Enzyme Catalysis of 1,2-Amino Shifts: The Cooperative Action of B_6, B_{12}, and Aminomutases

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Abstract: Ab initio molecular orbital theory is used to investigate 1,2-amino shifts catalyzed by aminomutases, coenzyme B_{12}, and vitamin B_6 (in the form of pyridoxal 5′-phosphate or PLP). Our calculations suggest essential catalytic roles for each of B_{12}, B_6, and the enzyme in aminomutase-catalyzed reactions. In the first place, coenzyme B_{12} provides a source of abstracting radicals, allowing the rearrangement reaction to take place on the radical surface. The involvement of radicals is supported by comparison of experimental and theoretical electron paramagnetic resonance parameters. Next, B_6 allows the enzyme to lower the barrier height by introducing a double bond (allowing a low-energy intramolecular rearrangement pathway) and by providing a suitable site for partial protonation (preventing overstabilization of the reaction intermediate which could lead to enzyme inactivation). The PLP hydroxyl group is also identified as an important participant in these reactions. Finally, the enzyme holds the various reaction components in place and is the source of acidic functional groups that can provide partial protonation.

Introduction

An abundance of literature, outlined in numerous reviews, is dedicated to the workings of coenzyme B_{12} (5′-deoxyadenosylcobalamin).[1−5] Coenzyme B_{12} catalyzes many biologically important reactions, most of which involve the interchange of a migrating group (X) and a hydrogen atom (eq 1). The

(1)

mechanism by which this transformation occurs has been scrutinized in attempts to understand how B_{12} assists these otherwise difficult rearrangements. It is generally believed that the main role of B_{12} is to provide a source of 5′-deoxyadenosyl radicals, which abstract hydrogen from the substrate and result in a radical rearrangement mechanism.

One class of coenzyme B_{12}-dependent enzymes,[6] the aminomutases, catalyzes the 1,2-shift of an amino group ($X = NH_2$ in eq 1). The main goal of these enzymes is to help prepare the carbon skeleton of their substrates, typically amino acids, for subsequent cleavage into easily metabolized intermediates. β-Lysine aminomutase, α-lysine 5,6-aminomutase, and ornithine 4,5-aminomutase are three of many aminomutases that have been determined to be coenzyme B_{12}-dependent (Scheme 1).[6]

In addition to coenzyme B_{12}, aminomutases require pyridoxal 5′-phosphate (PLP), the phosphate ester of the aldehyde form of vitamin B_6 (Chart 1).[7,8]The currently accepted mechanism[6,9] for 1,2-amino shifts catalyzed by B_6, B_{12}, and aminomutases is outlined in Scheme 2. Within enzymes, PLP is covalently bound as a Schiff base (imine) to a lysine residue (Scheme 2, **A**).[7] During an aminomutase-catalyzed reaction, a transimination reaction is believed to occur upon substrate binding whereby PLP forms an imine (Schiff base, **C**) with the amino group of the substrate (**B**) and the lysine residue is released from PLP. Schiff base formation with PLP is also an important step in other reactions, such as decarboxylation and transamination reactions of amino acids.[10] Coenzyme B_{12} then abstracts a hydrogen atom from the PLP-bound substrate to generate a radical intermediate (**D**). The PLP-bound substrate radical in turn rearranges to the product radical (**E**), which regains hydrogen from 5′-deoxyadenosine (**F**). The transformed amino acid (**G**) is released when PLP rebinds with the enzyme.

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Scheme 1. Examples of Reactions Catalyzed by B_{12}-Dependent Aminomutases

