Computational Investigation of Hydrogen Abstraction from 2-Aminoethanol by the 1,5-Dideoxyribose-5-yl Radical: A Model Study of a Reaction Occurring in the Active Site of Ethanolamine Ammonia Lyase

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Dedicated to Professor Henry F. Schaefer III on the occasion of his 60th birthday

Abstract: Hydrogen abstraction from 2-aminoethanol by the 5′-deoxyadenosyl radical, which is formed upon Co–C bond homolysis in coenzyme B_{12}, was investigated by theoretical means with employment of the DFT (B3LYP) and ab initio (MP2) approaches. As a model system for the 5′-deoxyadenosyl moiety the computationally less demanding 1,5-dideoxyribose was employed; two conformers, which differ in ring conformation (C2- and C3-*endo*), were considered. If hydrogen is abstracted from "free" substrate by the C2-*endo* conformer of the 1,5-dideoxyribose-5-yl radical, the activation enthalpy is 16.7 kcal mol^{-1}; with the C3-*endo* counterpart, the value is 17.3 kcal mol^{-1}. These energetic requirements are slightly above the activation enthalpy limit (15 kcal mol^{-1}) determined experimentally for the rate-determining step of the sequence, that is, hydrogen delivery from 5′-deoxyadenosine to the product radical. The activation enthalpy is lower when the substrate interacts with at least one amino acid from the active site. According to the computations, when a His model system partially protonates the substrate the activation enthalpy is 4.5 kcal mol^{-1} for the C3-*endo* conformer and 5.8 kcal mol^{-1} for the C2-*endo* counterpart. As hydrogen abstraction from the fully as well as the partially protonated substrate is preceded by the formation of quite stable encounter complexes, the actual activation barriers are around 13–15 kcal mol^{-1}. A synergistic interaction of 2-aminoethanol with two amino acids where His partially protonates the NH_2 group and Asp partially deprotonates the OH group of the substrate results in an activation enthalpy of 12.4 kcal mol^{-1} for the C3-*endo* conformer and 13.2 kcal mol^{-1} for the C2-*endo* counterpart. However, if encounter complexes exist in the active site, the actual activation barriers are much higher (>25 kcal mol^{-1}) than that reported for the rate-determining step. These findings together with previous computations suggest that the energetics of the initial hydrogen abstraction decrease with an interaction of the substrate with only a protonating auxiliary, but for the rearrangement of the radical the synergistic effects of *two* auxiliaries are essential to pull the barrier below the limit of 15 kcal mol^{-1}.

Keywords: ab initio calculations · coenzyme B_{12} · density functional calculations · lyases · radical reactions

Introduction

The enzymes dependent on the vitamin B_{12} coenzyme catalyze homolytic cleavages of various C–H bonds,[1,2] and radical intermediates are involved in the subsequent 1,2-migration of hydrogen, alkyl, carbonyl, hydroxyl, or amide groups.[1–3] Ethanolamine ammonia lyase[4] is a bacterial enzyme that metabolizes the substrate 2-aminoethanol[5] (**1**) to ethanal (**4**) and ammonia (Scheme 1).[6] The ethanolamine ammonia lyase requires the presence of the vitamin B_{12} coenzyme for its catalytic activity. In the first step of the reaction, the homolytic cleavage of the C–Co^{III} bond in the vitamin B_{12} coenzyme is assumed to generate the low-spin cob(II)alamin and a 5′-deoxyadenosyl radical, and it is the latter radical that has been proposed to abstract a hydrogen atom from aminoethanol (Scheme 1; **1**→**2**).[7] As for the initially formed intermediates involved in the subsequent rearrangement of **2**, extensive computational studies[8–10] provided a quite plausible picture of the possible rearrangement details of **2**. It was concluded that the activation enthalpy for the intramolecular migration of the NH_2 group (**2**→**3**) is only compatible with experimental data if a synergistic action of partial protonation of the NH_2 group and partial deprotonation of the OH group of **2** by two conceivable catalytic auxiliaries (for example, Asp/Glu and

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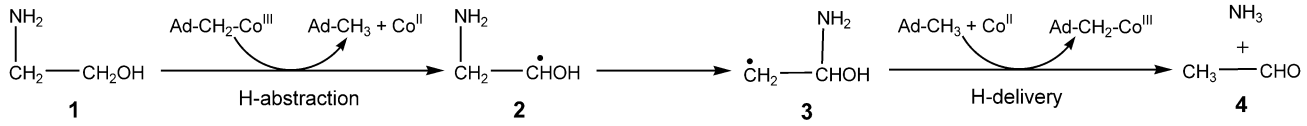


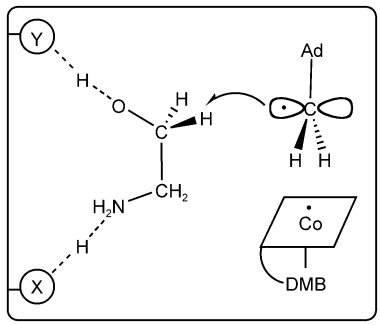
Scheme 1. Reaction sequence for the deamination of 2-aminoethanol (**1**) as catalyzed by ethanolamine ammonia lyase. Ad = 5′-deoxyadenosyl moiety.

