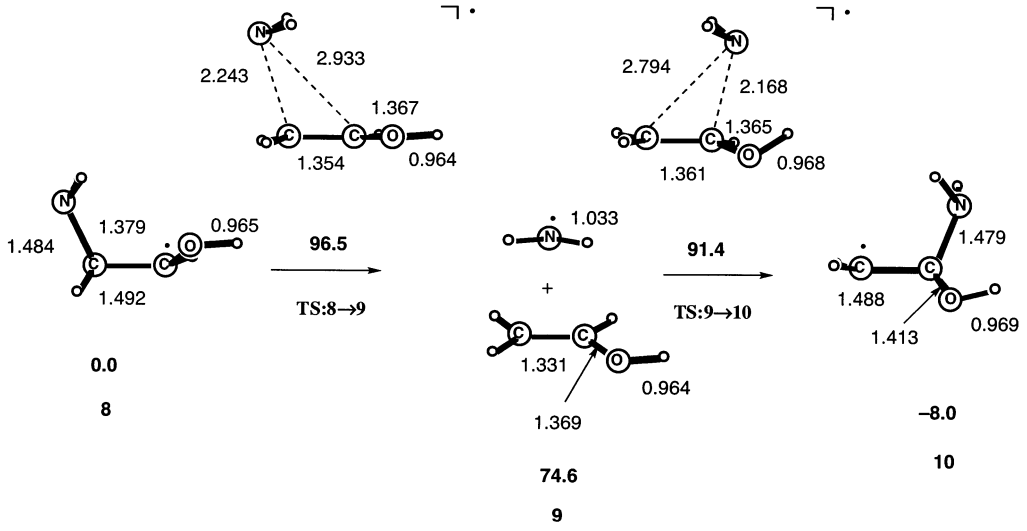


Figure 2. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in NH_2 migration in the (neutral) 2-amino-1-hydroxyethyl radical (**8**). Relative energies (kJ mol^{-1}) obtained with G3(MP2)-RAD(p) (bold type).

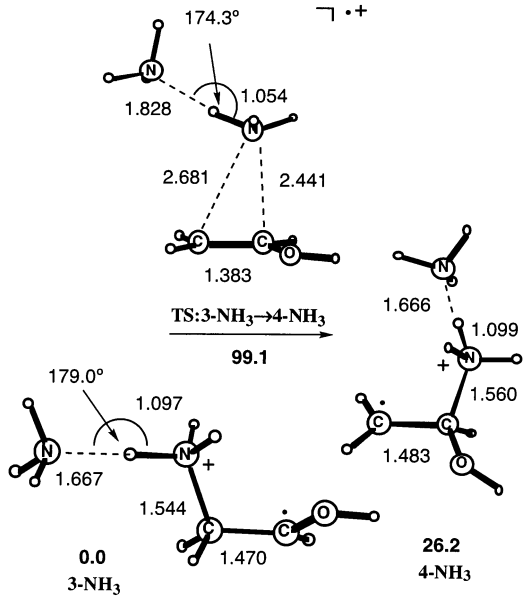


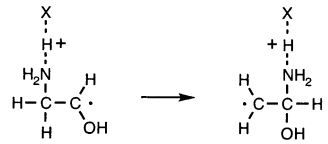
Figure 3. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in NH_3^+ migration in the protonated 2-amino-1-hydroxyethyl radical (**3**) mediated by NH_3 (see eq 6). Relative energies (kJ mol^{-1}) obtained with G3(MP2)-RAD(p) (bold type).

the active site of ethanolamine ammonia-lyase on the migration are best taken into account by considering varying degrees of deprotonation of the amino group. It is most convenient to begin with the extreme case of full deprotonation.

Previous calculations on the 1,2-amino shift in the 2-aminoethyl radical indicate that the barrier for migration of an NH_2 group (90.5 kJ mol^{-1}) is lower than the barrier for migration of an NH_3^+ substituent (104.8 kJ mol^{-1}).[10e] Thus, in this related system, full deprotonation of the migrating group is able to reduce the rearrangement barrier. We have also previously shown that the rearrangement of (*S*)-glutamate catalyzed by glutamate mutase can benefit from deprotonation of the NH_3^+ substituent in the migrating group.[10c]

In contrast to the results for these related systems, we find the barrier to migration for an NH_2 group (96.5 kJ mol^{-1}) in the (neutral) 2-amino-1-hydroxyethyl radical (**8**, Figure 2) to be 30.7 kJ mol^{-1} *higher* than the barrier for migration of the protonated amino group. Additionally, in contrast to the migration of NH_3^+, the migration of NH_2 in **8** is predicted to occur through a fragmentation−recombination pathway where the reaction intermediate is essentially the fully separated amino radical plus vinyl alcohol (collectively referred to as **9**). Semialjac and Schwarz report a barrier of 327.6 kJ mol^{-1} for the direct migration of the neutral NH_2 group in **8**.[12] Although we were unable to characterize this pathway, clearly a step with such a high barrier is unlikely to play a role in the enzyme-catalyzed reaction. In comparison, migration of a (neutral) hydroxyl substituent in the diol-dehydratase-catalyzed reaction does occur through a direct intramolecular pathway. [10d]

Despite the fact that full deprotonation of the migrating NH_3^+ group significantly increases the rearrangement barrier, it is unlikely that a putative hydrogen-bond acceptor would be able to fully deprotonate the NH_3^+ group. Therefore, calculations involving *partial* deprotonation are perhaps more relevant. To address this issue, we have investigated the rearrangement in a series of models where the NH_3^+ migrating group interacts with bases (X) of varying strength (equation 6):



(6)