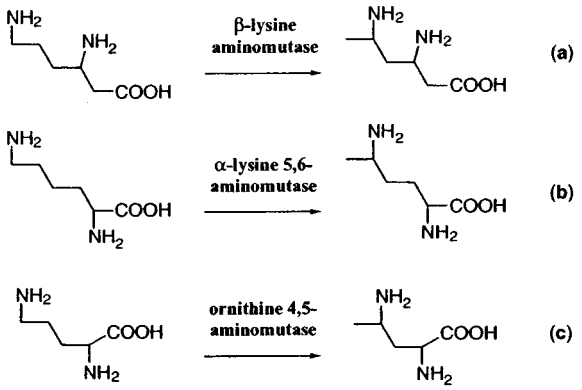
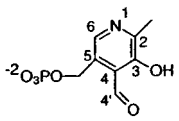
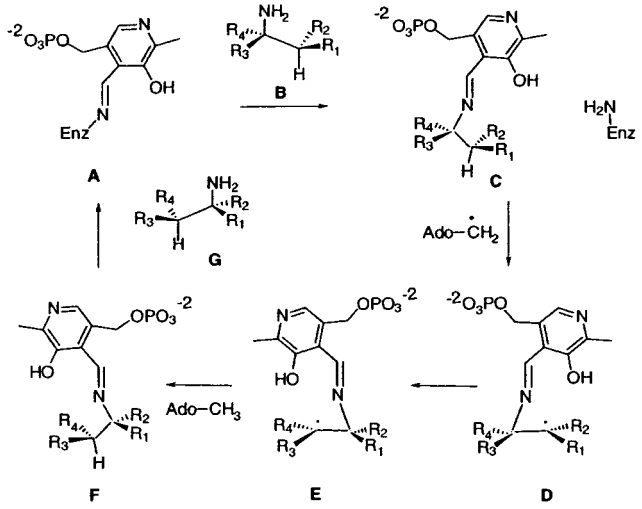


Chart 1. Structure of Pyridoxal 5′-Phosphate (PLP)



Scheme 2. Currently Accepted Mechanism[6,9] for 1,2-Amino Shifts Catalyzed by B_6, B_{12}, and Aminomutases



The involvement of radicals in aminomutase-catalyzed reactions has been verified through electron paramagnetic resonance (EPR) studies.[9,11−16,17] However, little definitive information has been obtained about the radical rearrangement step (**D** → **E** in Scheme 2) due to difficulties in probing these reactions experimentally. The currently accepted rearrangement mechanism involves intramolecular addition of the unpaired electron in the substrate radical to the imine C=N double bond. The ring of the resulting substituted azacyclopropylcarbinyl radical may then open through homolytic fission of the alternate bond to yield the product radical. This (addition−elimination) mechanism has also been discussed for the reactions catalyzed by 2-methyleneglutarate mutase and methylmalonyl-CoA mutase, two B_{12}-dependent carbon-skeleton mutases.[3,4,18−20] A chemical model for the radical rearrangement of an aldimine has also been found,[21] which provides support for both the involvement of PLP and the radical mechanism.

Due to the lack of experimental evidence concerning the details of the radical rearrangement mechanism, theoretical investigations are potentially extremely valuable. The goal of the present work is to gain a greater understanding of the mechanism associated with 1,2-amino shifts catalyzed by aminomutases through the use of molecular orbital theory.[22] We attempt to shed light on why B_{12}-dependent aminomutases require pyridoxal 5′-phosphate and how the enzyme may serve to facilitate these reactions. This is most easily accomplished by studying the energetics of the rearrangement reaction. The B_{12}-dependent reactions typically proceed with a k_{cat} between 40 and 150 s^{-1}.[23,24] Experimental rate data for B_{12}-independent lysine 2,3-aminomutase are also consistent with this range.[9,25] Using techniques employed previously, we estimate that the barrier for the rate-limiting step in B_{12}-catalyzed 1,2-amino shifts should lie between approximately 55 and 75 kJ mol^{-1}.[26] The barrier for the radical rearrangement step must therefore fall within or below this range. This provides an approximate reference point to help assess the proposed mechanism of 1,2-amino shifts catalyzed by B_6, B_{12}, and aminomutases.

Computational Details

In accord with our most recent theoretical investigation of a B_{12}-mediated rearrangement,[27] geometries for small model systems were optimized with B3-LYP/6-31G(d,p). Larger models were optimized at the B3-LYP/6-31G(d) level. The differences in relative energies obtained through higher-level calculations performed on these respective geometries is found to be negligible.[28]

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In studies of related B_{12}-dependent carbon-skeleton rearrangements[18−20,27] and the eliminase reaction catalyzed by diol dehydratase,[29] improved relative energies were obtained from high-level, single-point calculations on B3-LYP optimized geometries performed with a variety of techniques based on the complete basis set (CBS)[30] and Gaussian-*n*[31] formalisms. In the present study, improved energy profiles were generally obtained with the previously defined G3(MP2)-RAD(p) technique.[27] This technique is a modification of the G3(MP2) method 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 calculated with restricted-open-shell perturbation theory (RMP2) rather than the unrestricted formalism (UMP2). The geometries and frequencies are obtained with UB3-LYP/6-31G(d,p) rather than UHF/6-31G(d) or UMP2/6-31G(d). Unless otherwise noted, relative energies in the text refer to the G3(MP2)-RAD(p) level of theory.