His)[10] from the enzyme's active site is operative. The study of deuterium kinetic isotope effects suggests that hydrogen delivery from the 5′-deoxyadenosine to radical **3** constitutes the rate-limiting step in the overall reaction sequence,[11] and the estimated energy barrier equals 15 kcal mol^{-1} at 298 K.[12] The computed activation enthalpy for the rearrangement of **2** amounts to 13.7 kcal mol^{-1} when a synergistic interaction exists between **2** and Asp/Glu and His residues in the model systems. Interestingly, this "push–pull" action of the two catalytic groups is expected to take place in a physiologically realistic pH range of 6.0–9.5.[10]

Important questions that remain unresolved for ethanolamine ammonia lyase, as well as other coenzyme B_{12} dependent enzymes, concern the mechanism by which these enzymes bring about the homolytic C–H bond activation and how they catalyze the cleavage of the Co–C bond; for the latter, bond homolysis is accelerated up to a factor of 10^{12} in the presence of the enzyme.[13] Quite a few different mechanisms have been proposed that could account for this enormous rate acceleration: these are enzyme-induced distortion of the corrin ring to sterically labilize the Co–C bond,[14] enzymatic compression of the axial Co–N bond that causes a weakening of the *trans*-located Co–C bond,[14c,15] corrin-ring distortion by twisting the axial Co–N bond to rotate the 5,6-dimethylbenzimidazole,[16] direct bending of the Co–C bond by interaction of the adenosyl ligand with the protein,[14b,d,16,17] or weakening of the Co–C bond by the substrate itself, as indicated by early studies because cob(II)alamin was not formed in significant quantity in the absence of the substrate.[18] Direct evidence that a substrate promotes Co–C bond homolysis has been provided for methylmalonyl-CoA mutase where it was shown that the rate of Co–C bond catalysis is sensitive on isotopic substitution in the substrate.[19]

Another intriguing aspect, which will form the subject of the present study, concerns the mechanism by which the 5′-deoxyadenosyl radical abstracts a hydrogen atom from the substrate. In the case of ethanolamine ammonia lyase, both steady-state hydrogen-isotope-exchange studies and EPR spectroscopy of trapped radical intermediates suggested the involvement of two different species, that is, the 5′-deoxyadenosyl radical itself and a protein radical formed in the reaction with the former.[20,21] Evidence for the direct interaction of the 5′-deoxyadenosyl radical with substrate **1** was provided by isotope-exchange experiments, where the incorporation of tritium from 1-[^{3}H]aminoethanol into the coenzyme was demonstrated as was the release of tritium from 5′-[^{3}H]adenosylcobalamin to radical **3**.[11,22] Recently, several different experiments, in particular, the exchange of deuterium between the 5′-deoxyadenosyl radical and the enzyme's deactivator, electron nuclear double-resonance studies, and electron spin-echo envelope-modulation spectroscopy provided additional support for this mechanism.[20] However, the isotope effect observed for the incorporation of tritium from 5′-[^{3}H]deoxyadenosylcobalamin to form ethanal was contradictory to the conclusion that the three hydrogen atoms at the C5′ position in 5′-deoxyadenosine (the one abstracted from the substrate and the two from the intact coenzyme) were equivalent with respect to the probability of incorporation into the product. Similar observations were made for the related diol-dehydrase.[23] The anomalous tritium isotope effects observed for both enzymes have been rationalized by a model that rests on the assumption that two radical species, the 5′-deoxyadenosyl radical itself and a protein radical, interact with the substrate.[24] In this "two-radical" model the major part of hydrogen exchange proceeds through a protein radical, while only in approximately 11% of cases the 5′-deoxyadenosyl radical directly abstracts a hydrogen atom from the substrate. Even though the substrate and product radicals for the reaction catalyzed by ethanolamine ammonia lyase have been detected by EPR spectroscopy,[25] a possible candidate for the protein radical has not yet been identified, in contrast to the related coenzyme B_{12} dependent ribonucleotide triphosphate reductase for which the protein radical has been characterized as a cysteine thiyl radical.[26] However, the 5′-deoxyadenosyl radical appears to react directly with the substrate for the base-off coenzyme B_{12} dependent enzymes (class I) methylmalonyl-CoA mutase and glutamate mutase.

In the present study we will focus on the "nonprotein radical hypothesis", that is, the assumption that the initially formed C-centered 5′-deoxyadenosyl radical acts as a hydrogen-atom abstractor from substrate **1** (Scheme 2). In addition, we will deal with the question of whether the substrate is "free" in the active site prior to hydrogen abstraction or whether its position is fixed and the C–H bond homolysis



Scheme 2. Abstraction of a hydrogen atom by the 5′-deoxyadenosyl radical from substrate **1** interacting with two amino acids X and Y in the active site of ethanolamine ammonia lyase. DMB = 5,6-dimethylbenzimidazole.

facilitated by interaction with amino acids from the enzyme's active site (residues X and Y as depicted in Scheme 2), a scenario that seems more appropriate for an enzymatic catalysis. We believe that the insight derived from this computational model study proves useful in a further clarification of those factors that affect the hydrogen-abstraction reactions in coenzyme B_{12} dependent enzymes.