Models of the form shown in eq 6 correspond to the migration of NH_3^+ under the influence of partial deprotonation. In a manner similar to that for the isolated migration of NH_3^+, we find that the reactions described by eq 6 proceed through intramolecular rearrangement routes involving cyclic transition structures (see, for example, the rearrangement with X = NH_3, Figure 3). In all cases, the proton involved in the hydrogen bond with the base X (equation 6) remains localized on the migrating group throughout the reaction (see, for example, Figure 3). We note a contraction of the appropriate N−H bond in the migrating group in the transition structures, compared with the reactant and product radicals. This demonstrates the higher proton affinity of the migrating group at the transition structure, a characteristic typical of partial-proton transfer.[10h]

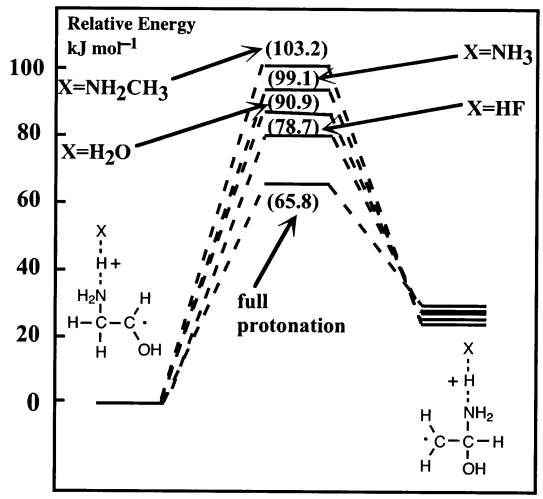


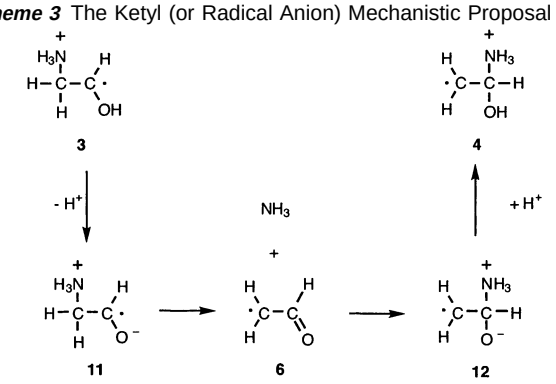
Figure 4. Schematic energy profile for the 1,2-shift of the NH_3^+ group in the protonated 2-amino-1-hydroxyethyl radical (**3**) mediated by interactions between the migrating group and bases (X) of varying strengths (see eq 6). Relative energies (kJ mol^{-1}) are obtained with G3(MP2)-RAD(p).

For the series of models represented by eq 6, the rearrangement barrier depends on the strength of the base interacting with the migrating group (Figure 4 and Table 1). Additionally, even the weakest base (HF) leads to a significant increase (12.9 kJ mol^{-1}) in the rearrangement barrier over the case of no protonation. It is interesting that the barriers increase so substantially from the fully protonated value despite the fact that, even in the interaction with the strongest base, the structure of the migrating group appears to closely resemble that involved in the fully protonated rearrangement.

Interestingly, the rearrangement barriers for **3** when it interacts with the strongest bases investigated in the present work (NH_3 and NH_2CH_3), are larger than the barrier associated with NH_2 migration in **8** (Table 1). That is, partial deprotonation leads to a *larger* increase in barrier than full deprotonation. This apparent discrepancy may be associated with differences in the rearrangement pathway, that is, fragmentation−recombination in the unprotonated pathway (Figure 2) versus an intramolecular pathway for partially or fully protonated migrating groups (Figure 3). We were not successful in locating transition structures for the alternative pathway in either case. This presumably indicates that these reactions proceed *either* through a two-step fragmentation−recombination type mechanism (as for **8**) *or* a one-step intramolecular rearrangement mechanism (as for models based on eq 6, e.g., **3-NH$_3$**).

Despite expectations that the enzyme binds the charged amino group with a hydrogen-bond acceptor, leading to partial deprotonation, our results suggest that the most efficient rearrangement of the substrate will occur when the migrating amino group is fully protonated. This implies that such binding to a hydrogen-bond acceptor would be anticatalytic, even with a very weak acceptor. Indeed, our calculated barriers quickly become too high to be consistent with experimental results upon consideration of interactions at the migrating group. This unfavorable state of affairs is in obvious contrast to the suggested mechanisms of action of methylmalonyl-CoA mutase,[10f] glutamate mutase,[10c] and diol dehydratase,[10d] where the most beneficial mode of binding also contributes to the catalysis of the radical rearrangement step. For example, in methylmalonyl-CoA mutase, the electronegative carbonyl oxygen of the thioester group

Scheme 3 The Ketyl (or Radical Anion) Mechanistic Proposal



is an ideal candidate for binding by a hydrogen-bond donor. Not only do our calculations indicate that such an interaction would be beneficial for catalysis of the radical rearrangement step,[10f] but experimental studies have confirmed precisely such a role for His244.[25] Similarly, the positively charged amine group of (*S*)-glutamate would seem to be best bound by a hydrogen-bond acceptor. Once again, our calculations indicate that this type of binding lowers the barrier for the radical rearrangement step in the glutamate-mutase-catalyzed reaction,[10c] while experimental studies have confirmed that Glu171 appears to do just that.[26] Finally, our calculations on the diol-dehydratase-catalyzed reaction show that a combination of hydrogen-bond donation to the migrating hydroxyl group and hydrogen-bond acceptance from the spectator hydroxyl group should dramatically accelerate the radical rearrangement step.[10d] The crystal structure of this enzyme[27] shows that His143 and Glu170 are in ideal positions to accomplish this catalysis and at the same time provide efficient binding. In light of these observations, how can we explain the apparently contrasting behavior in ethanolamine ammonia-lyase? A possible solution, which we now explore, is that, by analogy with diol dehydratase, EAL utilizes another reactive site to lower the rearrangement barrier.