Under the constraints of our existing computer resources, the G3-(MP2)-RAD(p) method is restricted to systems with less than ∼10 heavy atoms. For larger models investigated in the present work, the energy profiles were evaluated at the RMP2/G3MP2large level, which for simplicity will be referred to as RMP2. RMP2, with a variety of basis sets, has described radical reactions successfully in the past[32] and yields results comparable to those obtained with G3(MP2)-RAD(p) for the smaller models investigated in the present work (see below). We note, however, that RMP2 generally slightly overestimates the barriers for radical addition reactions,[33] such as those involved in the addition−elimination pathway.

In addition to examining their energies, we can also study radical intermediates by comparing experimental EPR data with results obtained from quantum chemical methods. In the present study, the hyperfine coupling constants (HFCCs) were calculated with B3-LYP/6-311G-(2d,p). This combination has previously been successfully employed for a variety of organic[34] and biologically relevant radicals.[27,35] In general, B3-LYP/6-311G(2d,p) slightly overestimates experimental HFCCs.[34]

All calculations were performed with the GAUSSIAN 98,[36] MOLPRO 98[37a] or MOLPRO 2000[37b] program suites.

Results and Discussion

A. 1,2-Amino Shifts in the Absence of B_6. In addition to those reactions catalyzed by aminomutases, migration of an amino group from one carbon to another is important in many other biochemical reactions. For example, ethanolamine ammonia lyase (ethanolamine deaminase) is a coenzyme B_{12}-dependent enzyme which transforms 2-aminoethanol into ammonia and acetaldehyde.[23] The first step in the rearrangement sequence, following hydrogen abstraction by B_{12}, is believed to be a 1,2-shift of the amino group from the 1-hydroxy-2-aminoethyl radical to form the highly unstable 2-hydroxy-2-aminoethyl radical. Amino group migrations are also of interest outside the biochemistry field. For example, a 1,2-amino shift is a key step in the Audier mechanism for skeletal isomerization of long-chain alkanamine radical cations.[38]

Scheme 3. Mechanistic Possibilities for the Degenerate Rearrangement of the 2-Aminoethyl Radical (**1**)

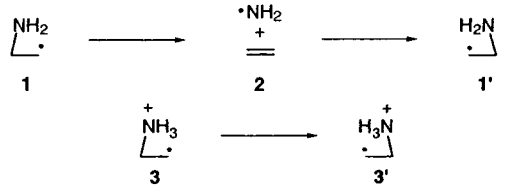

Table 1. Relative Energies (kJ mol^{-1})[a] for the Species Involved in the Degenerate Rearrangement of the 2-Aminoethyl Radical (**1**) (See Scheme 2)[b]

	G3(MP2)-RAD(p)	RMP2/ G3MP2large
1	0.0	0.0
TS:**1**→**2**	90.5	110.2
2	67.8	85.6
3	0.0	0.0
TS:**3**→**3′**	104.8	118.7

[a] Relative energies with respect to either **1** or **3**. [b] Calculations performed on B3-LYP/6-31G(d,p) geometries.

To determine the tendency of an amino group to migrate to an adjacent radical center, we initially examined the degenerate rearrangement of the 2-aminoethyl radical (**1**, Scheme 3). Although a transition structure describing a concerted migration between **1** and **1′** could not be located, a stepwise pathway (fragmentation−recombination) involving separation into the amino radical and ethylene (**2**), followed by recombination of the fragments, was characterized (Scheme 3). This pathway is associated with a barrier of 90.5 kJ mol^{-1} and the separated species lie 67.8 kJ mol^{-1} above the reactant at the G3(MP2)-RAD(p) level (Table 1). The RMP2 values somewhat overestimate the G3(MP2)-RAD(p) results (by 17.8−19.7 kJ mol^{-1}). As with the fragmentation−recombination mechanisms of several other B_{12}-assisted rearrangements,[19,20] this route appears to be associated with a barrier too large to be considered biologically relevant.