Computational Methods

All calculations were performed with the Gaussian 98 suite of programs[27] by using both DFT and an ab initio approach. The use of the DFT formalism was a natural choice because of the balance between accuracy and computational time required by the calculations, and the B3LYP functional was employed.[28,29] Geometry optimizations were performed with Pople's polarized double-ζ 6–31G* basis set.[30] In order to characterize the optimized structures, frequency analysis was performed at the same level of theory. Minima were characterized by the absence of imaginary vibrational frequencies, while transition structures (TSs) exhibited one imaginary frequency. A uniform scaling factor of 0.9806 was used for the zero-point energy (ZPE) corrections calculated at the B3LYP level of theory.[31] Computations of reaction pathways, that is, calculations of the intrinsic reaction coordinates (IRCs),[32] were carried out at the same level of theory.

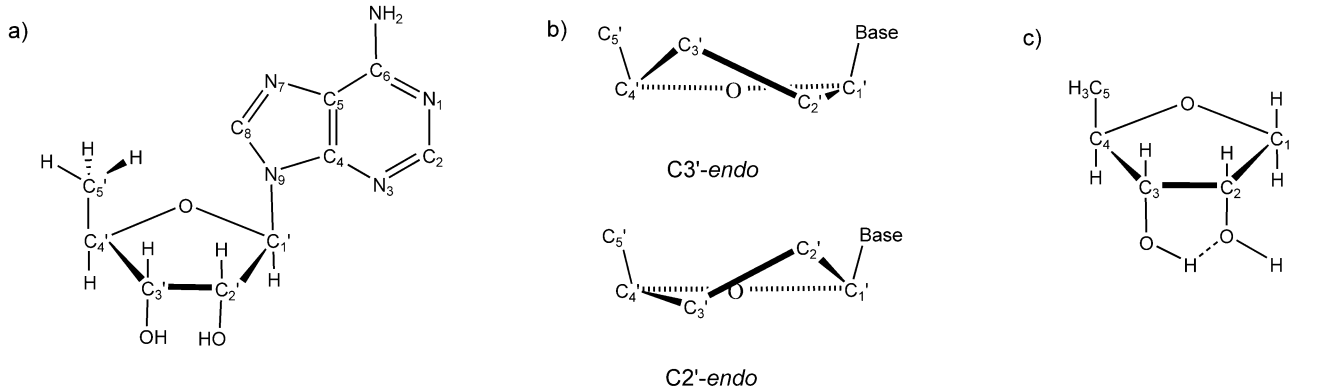
To obtain more reliable energetic profiles of the reactions in question, single-point calculations with triple-ζ basis sets with diffuse functions (6–311++G**) were performed by employing DFT (B3LYP) and ab initio theory (MP2). The relative energies of the stationary points were calculated at the B3LYP/6–311++G**//B3LYP/6–31G* and MP2/6–311++G**//B3LYP/6–31G* levels of theory, where the ZPEs calculated with B3LYP/6–31G* were used in the conversion into relative energies at 0 K. Overall, quite a good agreement between the two methods is observed, and the well-known problem of underestimation of the transition barriers by DFT is only notable for the transition structures. For the sake of briefness, throughout the text only results obtained at the MP2 level of theory are discussed, while the B3LYP/6–311++G** energies can be obtained from the authors upon request. The spin contamination was found to be negligible; the expectation values of $<S>^2$ for the doublet states are between 0.761 and 0.791. Due to the size of the system under investigation, we had to refrain from performing single-point calculations at some more accurate level of theory, as we have done in previous studies on related problems (that is, QCISD).[8,10] However, as reported by Morokuma and co-workers,[33] the B3LYP and MP2 results are of sufficient accuracy even for the hydrogen-bonding energies in the 5′-deoxyadenosyl radical, and we therefore believe that these methods are helpful in answering the questions addressed in the present study.

Relative energies (given in kcal mol^{-1}) discussed in the text correspond to the enthalpies at 298 K calculated at the MP2 level of theory (single-point calculations),[34] unless otherwise specified. Electronic energies, ZPEs, and the enthalpies of stationary points are available from the authors upon request.

Results and Discussion

Scenarios for hydrogen-abstraction from aminoethanol by 1,5-dideoxyribose-5-yl radical: While the amino acid sequence of the ethanolamine ammonia lyase has been determined,[35] the X-ray crystal structure of the enzyme is not yet known. However, as the active sites of several coenzyme B_{12} dependent enzymes were shown to exhibit high similarities, pertinent results obtained from the X-ray crystal structure of coenzyme B_{12} dependent glutamate mutase[36] will be used. In the active site of this enzyme two different conformers of the 5′-deoxyadenosyl moiety have been observed with the major conformational difference concerning the backbone of the ribose part. While a C2′-*endo* conformation is adopted by the 5′-deoxyadenosyl radical formed shortly after homolysis of the Co–C bond, a C3′-*endo* conformer is favored by the 5′-deoxyadenosine precursor (Scheme 3).