D. The 1,2-Shift Assisted by Interactions with the Spectator Hydroxyl Group. (1) Migration of NH_2. Our previous work on the reaction catalyzed by B_{12}-dependent diol dehydratase[10d] shows that interactions between the spectator hydroxyl moiety and a basic group can catalyze the rearrangement of the 1,2-dihydroxyethyl radical. Furthermore, a proposed rearrangement mechanism involving ketyl (or radical anion) intermediates,[28] based on the loss of the hydroxyl proton, can easily be applied to the ethanolamine-ammonia-lyase-catalyzed reaction (Scheme 3). These two factors suggest that it is indeed worthwhile to consider this type of mechanism in the context of the 1,2-amino shift currently under investigation. In the interest of computational simplicity, we initially examine the effects of a basic group interacting with the hydroxyl substituent of the deprotonated substrate-derived radical (**8**). We will return, in a later section, to determine the effects of basic catalysis at the spectator hydroxyl group when the migrating group is (partially) protonated.

The extreme of full deprotonation of the spectator hydroxyl group is represented by the migration of NH_2 in the radical anion

(25) (a) Maiti, N.; Widjaja, L.; Banerjee, R. *J. Biol. Chem.* **1999**, *274*, 32733−32727. (b) Thomä, N.; Evans, P. R.; Leadlay, P. F. *Biochemistry* **2000**, *39*, 9213−9221.
(26) Madhavapeddi, P.; Marsh, N. G. *Chem. Biol.* **2001**, *145*, 1−7.
(27) Shibata, N.; Masuda, J.; Tobimatsu, T.; Toraya, T.; Suto, K.; Morimoto, Y.; Yasuoka, N. *Structure* **1999**, *7*, 997−1008.
(28) Buckel, W.; Golding, B. T. *FEMS Microbiol. Rev.* **1998**, *22*, 523−541.

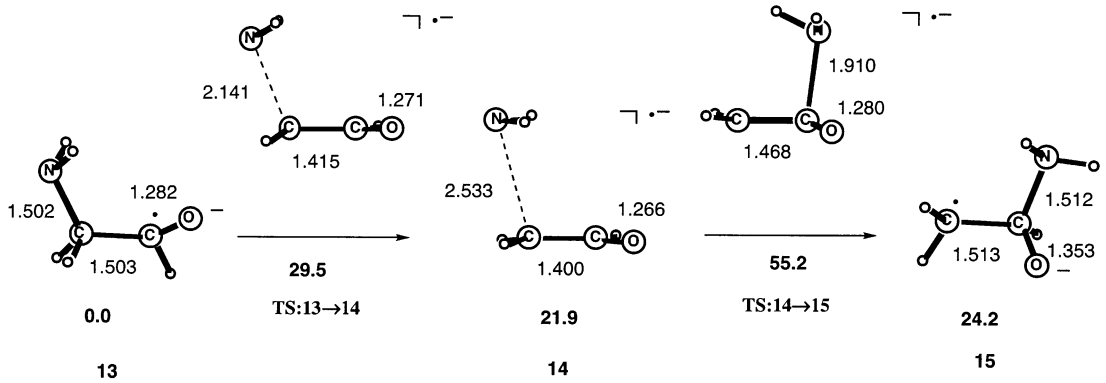


Figure 5. B3-LYP/6-31+G(d,p) structures and selected bond lengths (Å) for species involved in NH_2 migration in the radical anion of 2-aminoacetaldehyde (**13**). Relative energies (kJ mol^{-1}) obtained with G3(MP2)(+)-RAD(p) (bold type).

of 2-aminoacetaldehyde (**13**, Figure 5). Ab initio investigations of the ketyl mechanism are complicated by the fact that the 2-aminoacetaldehyde radical is calculated to have a negative electron affinity in the gas phase (−95.3 kJ mol^{-1}). This implies that the isolated radical anion would benefit from the loss of an electron. However, the radical anion of 2-aminoacetaldehyde may be stabilized at the active site within the enzyme. Despite the difficulties associated with the stability of the radical anion, we have characterized a fragmentation−recombination pathway for the 1,2-amino shift in **13** (Figure 5). Clearly, deprotonation of the hydroxyl group significantly reduces the energies of the transition structures for fragmentation (by 67.0 kJ mol^{-1}) and recombination (by 36.2 kJ mol^{-1}) compared with the NH_2 migration in the 2-amino-1-hydroxyethyl radical (Figure 2). The intermediate along the fragmentation−recombination pathway for the radical anion of 2-aminoacetaldehyde (**13**) is a weakly complexed species (**14**) rather than the fully separated fragments (**9**) characterized on the rearrangement surface associated with **8**. Formation of the complex **14** is likely to be associated with the beneficial effects of charge delocalization in the gas phase. This complex is calculated to be bound by 30.9 kJ mol^{-1} with respect to the $\cdot NH_2$ radical plus the allyloxy anion and by 143.9 kJ mol^{-1} with respect to the amino anion plus an allyloxy radical. The analogous pathway in the diol-dehydratase-catalyzed reaction was a single-step intramolecular rearrangement, with no minumum-energy point corresponding to the intermediate **14**. However, given that **14** lies in a relatively shallow well (of depth 7.6 kJ mol^{-1}) with respect to **TS:13→14**, this does not reflect a major difference between the two rearrangements.

Although full deprotonation of the spectator hydroxyl group clearly assists a 1,2-amino shift in **8**, it is unreasonable to expect that full deprotonation will occur in the enzymatic environment or to expect sufficient stabilization of the related radical anion (i.e., **11** or model **13**). Therefore, we investigate the effects of interactions between the hydroxyl group in **8** and bases of varying strengths (Y, eq 7) on the rearrangement barrier:

(7)

The interaction of OH^- with the hydroxyl group (Figure 6a) decreases the extent to which the additional electron is unbound compared with **13** (to −19.2 kJ mol^{-1}). Furthermore, we see that the proton is localized on the base (Figure 6a) and the system could perhaps be better described as the radical anion of 2-aminoacetaldehyde interacting with H_2O. There is a clear lengthening of the O···H−O hydrogen bond in the associated transition structures, compared with the reactant and product radicals.