We previously found that protonation of the migrating group facilitates many 1,2-shifts.[18−20,27,29] Additionally, experimental evidence suggests that NH_3^+ migrations in the gas phase are rapid and common reactions of protonated β-aminoalkyl radicals.[39] However, the calculated barrier (104.8 kJ mol^{-1}) for the

(28) For example, the differences between the G3(MP2)-RAD relative energies obtained for the species involved in the degenerate rearrangement of the 2-(*N*-methylidine)ethyl radical (eqn 2) when the geometry optimizations are carried out with the 6-31G(d) (denoted RAD) or 6-31G(d, p) (denoted RAD(p)) basis set are just 0.1−0.2 kJ mol^{-1}.

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(37) (a) MOLPRO 98 is a package of ab initio programs written by Werner, H.-J. and Knowles, 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.

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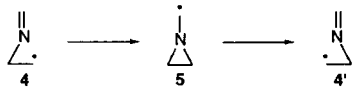
rearrangement involving a protonated migrating amino substituent (**3** → **3′**, Scheme 3) is even higher than that of the fragmentation−recombination pathway. Our calculated barrier height is consistent with previously reported values.[2,40] Once again, we note that the RMP2 results slightly overestimate the G3(MP2)-RAD(p) reaction barrier (by 13.9 kJ mol^{-1}).

With the avenues of both direct migration and migration assisted by protonation closed, nature is forced to find another way to accomplish 1,2-shifts of amino groups. This is apparently achieved through judicious use of vitamin B_6. The remainder of our work is concerned with elucidating how B_6 helps to catalyze 1,2-amino shifts.

B. 1,2-Amino Shifts in the Presence of B_6. (1) Degenerate Rearrangement of the 2-(*N*-methylidine)ethyl Radical. Since it is understood that PLP forms a Schiff base with a variety of substrates, we have examined the effect of modifying the migrating amino substituent through formation of an imine by considering the degenerate rearrangement of the 2-(*N*-methylidine)ethyl radical (**4**, eq 2). The introduction of an imine

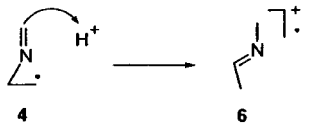


(2)

functionality allows the reaction to proceed via a three-membered cyclic intermediate (the 1-aziridinylcarbinyl radical, **5**), as discussed for other unsaturated migrating groups.[19,20] The preferred conformation of **5** leads to maximum overlap between the orbital containing the unpaired electron and the nitrogen lone pair (Figure 1). This intermediate lies 51.8 kJ mol^{-1} higher in energy than the open-chain reactant (**4**) and the barrier to its formation is 77.8 kJ mol^{-1} at the G3(MP2)-RAD(p) level (Table 2).[41,42] Particular note should be taken of the good agreement between the relative energies calculated with G3(MP2)-RAD(p) and RMP2 for reaction 2, which supports our later use of RMP2 energetics for similar reactions of larger models. While the energy requirement for this reaction is significantly lower than that described for the rearrangement of the 2-aminoethyl radical (by 34 kJ mol^{-1} at G3(MP2)-RAD(p)), it is desirable to examine mechanisms whereby the barrier might be further lowered.

Led by our previous studies,[5,18−20,27,29] we considered protonation of the migrating group. The migrating group in **4** has two possible protonation sites. Protonation at nitrogen (**4**-NH$^+$) yields barriers slightly larger than those discussed for the nonassisted pathway (Table 2).[43] The failure of protonation at this site to reduce the reaction barrier is associated with the fact that the cyclic intermediate and transition structure have smaller proton affinities at nitrogen (891.6 and 895.2 kJ mol^{-1} at G3(MP2)-RAD(p), respectively) than the ring-opened imine (899.7 kJ mol^{-1}).