Theory has characterized no less than 34 conformers of the 5′-deoxyadenosyl radical. As to the global minimum, the solid-state structure[37] of the 5′-deoxyadenosyl moiety does not correspond to the one obtained by the computational studies.[33] In the latter, the global minimum of the free radical contains a hydrogen bond between the OH group on the C2′ position and the N3 atom from adenine.[33] Even though the X-ray crystal structure of the enzyme does not reveal this particular hydrogen bond, there are two water molecules situated suitably to form hydrogen bonds with the N3 atom from adenine and the OH group at the C2′ center of the ribose through a network of water molecules. Concerning the relative orientation of the ribose and adenine moieties, the X-ray crystal structure points to an arrangement that is disfavored by the computational work.[33] It has been concluded that this particular orientation of the ribose and adenine ring in the solid state is a result of several stabilizing in-



Scheme 3. a) The 5′-deoxyadenosyl moiety, b) different ribose conformations, c) 1,5-dideoxyribose, which was employed as a model system for the 5′-deoxyadenosyl moiety.

teractions between the 5′-deoxyadenosyl moiety, additional water molecules, and the corrin ring. Moreover, the computationally preferred C2′-*endo* conformation of the ribose ring in the "liberated" 5′-deoxyadenosyl radical is triggered exactly by the hydrogen-bond interaction between the OH group at the C2′ position and the N3 atom from adenine. In view of these conflicting results, we have decided to focus on calculating the energetics of the hydrogen-abstraction step by employing a more simplified model system for the 5′-deoxyadenosyl radical. Clearly, quantum mechanics/molecular mechanics studies would represent an attractive alternative for obtaining more accurate computational results. However, as long as the X-ray crystal structure of ethanolamine ammonia lyase remains unknown, one has to refrain from such an approach.

The model chosen, that is, 1,5-dideoxyribose[38] (see Scheme 3c), is reduced to the ribose part of 5′-deoxyadenine; the adenine fragment has not been included on the ground that its presence is not likely to affect energetically the hydrogen abstraction from substrate **1** by the C5′-centered radical. For the 1,5-dideoxyribose we have used a conformation that contains one intramolecular hydrogen bond (Scheme 3c), and two different conformations of the "ribose" ring (C3-*endo* and C2-*endo*) have been considered in the computations; structural details can be found in Figure 1.

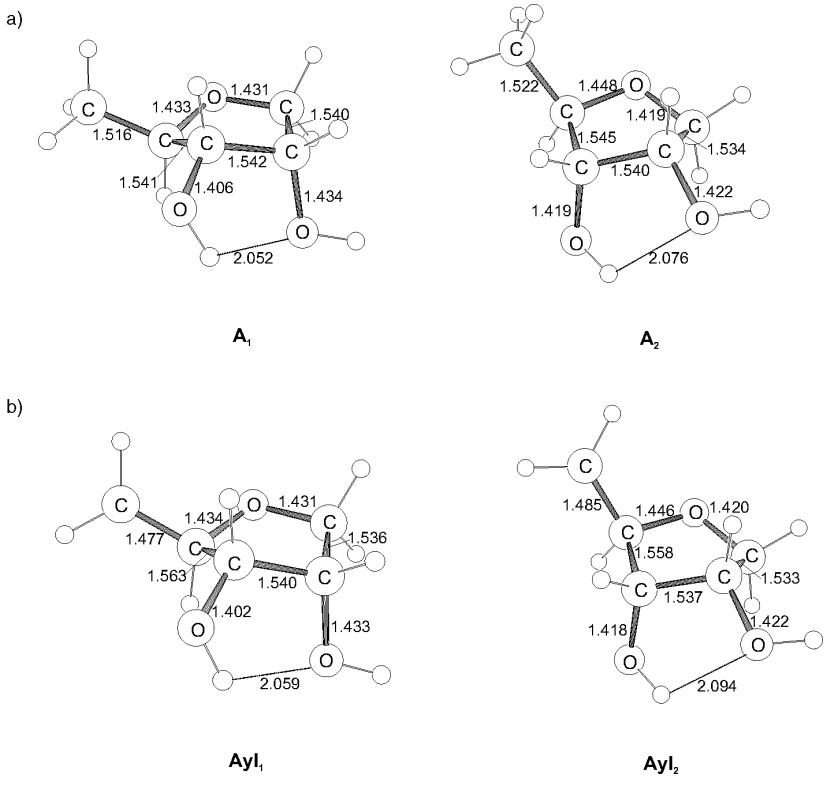


Figure 1. Optimized geometries (B3LYP/6–31G*) of a) 1,5-dideoxyribose (C3- and C2-*endo* conformers) and b) the 1,5-dideoxyribose-5-yl radical (C3- and C2-*endo* conformers). All bond lengths are given in Å.

Furthermore, in view of the findings[9,10] that the energetics of the rearrangement **2**→**3** (Scheme 1) are crucially dependent on the simultaneous operation of partially protonating and deprotonating auxiliaries X and Y (Scheme 2), we wondered if the barrier of the hydrogen abstraction itself from substrate **1** is also affected by the presence or absence of amino acid residues. To this end four different scenarios have been investigated: the hydrogen abstraction from the "free" substrate **1** (Scheme 2, without X and Y), from a fully protonated substrate (Scheme 2, $X=H^+$ and without Y), from a substrate interacting with a His equivalent (Scheme 2, X = His and without Y), and finally from a substrate interacting simultaneously with His and Asp models (Scheme 2, X = His and Y = Asp).