If OH^- is replaced by the weaker base CN^-, then the resulting complex has a positive electron affinity (+28.3 kJ mol^{-1}) and we find that the proton is now localized on the substrate hydroxyl group throughout the rearrangement (Figure 6b). We again note a lengthening of the spectator O−H bond in the transition structures relative to the appropriate reactant (Figure 6b).

Figure 6 shows that the amino shifts mediated by Y = OH^- and CN^- (equation 7) proceed through fragmentation−recombination-type pathways involving weakly bound intermediate complexes (**9-OH**$^-$ and **9-CN**$^-$, respectively), similar to the reaction in which the hydroxyl is fully deprotonated (**14**, Figure 5). Although the complex for the OH^--mediated surface is lower in energy than the associated fragmentation transition structure at the B3-LYP level (by 3.2 kJ mol^{-1}), G3(MP2)(+)-RAD(p) calculations predict this complex to be slightly higher in energy than the transition structure (by 0.6 kJ mol^{-1}), in closer analogy with the diol-dehydratase-catalyzed reaction.[10d]

We also consider interactions between a neutral base (ammonia) and the hydroxyl moiety in **8** (Figure 7). The proton in the complexes with ammonia remains localized on the substrate throughout this rearrangement. The intermediate complex **9-NH_3** is bound by 3.2 kJ mol^{-1} with respect to the separated fragments (vinyl alcohol···NH_3 plus $\cdot NH_2$) and lies in a deeper potential energy well (with respect to **TS:9-NH_3→10-NH_3**) because of the possibility of a hydrogen bond between the migrating group and the interacting base in this case. Although it was possible to characterize the pathway linking **9-NH_3** with **TS:9-NH_3→10-NH_3**, we were not able to establish the pathway linking **9-NH_3** with **TS:8-NH_3→9-NH_3**.

Schematic energy profiles for the results from this part of our investigation are displayed in Figure 8 and are supplemented by the data presented in Table 2. Focusing initially only on the fragmentation barriers, we see barriers ranging from that associated with full deprotonation of the hydroxyl hydrogen (29.5 kJ mol^{-1}, **13**) to that associated with no deprotonation as for the amino migration in the 2-amino-1-hydroxyethyl radical (96.5 kJ mol^{-1}, **8**), depending on the strength of the base Y (equation 7). Furthermore, consideration of the overall reaction