Protonation at carbon in the migrating group of **4** is also unsatisfactory. Specifically, protonation at the exocyclic carbon in **5** (**5**-CH$^+$, proton affinity of 875.5 kJ mol^{-1} at G3(MP2)-RAD(p)) is disfavored over protonation at nitrogen by ∼16 kJ mol^{-1} (**5**-NH$^+$, proton affinity of 891.6 kJ mol^{-1} at G3(MP2)-RAD(p)). Additionally, attempts to optimize a structure equivalent to **4** protonated at the migrating group carbon lead to the 2-azabut-2-ene radical cation (**6**, eq 3).[44] This result suggests



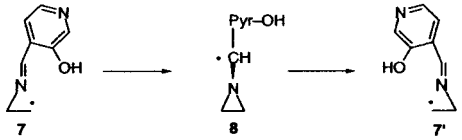
(3)

that following protonation at the carbon of the migrating group in **4**, a 1,2-hydrogen shift occurs spontaneously and is clearly favored over the 1,2-imino migration.

Thus, although protonation at a double bond reduces the barrier height in models of several other rearrangements,[18−20,27,29] protonation at the double bond does not appear to be the answer for the **4** → **4′** rearrangement. More specifically, the reaction barrier is not reduced upon protonation at nitrogen, while protonation at carbon leads to alternative products. Before consideration of larger models, it is important to again stress the relatively good agreement between the reaction barriers calculated at G3(MP2)-RAD(p) and RMP2 for the rearrangements considered thus far (Tables 1 and 2).

(2) Effects of the Pyridoxal Ring. From our results on the small model systems, we see that PLP can in principle assist 1,2-amino shifts by introducing unsaturation into the migrating group, thereby permitting an intramolecular mechanism. However, since the resulting barrier height (77 kJ mol^{-1}) is still slightly above the range estimated for the rate-limiting step in B_{12}-assisted 1,2-amino shifts (55−75 kJ mol^{-1}), it is desirable to explore additional roles for B_6 that might provide an explanation for its importance.

In attempting to determine whether the sole significance of this coenzyme form of vitamin B_6 is to introduce unsaturation into the migrating group of the substrate, we extend our model system to account for the pyridoxal functionality (eq 4). We



(4)

use a simplified pyridoxal ring that replaces the methyl group and the phosphate handle of PLP with hydrogen atoms.[45] In addition, we use RMP2 in place of G3(MP2)-RAD(p) in calculations on these larger systems.

The substituted cyclic intermediate that includes the simplified pyridoxal ring (**8**) is stabilized to a greater extent (by 3.6 kJ mol^{-1} at RMP2) than its ring-opened counterpart (**7**), relative to the model system that does not include the PLP ring (**5** and **4**). The barrier to ring closure is also reduced (by 14.9 kJ mol^{-1} at RMP2). Thus, the presence of the model PLP ring leads to

(39) (a) Hammerum, S.; Kuck, D.; Derrick, P. J. *Tetrahedron Lett.* **1984**, *25*, 893−896. (b) Bjornholm, T.; Hammerum, S.; Kuck, D. *J. Am. Chem. Soc.* **1988**, *110*, 3862−3869. (c) Hammerum, S.; Petersen, A. C.; Sølling, T. I.; Vulpius, T.; Zappey, H. *J. Chem. Soc., Perkin Trans. 2* **1997**, 391−395.

(40) (a) Yates, B. F.; Radom, L. *Org. Mass Spectrom.* **1987**, *22*, 430. (b) Hammerum, S. *Int. J. Mass Spectrom.* **2000**, *199*, 71−78.

(41) Similar barriers have been calculated with other levels of theory. See: Reference 42.

(42) Smith, D. M.; Nicolaides, A.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1998**, *120*, 10223−10233.

(43) The B3-LYP optimized structures for **4**-NH$^+$, TS:**4**-NH$^+$ → **5**-NH$^+$, **5**-NH$^+$, **5**-CH$^+$, **6**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, and **18** are provided in the Supporting Information.

(44) Although a structure equivalent to **4** protonated at carbon could be located with HF/6-31G(d, p), inclusion of electron correlation through the use of either B3-LYP or MP2 with the same basis set leads to **6**.

(45) It is believed that neither the methyl group nor the phosphate handle is required in model systems for catalysis (see ref 7b).

(46) The RMP2 radical stabilization energy of **5**, which was calculated as the enthalpy change of the appropriate isodesmic reaction with methane, is 29.5 kJ mol^{-1}. This value is reduced to 18.2 kJ mol^{-1} with rotation at the radical center by 90°.