According to our computations, the C3-*endo* conformer of 1,5-dideoxyribose is only slightly more stable (by 0.9 kcal mol^{-1}) than the C2-*endo* counterpart, and this difference gets even smaller (0.6 kcal mol) for the corresponding radicals. Concerning the labeling of the stationary points, throughout the text all structures with a C3-*endo* conformation are labeled with the subscript 1 (for example, 1,5-dideoxyribose $\mathbf{A_1}$ and its radical $\mathbf{Ayl_1}$) while the C2-*endo* conformers carry a subscript 2 (for example, 1,5-dideoxyribose $\mathbf{A_2}$ and its radical $\mathbf{Ayl_2}$).

*1. Nonprotonated substrate **1***: When a hydrogen atom is abstracted from the nonprotonated substrate **1** by the two conformers $\mathbf{Ayl_1}$ and $\mathbf{Ayl_2}$, the corresponding transition structures, $\mathbf{TS_1}$ (Figure 2) and $\mathbf{TS_2}$, respectively, are energetically almost equivalent (17.3 and 16.7 kcal mol^{-1}). Commencing from the corresponding TSs, IRC computations in the direction of reactants converged into complexes between the C5-centered radical and **1** ($\mathbf{1^*Ayl_1}$ and $\mathbf{1^*Ayl_2}$) that are slightly less stable than the reactants with energies of 0.9 kcal mol^{-1} for $\mathbf{1^*Ayl_1}$ and 1.8 kcal mol^{-1} for $\mathbf{1^*Ayl_2}$ (Table 1).[39,40] Irrespective of the conformation of the C5-centered radical, the energy demands to overcome the hydrogen-abstraction barrier exceed the activation enthalpy (15 kcal mol^{-1}) of the rate-determining step for the whole catalytic sequence. Therefore, hydrogen abstraction from a nonprotonated substrate is quite unlikely to occur. Moreover, the pK_a value for the conjugated acid of **1** equals 9.45 and it is consequently reasonable to assume that **1** is (partially) protonated by some of the amino acids present in the enzyme's active site. Therefore, hydrogen abstraction from fully and partially protonated substrates will be considered next.

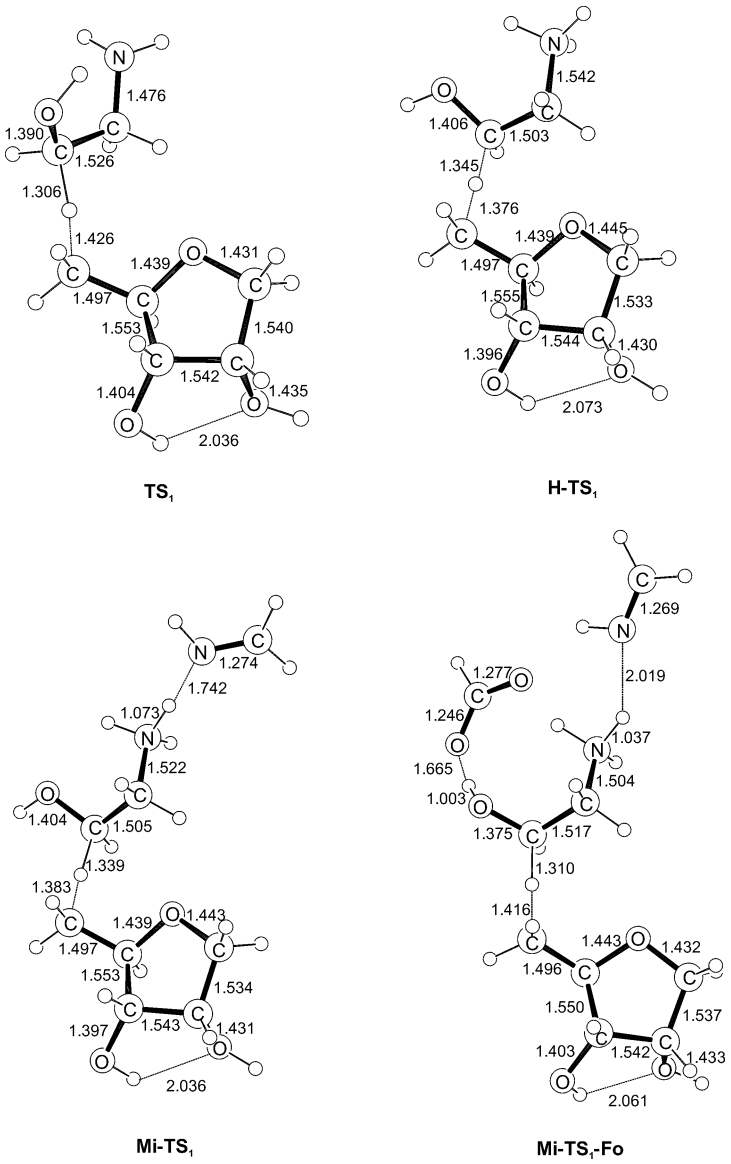


Figure 2. Optimized geometries (B3LYP/6–31G*) of transition structures for the hydrogen-abstraction reaction by C3-*endo* conformers of the 1,5-dideoxyribose-5-yl radical. All bond lengths are given in Å.

Table 1. Relative enthalpies [kcal mol^{-1}] at 0 K ($H_{rel,0}$) and 298 K ($H_{rel,298}$) of the stationary points relevant for the hydrogen-abstraction reaction from substrate **1**.