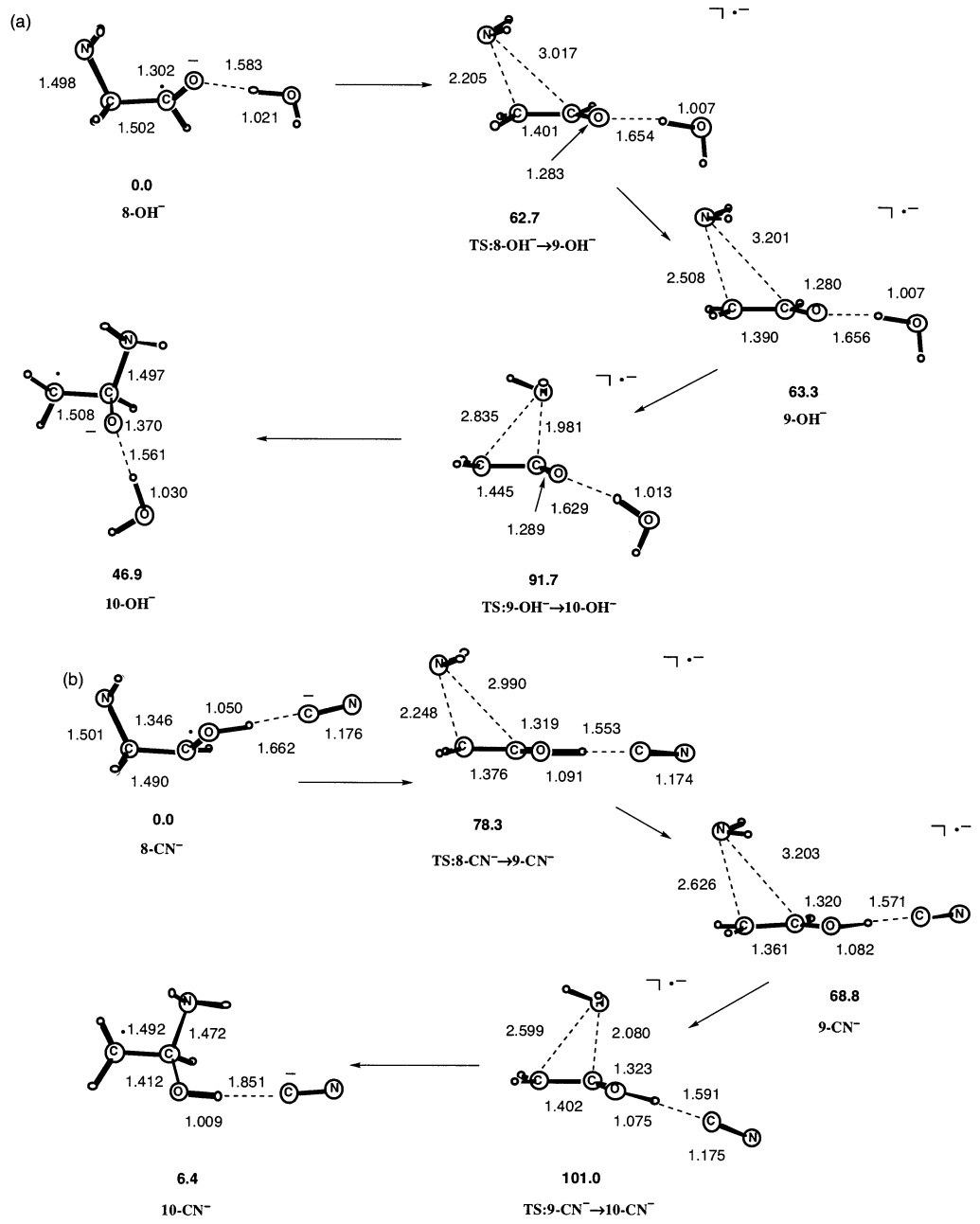


Figure 6. B3-LYP/6-31+G(d,p) structures and selected bond lengths (Å) for species involved in NH_2 migration mediated by OH^- and CN^- at the hydroxyl hydrogen (Y, eq 7) in the 2-amino-1-hydroxyethyl radical (**8**). Relative energies (kJ mol^{-1}) obtained with G3(MP2)(+)-RAD(p) (bold type).

in the reverse direction (i.e., **15** → **13)** shows a similar trend in the barrier heights, which range from that associated with full deprotonation of the spectator hydroxyl (31.0 kJ mol^{-1}) to no deprotonation, that is, the migration of NH_2 unassisted by a base Y (99.4 kJ mol^{-1}, Table 1). The fragmentation barriers in the reverse direction for the partially deprotonated systems increase according to OH^- (44.8 kJ mol^{-1}) < NH_3 (88.8 kJ mol^{-1}) < CN^- (94.6 kJ mol^{-1}). The relative magnitude of the assistance provided by CN^- and NH_3 is inverted for **15** → **13** compared with the **13** → **15** rearrangement. This reversal may arise because of the additional interactions in the transition structure for the reaction assisted by NH_3 (**TS:9-NH_3** → **10-NH_3**, Figure 7).

The clear trends that are observed for the fragmentation barriers in either direction are not transferable to the relative energies of the transition structures associated with recombination. Clearly, full deprotonation of the spectator hydroxyl facilitates NH_2 recombination. Similarly, the energy of the recombination transition structure is slightly reduced when ammonia interacts with the spectator hydroxyl group. However, the OH^- and CN^- mediated rearrangements have transition structures for recombination with energies (91.7 and 101.0 kJ mol^{-1}, respectively) that are slightly higher than the transition structure associated with unassisted NH_2 migration in the 2-amino-1-hydroxyethyl radical (**8**) (91.4 kJ mol^{-1}).

Interestingly, the rearrangement barrier calculated for the radical anion of 2-hydroxyacetaldehyde (**13**) is substantially increased (by 33.2 kJ mol^{-1} for the first step and by 36.5 kJ mol^{-1} for the higher-energy second transition structure) by the presence of the relatively weak acid, H_2O (i.e., the reaction

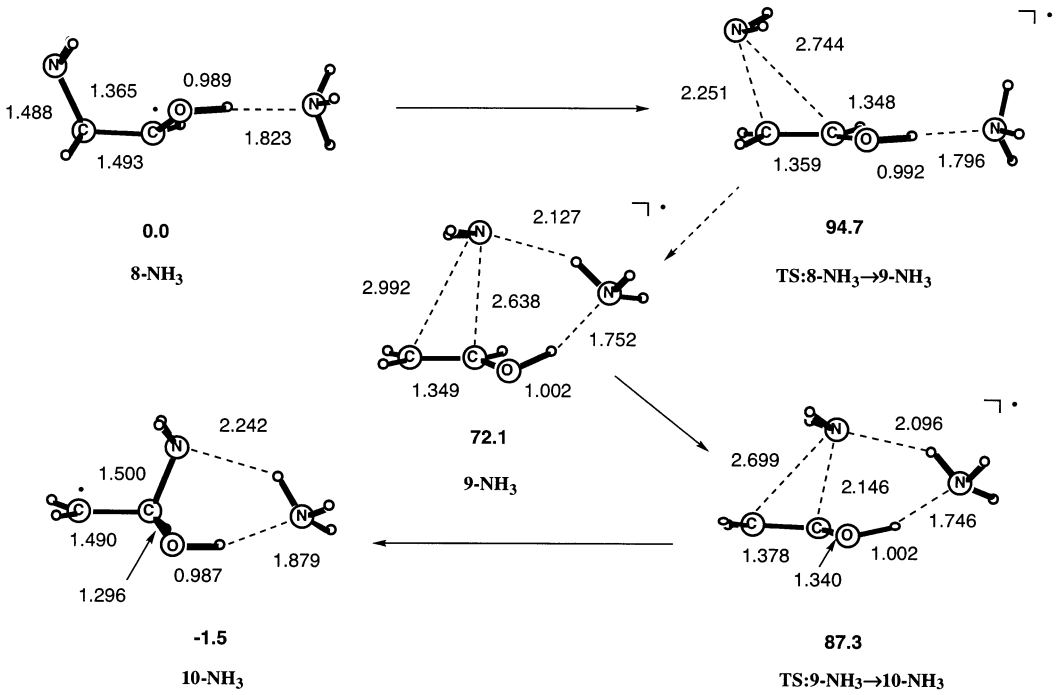


Figure 7. B3-LYP/6-31+G(d,p) structures and selected bond lengths (Å) for species involved in NH_2 migration mediated by NH_3 at the hydroxyl hydrogen (Y, eq 7) in the 2-amino-1-hydroxyethyl radical (**8**). Relative energies (kJ mol^{-1}) obtained with G3(MP2)-RAD(p) (bold type).