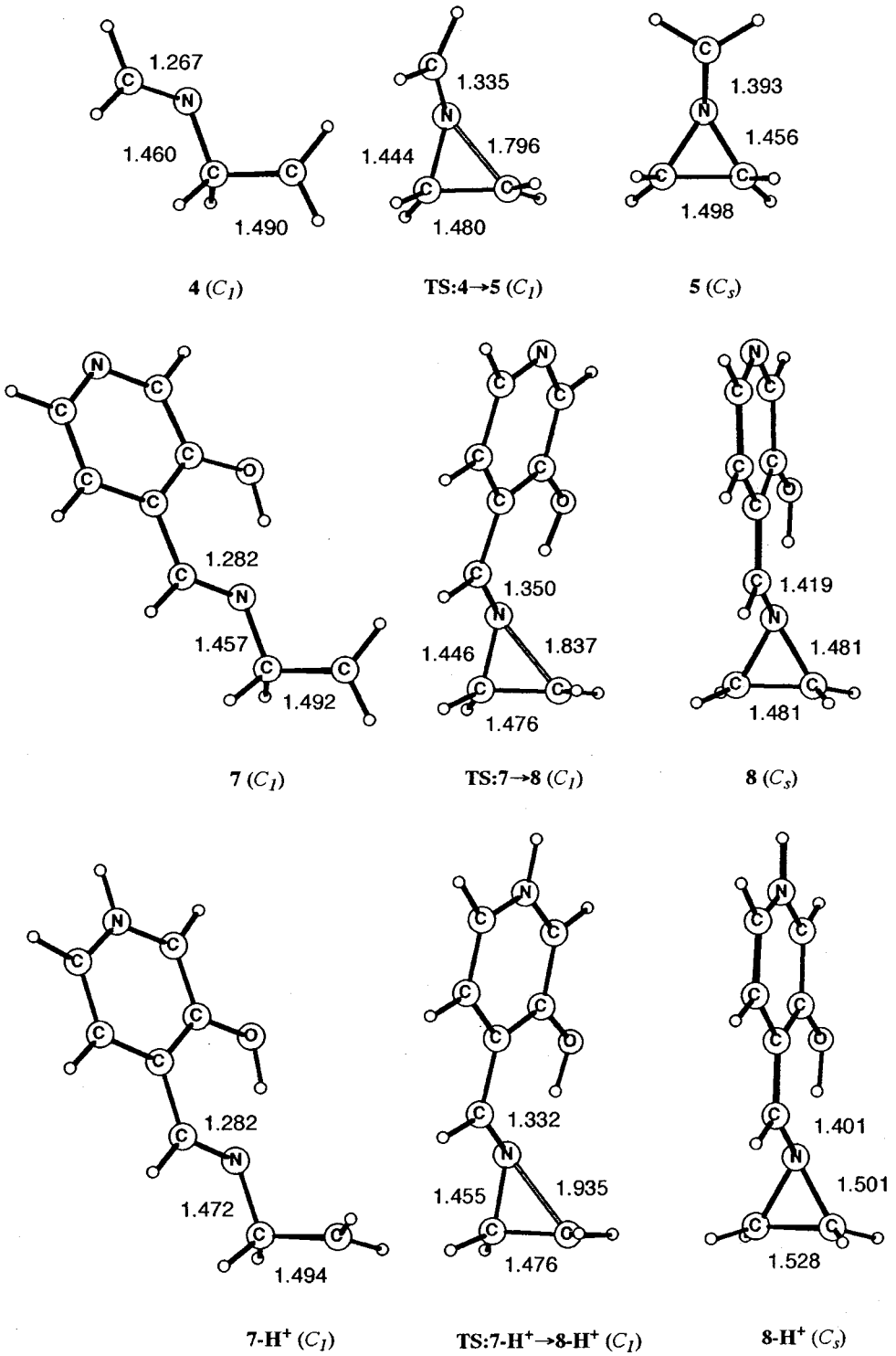


Figure 1. B3-LYP optimized structures and selected bond lengths (Å) for the species involved in the degenerate rearrangement of **4**, **7**, and **7**-H$^+$. See also eqs 2, 4, and 5.

a small barrier reduction compared with the barrier reduction associated with the introduction of a double bond to the migrating group.