	B3LYP/6–31G*		MP2/6–311++G**//B3LYP/6–31G*	
	$H_{rel,0}$	$H_{rel,298}$	$H_{rel,0}$	$H_{rel,298K}$
1 + Ayl$_1$	0.0	0.0	0.0	0.0
1*Ayl$_1$	−2.8	1.5	−4.4	0.9
TS$_1$	10.1	13.6	15.1	17.3
2*A$_1$	−10.7	−6.2	−10.0	−4.5
2 + A$_1$	−10.2	−6.8	−9.1	−4.7
1 + Ayl$_2$	0.9	1.0	0.6	0.6
1*Ayl$_2$	−1.6	2.6	−3.4	1.8
TS$_2$	9.7	13.0	14.4	16.7
2*A$_2$	−10.1	−5.8	−9.3	−3.6
2 + A$_2$	−10.1	−5.8	−8.3	−3.8

*2. Fully protonated substrate **H-1***: For both conformers of the 1,5-dideoxyribose-5-yl radical, transition structures for hydrogen abstraction from the fully protonated substrate, **H-1**, were located. When compared to the separate reactants, both TSs lie very low in energy, with **H-TS$_1$** (Figure 2) only 2.2 kcal mol^{-1} and **H-TS$_2$** 3.7 kcal mol^{-1} above the reactant pair **H-1**/**Ayl$_1$** (see Table 2). However, from the corresponding TSs in the direction of reactants, the IRC computations converged in complexes of **H-1** with **Ayl$_1$** or **Ayl$_2$**. If it is assumed that these complexes are present in the active site, then the actual activation barriers for the hydrogen abstraction amount to 13.1 kcal mol^{-1} when the **Ayl$_1$** radical is involved and 13.8 kcal mol^{-1} for the **Ayl$_2$** counterpart.

Table 2. Relative enthalpies [kcal mol^{-1}] at 0 K ($H_{rel,0}$) and 298 K ($H_{rel,298}$) of the stationary points relevant for the hydrogen-abstraction reaction from the fully protonated substrate **H-1**.

	B3LYP/6–31G*		MP2/6–311++G**//B3LYP/6–31G*	
	$H_{rel,0}$	$H_{rel,298}$	$H_{rel,0}$	$H_{rel,298}$
H-1 + Ayl$_1$	0.0	0.0	0.0	0.0
H-1*Ayl$_1$	−10.2	−10.2	−11.8	−10.9
H-TS$_1$	−0.6	−0.9	3.9	2.2
H-2*A$_1$	−30.0	−29.5	−28.8	−27.4
H-2 + A$_1$	−9.6	−9.4	−5.9	−5.7
H-1 + Ayl$_2$	0.9	1.0	0.6	0.6
H-1*Ayl$_2$	−9.0	−9.1	−11.0	−10.1
H-TS$_2$	0.9	0.5	5.3	3.7
H-2 + A$_2$	−8.4	−8.3	−5.1	−4.8

When compared to the hydrogen abstraction from the nonprotonated substrate **1**, the decreased activation enthalpy is quite probably the result of stabilization of the emerging radical, where the radical center is better delocalized through the C–C bond by the presence of the electron-withdrawing NH_3 group in the TSs. This is already indicated in some of the structural features of **TS$_1$** versus **H-TS$_1$** (Figure 2). In the latter, the relevant C–C bond is shorter (1.503 Å) than in **TS$_1$** (1.526 Å), while the C–N bond is elongated (1.542 versus 1.476 Å).[40] For **H-TS$_1$** the IRC computations in the direction of a product converged into a complex, **H-2*A$_1$**, between the protonated product radical, **H-2**, and **A$_1$**.[41] This complex is much more stable (21.7 kcal mol^{-1}) than the separate species **H-2** and **A$_1$** and extensive charge delocalization can be held responsible for this effect.

Even though hydrogen-atom abstraction from a fully protonated substrate is feasible from an energetic point of view, it is quite unrealistic to expect that **1** will exist as the "free", fully protonated species **H-1** in the active site. A more likely scenario is one in which the substrate is captured in the enzyme's active site and its position fixed by interaction with some amino acids that may result in *partial* protonation of the NH_2 group and, depending on structural details, partial deprotonation of the OH group as well. The implications of these features for the energetics of the C–H bond homolysis will be considered next.

*3. Partially protonated substrate **Mi-1***: The concept of partial protonation of a migrating group has been shown to play

an important role for several coenzyme B_{12} dependent enzymes[42] because it lowers the barriers for substrate rearrangement. As a model for an amino acid that might partially protonate **1**, a quite simple imine (methanimine, CH_2=NH) was used to mimic His, one of the natural choices to interact with the substrate. While the X-ray crystal structure of ethanolamine ammonia lyase has not been determined yet, in the case of the rearrangement catalyzed by the related methylmalonyl-CoA mutase it was concluded that His244 is the amino acid that partially protonates the substrate.[43] As shown in a previous study,[10] the energetics of the reaction **2**→**3** for partially protonated **2** were almost identical for different His models employed, and the structurally most simple system, CH_2NH, was used here to save computational time.