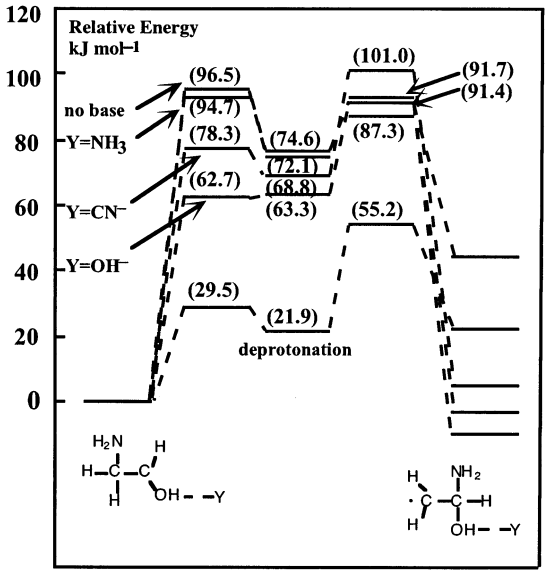
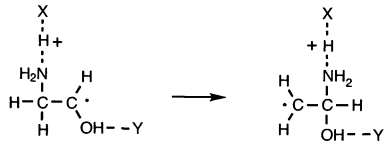


Figure 8. Schematic energy profile for the mediation of the 1,2-shift of the NH_2 group in neutral 2-amino-1-hydroxyethyl radical (**8**) through interactions between the hydroxyl group and bases (Y) of varying strengths (see eq 7). Relative energies (kJ mol^{-1}) are obtained with G3(MP2)-RAD-(p) (or G3(MP2)(+)-RAD(p) for full deprotonation).

displayed in Figure 5 compared with that in Figure 6a). This is analogous to the significant increase in the barrier for NH_3^+ migration when the migrating group interacts with a weak base (HF). Thus, although full protonation (of the migrating NH_2) and full deprotonation (of the spectator hydroxyl) greatly reduce the barrier to (neutral) NH_2 migration, these effects are significantly reduced in the presence of even the weakest interactions.

(2) Migration of NH_3^+. Although it is instructive to consider (as we have just done) the effects of basic interactions with the hydroxyl group on the barrier for NH_2 migration in the (neutral) 2-amino-1-hydroxyethyl radical (**8**), it is desirable to extend these results to encompass the situation where the substrate-derived radical is (at least partially) protonated, to be consistent with the known pK_a for ethanolamine. In the present section, we consider the combined effects of residues interacting with both the migrating amino group and the spectator hydroxyl group. For this investigation, we restrict our attention to systems in which ammonia interacts with the NH_3^+ migrating group and bases (Y) of varying strength interact with the hydroxyl group (equation 8):



(8)

Rearrangements in models of the type represented by eq 8 are found to proceed through one-step intramolecular pathways (see, for example, the combined effects of NH_3 interacting at the migrating group and at the hydroxyl group, Figure 9). Inspection of Figure 9 shows that the protons are located on the migrating group and the hydroxyl moiety of the substrate rather than on the associated bases. The calculated shortening in the N−H bond in the migrating group (0.039 Å, Figure 9) at the transition structure, compared with the reactant, is similar to that for the reaction involving only NH_3 interacting with the migrating substituent (0.043 Å, Figure 3). The O−H···N hydrogen bond between the hydroxyl group and ammonia exhibits a greater lengthening of the O−H component (0.035 Å) and a greater shortening of the H···N component (0.105 Å) in the transition structure (relative to the reactant) when the migrating group is (partially) protonated, compared with a fully deprotonated (NH_2) migrating group (Figure 7). This suggests a stronger influence of the base at the hydroxyl group when

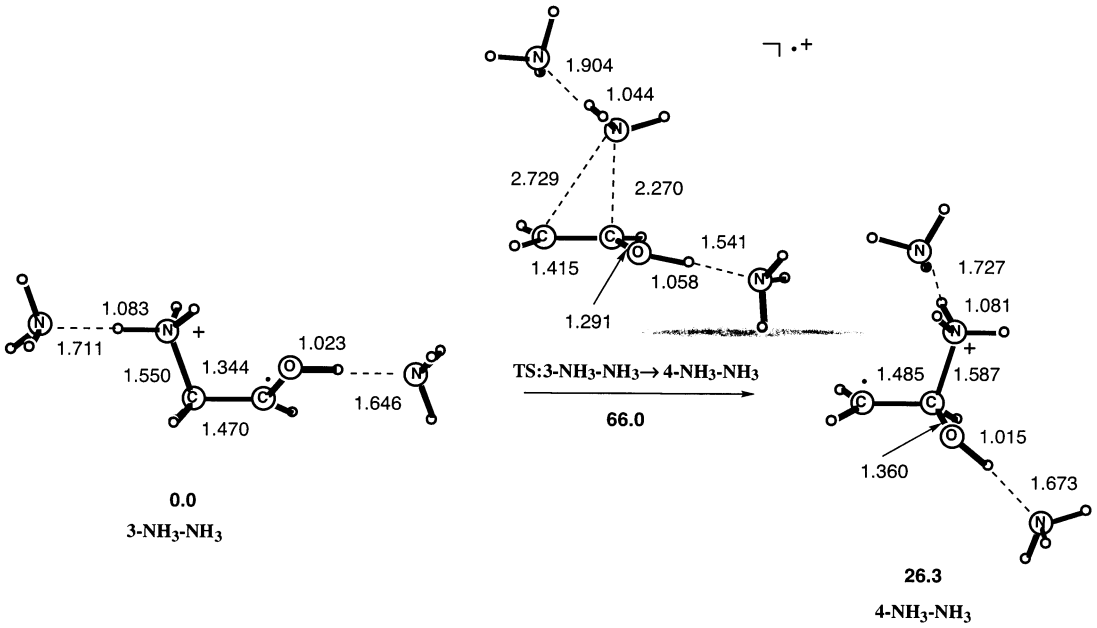


Figure 9. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in NH_3^+ migration in the protonated 2-amino-1-hydroxyethyl radical (**3**) mediated by interactions with ammonia at the migrating group and at the spectator hydroxyl moiety. Relative energies (kJ mol^{-1}) obtained with G3(MP2)-RAD(p) (bold type).

Table 2. G3(MP2)(+)-RAD(p) Relative Energies (kJ mol^{-1}) for the 1,2-Amino Shift in the (neutral) 2-Amino-1-hydroxyethyl Radical (**8**) Mediated by Bases (Y) of Varying Strength at the Hydroxyl Moiety (Eq 7 and Figures 5−8)[a]

species	relative energy	species	relative energy
13	0.0	**8-CN$^-$**	0.0
TS:13→14	29.5	**TS:8-CN$^-$→9-CN$^-$**	78.3
14	21.9	**9-CN$^-$**	68.8
TS:14→15	55.2	**TS:9-CN$^-$→10-CN$^-$**	101.0
15	24.2	**10-CN$^-$**	6.4
8-OH$^-$	0.0	**8-NH$_3$**[a]	0.0
TS:8-OH$^-$→9-OH$^-$	62.7	**TS:8-NH$_3$→9-NH$_3$**	94.7
9-OH$^-$	63.3	**9-NH$_3$**	72.1
TS:9-OH$^-$→10-OH$^-$	91.7	**TS:9-NH$_3$→10-NH$_3$**	87.3
10-OH$^-$	46.9	**10-NH$_3$**	−1.5

[a] The relative energies for the species related to the rearrangement of **8-NH$_3$** were obtained with G3(MP2)-RAD(p) because they do not involve anionic systems.

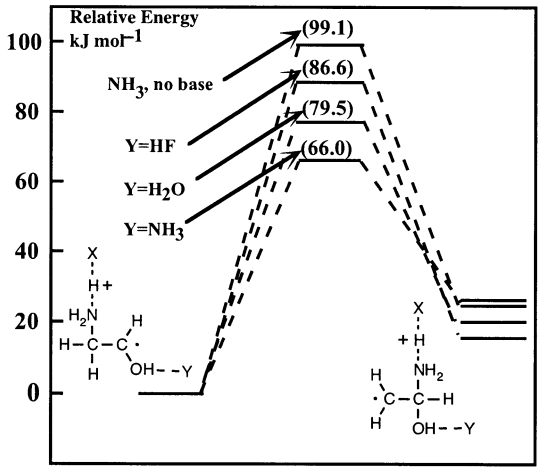


Figure 10. Schematic energy profile for the 1,2-shift of the NH_3^+ group in protonated 2-amino-1-hydroxyethyl radical (**3**) mediated by NH_3 together with various bases (Y, see eq 8). Relative energies (kJ mol^{-1}) are obtained with G3(MP2)-RAD(p).

the migrating group of the substrate is (partially) protonated compared with a neutral (NH_2) migrating group.

Figure 10 displays schematic energy profiles for NH_3^+ migration for systems in which an NH_3 interacts with the migrating group and bases (Y) of varying strength interact with the hydroxyl group (see also eq 8 and Table 3). There are unambiguous benefits of interactions with the hydroxyl group when the migrating group is partially protonated. Even interactions with the weakest base (HF) at the hydroxyl moiety significantly reduce (by 12.5 kJ mol^{-1}) the rearrangement barrier compared with the rearrangement in the absence of a base at the hydroxyl moiety. Furthermore, the effect of ammonia interacting with the hydroxyl group is much greater for the migration of the partially protonated NH_3^+ group (33.1 kJ mol^{-1}, Y = NH_3, eq 8) compared with the migration of NH_2 (1.8−4.1 kJ mol^{-1}, Y = NH_3, eq 7). This clearly demonstrates the combined benefits of partially protonated migrating group and a basic residue at the hydroxyl moiety.

The synergism between the interactions at the migrating group and at the spectator hydroxyl is usefully illustrated by starting with migration of the neutral amino group in the 2-amino-1-hydroxyethyl radical (**8**) as a reference point, for which the rearrangement barrier is 96.5 kJ mol^{-1} (Figure 2). If the migrating amino group interacts with an NH_4^+ moiety, that is, we have a partially protonated migrating group, the barrier is actually increased slightly to 99.1 kJ mol^{-1} (although it is decreased with stronger acids, Figure 4). If instead we have an NH_3 group interacting at the spectator hydroxyl, the barrier is reduced, but only slightly to 94.7 kJ mol^{-1} (Figure 7). However, if *both* of these interactions take place simultaneously, then the barrier is reduced substantially to 66.0 kJ mol^{-1} (Figure 9). When examined from this perspective, the behavior of the ethanol ammonia-lyase system shows similarities to the reaction catalyzed by diol dehydratase (see below).[10d]

We conclude that although neither interaction with base of the migrating NH_3^+ group nor the interaction with base of the spectator hydroxyl group are, on their own, able to provide

Table 3. G3(MP2)-RAD(p) Relative Energies (kJ mol^{-1}) for the 1,2-Amino Shift in the Protonated 2-Amino-1-hydroxyethyl Radical (**3**) Mediated by Interaction of Ammonia with the Migrating Group and with Bases (Y) of Varying Strength at the Hydroxyl Moiety (See Eq 8 and Figures 9 and 10)

species	relative energy
3-NH_3-HF	0.0
TS:3-NH_3-HF→4-NH_3-HF	86.6
4-NH_3-HF	16.4
3-NH_3-H_2O	0.0
TS:3-NH_3-H_2O→4-NH_3-H_2O	79.5
4-NH_3-H_2O	19.9
3-NH_3-NH_3	0.0
TS:3-NH_3-NH_3→4-NH_3-NH_3	66.0
4-NH_3-NH_3	26.3

suitable catalysis, the combination of the two produces a plausible mechanism. We believe that this synergistic interaction plays a key role in the reaction mechanism.