If the $\mathbf{Ayl_1}$ radical abstracts the hydrogen atom from **Mi-1**, the corresponding TS, $\mathbf{Mi\text{-}TS_1}$ (Figure 2), lies 4.5 kcal mol^{-1} above the separate reactants, $\mathbf{Ayl_1}$ and **Mi-1**. $\mathbf{Mi\text{-}TS_2}$ is slightly less stable, lying 5.8 kcal mol^{-1} above the reactants (Table 3). This small difference in the stability of the two

Table 3. Relative enthalpies [kcal mol^{-1}] at 0 K ($H_{rel,0}$) and 298 K ($H_{rel,298}$) of the stationary points relevant for the hydrogen-abstraction reaction from substrate **Mi-1** interacting with a His model system.

	B3LYP/6–31G*		MP2/6–311++G**//B3LYP/6–31G*	
	$H_{rel,0}$	$H_{rel,298}$	$H_{rel,0}$	$H_{rel,298}$
Mi-1 + Ayl$_1$	0.0	0.0	0.0	0.0
Mi-1*Ayl$_1$	−8.8	−8.1	−10.8	−9.1
Mi-TS$_1$	2.0	1.9	6.0	4.5
Mi-2*A$_1$	−8.6	−15.8	−16.5	−14.7
Mi-2 + A$_1$	−9.0	−8.7	−6.0	−5.7
Mi-1 + Ayl$_2$	0.9	1.0	0.6	0.6
Mi-1*Ayl$_2$	−8.1	−8.1	−10.0	−9.0
Mi-TS$_2$	3.2	3.0	7.2	5.8
Mi-2 + A$_2$	−7.8	−7.6	−5.1	−4.8

TSs, $\mathbf{Mi\text{-}TS_1}$ and $\mathbf{Mi\text{-}TS_2}$, reflects the energetic difference that already exists for the 1,5-dideoxyribose, where the C3-*endo* form was found to be 0.9 kcal mol^{-1} more stable. By taking into account the existence of complexes $\mathbf{Mi\text{-}1*Ayl_1}$ and $\mathbf{Mi\text{-}1*Ayl_2}$, which were obtained in the IRC computations from the corresponding TSs in the direction of the reactants, the activation enthalpy for hydrogen abstraction from **Mi-1** can be calculated to be 13.6 kcal mol^{-1} with $\mathbf{Ayl_1}$ and 14.8 kcal mol^{-1} with $\mathbf{Ayl_2}$; this is only slightly higher than for the analogous reaction in which the substrate is fully protonated (13.1 kcal mol^{-1}), but still below the limiting value of 15 kcal mol^{-1}. The IRC calculations from $\mathbf{Mi\text{-}TS_1}$ in the direction of a product led to a complex between **Mi-2** and $\mathbf{A_1}$;[44] the formation of this complex is highly exothermic (−14.7 kcal mol^{-1}) and the complex itself is 9.0 kcal mol^{-1} more stable than the separate constituents **Mi-2** and $\mathbf{A_1}$.

As to the role of substrate protonation on the activation enthalpy for the C–H bond homolysis of **1**, the computational findings clearly point to the operation of a catalytic effect. However, in view of previous findings[9,10] that the synergistic operation of a simultaneous partial protonation of the NH_2 group and a partial deprotonation of the OH group of **1** brings about a dramatic acceleration of the intramolecular rearrangement **2**→**3**, we wondered if this effect also holds true for the C–H bond activation step **1**→**2**; the computational findings in this area are discussed next.

4. Substrate captured by two amino acids from the active site ***Mi-1-Fo***: In addition to His partially protonating the NH_2 group of **1**, Asp (in its carboxylate form) is assumed to partially deprotonate the OH group of the substrate; this kind of synergistic interaction of the two amino acids can take place in a physiologically realistic pH range of 6.0–9.5.[10] As a model system for Asp we employed formate, which was shown earlier to serve well for the computational investigation of the rearrangement reactions.[10] The TS involving the C3-*endo* conformation of the 1,5-dideoxyribose-5-yl moiety ($\mathbf{Mi\text{-}TS_1\text{-}Fo}$, Figure 2; 12.4 kcal mol^{-1}) is energetically 0.8 kcal mol^{-1} less demanding than the one involving the C2-*endo* conformer ($\mathbf{Mi\text{-}TS_2\text{-}Fo}$; 13.2 kcal mol^{-1}). While IRC computations from the TSs in the direction of reactants did not converge into the expected complexes between the reactant species, by means of exhaustive geometry optimization we managed to locate two relevant complexes between the reactants. The complex $\mathbf{Mi\text{-}1\text{-}Fo*Ayl_1}$ lies 13.2 kcal mol^{-1} below the separate reactants, while $\mathbf{Mi\text{-}1\text{-}Fo*Ayl_2}$ is 7.7 kcal mol^{-1} more stable then the noninteracting reactants. With the assumption that such complexes can be formed in the active site, the activation enthalpy for the hydrogen abstraction would amount to 25.6 kcal mol^{-1}, which clearly exceeds the upper limit of 15 kcal mol^{-1}. Such a high activation enthalpy is presumably the result of an unfavorable delocalization of the emerging radical center at the C1 position in the presence of the partially developing negative charge on the adjacent oxygen atom from the OH group. A comparison of the relevant C–O and C–C bond lengths of the substrates (**Mi-1** and **Mi-1-Fo**) in the transition structures $\mathbf{Mi\text{-}TS_1}$ and $\mathbf{Mi\text{-}TS_1\text{-}Fo}$ (Figure 2) lends qualitative support to this suggestion. Consequently, for the C–H bond activation step, the synergistic interaction of **1** with two amino acids does not lower the barrier; rather, the effect of only one partially protonating amino acid suffices.