It is instructive to compare the mechanistic behavior of ethanolamine ammonia-lyase with that of diol dehydratase.[10d] In the diol-dehydratase-catalyzed reaction, partial protonation of the neutral migrating hydroxyl group unambiguously reduces the barrier for rearrangement. We have termed this effect retro-push catalysis to indicate that the acid catalyst pushes the migrating hydroxyl group but in an opposite direction to the electron flow.[10d] If the neutral 2-amino-1-hydroxyethyl radical (**8**) is taken as the reference point for the EAL-catalyzed reaction, then apart from the discontinuity that accompanies the change from inter- to intramolecular rearrangement, retro-push catalysis can also be seen to be operative in this reaction, that is, the rearrangement is facilitated by partial protonation of the migrating group. Thus, in this respect, the migration of a neutral amino group is very similar to that of a neutral hydroxyl group. However, in contrast to hydroxyl, the more basic amino group is expected to *already* be in a protonated state at physiological pH. Because hydrogen-bonding interactions associated with the retro-push phenomenon will reduce the extent of protonation, they will be anticatalytic for amino migration. The retro-pull catalysis (in which the migrating group is pulled across by a base interacting with the spectator hydroxyl group)[10d] is operative for both hydroxyl and amino migration. Although the details in the amino migration are again somewhat complicated, it is clear that interaction with a base at the spectator hydroxyl is generally beneficial. Indeed, in the context of amino migration, retro-pull catalysis is possibly even more significant since it is able to counteract the detrimental anticatalytic effects of the retro-push interaction.

As a final point, the favored intramolecular pathway would generally be expected to be associated with a high energy. A common explanation for this expectation is the one-electron occupancy of an antibonding orbital in cyclic intermediates or transition structures on such pathways. Calculations on model cyclic structures in the rearrangement of neutral 2-amino-1-hydroxyethyl radical (**8**) do indeed show a high-energy singly occupied molecular orbital with antibonding characteristics, at least with respect to the interaction of the migrating group with the π system of the two-carbon fragment.[29] While protonation of the migrating group does not significantly alter the antibonding characteristics of this orbital, it does significantly reduce the relative energy of the cyclic structure.

Concluding Remarks

We have used a variety of ab initio quantum chemistry calculations to try to gain a better understanding of the mechanism of action of ethanolamine ammonia-lyase. Evaluation of the reactivity of the radical intermediates appearing in the various proposed mechanisms allows a distinction between the possibilities. In particular, the calculations for the step in which a product-related radical must abstract an unactivated hydrogen atom from 5′-deoxyadenosine indicate that a more reactive radical is preferred. On this basis, we conclude that a mechanism involving 1,2-migration of the amino group is more likely than the alternative in which the amino group is directly eliminated.

The migration of the amino group in its protonated form is associated with a barrier consistent with the experimental reaction rate. However, if one recognizes that the protonated amino group is likely to be bound to the enzyme by a hydrogen-bond acceptor (which in the process partially deprotonates the NH_3^+ group), an interesting dichotomy arises because even a very small amount of partial deprotonation accompanying binding is found to substantially *increase* the rearrangement barrier. The increased barrier that accompanies binding then becomes too high to be consistent with the experimental reaction rate. This situation is in stark contrast to other B_{12}-dependent reactions, in which the most obvious forms of binding are also found to offer some catalytic advantage.[10,11]

A resolution of this dilemma comes through an investigation of the interaction of a range of bases with the spectator hydroxyl group of the substrate-derived radical (the 2-amino-1-hydroxyethyl radical, **8**) that reveals a possible additional component to the catalytic mechanism available for the amino migration. Specifically, hydrogen bonding of this hydroxyl group to the enzyme leads to (partial) deprotonation of the spectator hydroxyl group, which results in a lowering of the rearrangement barrier. However, even a very small deviation from the extreme of full deprotonation (i.e., using a strong base such as OH^-) leads to a significant reduction in the lowering in the rearrangement barrier.

We believe that the combination of a protonated migrating group (bound by a hydrogen-bond acceptor) with a basic catalyst interacting with the spectator hydroxyl group constitutes the most plausible mechanism for the ethanolamine-ammonia-lyase-catalyzed rearrangements. In these instances, the effect of the base at the hydroxyl group is substantially amplified when compared with its effect for a neutral migrating group, indicating a strongly synergistic action. Once again, a relatively small change in the strength of the interacting base at the hydroxyl group leads to a relatively large change in the associated rearrangement barrier.

The calculations presented herein demonstrate the importance of accounting for the effect of possible interacting groups within the enzyme active site. If only the rearrangement of the protonated substrate-derived radical is considered, then the reaction barrier lies within the experimental range and the mechanism for the rearrangement appears clear. However, this is possibly a fortuitous result since we find that relatively weak interacting groups at the active site can dramatically alter this

(29) This is also true for the high-energy cyclic transition structure found for the hydroxyl migration in the neutral 1,2-dihydroxyethyl radical.[10d]

barrier to rearrangement. It is only when the appropriate combination of binding and catalytic moieties is included that a satisfactory mechanism emerges.

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Supporting Information Available: G3(MP2)-RAD(p) (neutral and positively charged species) or G3(MP2)(+)-RAD(p) (negatively charged species) total energies (Table S1) and GAUSSIAN 98 archive entries from RMP2/6-31G(d) or RMP2/6-31+G(d) single-point calculations for all relevant structures (Table S2) (PDF). This information is available free of charge via the Internet at http://pubs.acs.org.

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