IRC computations in the direction of the products result in quite stable complexes between the radical **Mi-2-Fo** and both conformers of 1,5-dideoxyribose, $\mathbf{A_1}$ and $\mathbf{A_2}$; if it is assumed that such complexes exist, hydrogen abstraction from **1** becomes highly exothermic, especially in the case of $\mathbf{Mi\text{-}2\text{-}Fo*A_1}$ which is 21.5 kcal mol^{-1} more stable than the separate products **Mi-2-Fo** and $\mathbf{A_1}$ (Table 4). If this product complex is really formed in the active site, the $\mathbf{A_1}$ moiety being kept close to the radical **2** in the course of the subsequent migration of the amino group (**2**→**3**) could immediately deliver a hydrogen atom back to **3**, thereby regenerating the 5′-deoxyadenosyl radical and closing the catalytic cycle.

Conclusion

According to our computations, the TSs in which the C3-*endo* conformer of the 1,5-dideoxyadenosyl radical is in-

Table 4. Relative enthalpies [kcal mol^{-1}] at 0 K ($H_{rel,0}$) and 298 K ($H_{rel,298}$) of the stationary points relevant for the hydrogen-abstraction reaction from substrate **Mi-1-Fo** interacting synergistically with the His and Asp model systems.

	B3LYP/6–31G*		MP2/6–311++G**//B3LYP/6–31G*	
	$H_{rel,0}$	$H_{rel,298}$	$H_{rel,0}$	$H_{rel,298}$
Mi-1-Fo + Ayl$_1$	0.0	0.0	0.0	0.0
Mi-1-Fo*Ayl$_1$	−12.7	−12.8	−16.1	−13.2
Mi-TS$_1$-Fo	9.1	8.6	14.0	12.4
Mi-2-Fo*A$_1$	−23.7	−24.2	−25.3	−22.1
Mi-2-Fo + A$_1$	−11.4	−10.8	−0.3	−0.6
Mi-1-Fo + Ayl$_2$	0.9	1.0	0.6	0.6
Mi-1-Fo*Ayl$_2$	−6.3	−6.2	−10.0	−7.7
Mi-TS$_2$-Fo	9.6	9.0	14.7	13.2
Mi-2-Fo*A$_2$	−14.3	−14.4	−15.6	−13.0
Mi-2-Fo + A$_2$	−10.3	−9.6	0.5	0.3

volved are only slightly energetically less demanding than those which involve the C2-*endo* counterpart, and the energy differences in the transition structures reflect, to a large extent, those that already exist for the free conformers **Ayl$_1$** and **Ayl$_2$**. Furthermore, since the transition structures for both 1,5-dideoxyribose-5-yl conformers are energetically and structurally quite similar in all cases investigated, it is difficult (if not impossible) to decide definitively which of the C5-centered radical conformers actually attacks the substrate.

As to the energetics, homolysis of the C–H bond from the nonprotonated substrate requires activation enthalpies greater than 16.7 kcal mol^{-1} for both conformers of the model radical. In contrast, all computed activation enthalpies for the hydrogen abstraction from a partially or fully protonated substrate **1** are energetically less demanding than the value (15 kcal mol^{-1}) that has been experimentally derived for the rate-determining step, that is, the hydrogen abstraction from 5′-deoxyadenosine by radical **3**.[11] As it is realistic to assume that the quite basic aminoethanol substrate is at least partially protonated in the active site, we conclude that, from an energetic point of view, the initially formed 5′-deoxyadenosyl radical can abstract a hydrogen atom directly from an appropriately "activated" substrate. It does not seem necessary to invoke the role of a protein radical ("two-radical hypothesis").[24]

Interestingly, in case of a synergistic interaction, when substrate **1** is captured by two amino acids (such as His and Asp), the activation enthalpy for the hydrogen abstraction exceeds 25 kcal mol^{-1}. Therefore, it is likely that in the homolysis, in distinct contrast to the rearrangement step **2**→**3**, a synergistic interaction of **1** with two activating auxiliaries is not essential; rather, partial protonation suffices.

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[39] Similarly, IRC computations from the TSs in the direction of a product led to complexes between **2** and 1,5-dideoxyribose (**2A$_1$** and **2A$_2$**; Table 1); these are slightly less stable (each 0.2 kcal mol) than the separate species **2** and 1,5-dideoxyribose (C3- or C2-*endo* conformers).

[40] Only those TSs involving the C3-*endo* conformer of the 1,5-dideoxyribose are depicted in Figure 2 because of the pronounced structural similarities between the TSs for the reactions with the C3- and C2-*endo* conformers.

[41] IRC computations commencing from **H-TS$_2$** in the direction of a product did not converge into a complex, but because of otherwise strong similarities between the two conformers involved in this reaction, such a complex might be postulated to exist. Since our main interest concerns the barriers to the hydrogen abstractions, we refrained from an exhaustive search for this kind of product complex.

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