Our previous work suggests that protonation or partial protonation of the migrating group by an amino acid residue at the active site in the enzyme can facilitate intrinsically difficult radical rearrangements. Thus, we investigate protonation of the migrating group in search of a mechanism by which the enzyme might further reduce the rearrangement barrier for the aminomutase-catalyzed reactions.

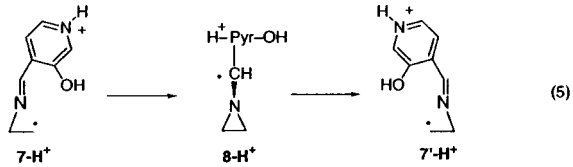
There are two obvious sites to protonate the migrating group of **7**: the imine nitrogen and the pyridine nitrogen. Protonation at the pyridine nitrogen is preferred by 33 kJ mol^{-1} (RMP2), mainly because it avoids disruption of the intramolecular hydrogen bond between the imine nitrogen and the hydroxyl group (Figure 1). Furthermore, we might expect from our calculations on the related small model (**4**) that the reaction will not benefit from protonation at the imine nitrogen (see previous section). Thus, a possible role of the hydroxyl group of PLP is to dissuade protonation at an unbeneficial site. We therefore

Table 2. Relative Energies (kJ mol^{-1})[a] for Models of B_6-Assisted 1,2-Amino Shifts (See Eqs 2−5)[b]

	G3(MP2)-RAD(p)	RMP2/ G3MP2large
4	0.0	0.0
TS:**4**→**5**	77.8	76.2
5	51.8	42.2
4-NH$^+$	0.0	0.0
TS:**4**-NH$^+$→**5**-NH$^+$	82.3	85.5
5-NH$^+$	60.0	50.5
5-CH$^+$	76.0	79.9
6	−0.3	14.4
7		0.0
TS:**7**→**8**		61.3
8		38.6
7-H$^+$		0.0
TS:**7**-H$^+$→**8**−H$^+$		37.2
8-H$^+$		−0.7
7		0.0
TS:**7**→**15**		119.8
15		87.4

[a] Relative energies with respect to **4**, **4**-NH$^+$, **7** or **7**-H$^+$. [b] Calculations performed on B3-LYP/6-31G(d,p) geometries.

consider the effects of protonation at the nitrogen in the 3-hydroxypyridine ring on the reaction barrier (eq 5). We find



(5)

that protonation of the pyridine nitrogen greatly stabilizes the cyclic intermediate (**8**-H$^+$), which is found to lie 0.7 kJ mol^{-1} below the relevant reactant radical (**7**-H$^+$). As a result of this stabilization, the barrier for ring closure (via TS:**7**-H$^+$ → **8**-H$^+$) is strikingly reduced to just 37.2 kJ mol^{-1}.

There are notable effects on the cyclic intermediate upon inclusion of the model PLP ring, and upon protonation of the ring, that may help explain the differences in the calculated barrier heights for reactions 2, 4, and 5. First, a change in the spin distribution at the primary radical center (the exocyclic carbon) is observed, with calculated RMP2 spin densities at this position in **5**, **8**, and **8**-H$^+$ of 0.91, 0.74, and 0.63, respectively. Thus, when the PLP model is included, and subsequently protonated, there is delocalization of spin density from the radical center and the cyclic intermediate becomes more stable with respect to the appropriate ring-opened reactant.

Second, there are significant differences in the geometries of the cyclic intermediates **5**, **8**, and **8**-H$^+$ (Figure 1). A coplanar arrangement between the singly occupied orbital and the nitrogen lone pair exists in **5,** which allows a maximum interaction between the two. In contrast, the radical center is twisted by 90° in **8** and **8**-H$^+$, due to the formation of an intramolecular hydrogen bond involving the hydroxyl group. Thus, the stabilizing effect of electron donation from the nitrogen lone pair to the radical center in **5** is not possible in either **8** or **8**-H$^+$. However, by analogy with the cyclopropylcarbinyl radical,[42] the conformations of both **8** and **8**-H$^+$ allow maximum overlap between the orbital containing the unpaired electron and the Walsh-type orbitals involving the C−N bonds of the aziridine ring. Although this type of donor stabilization is smaller than that provided by interactions with the nitrogen lone pair,[4] the stabilizing effects are still significant.

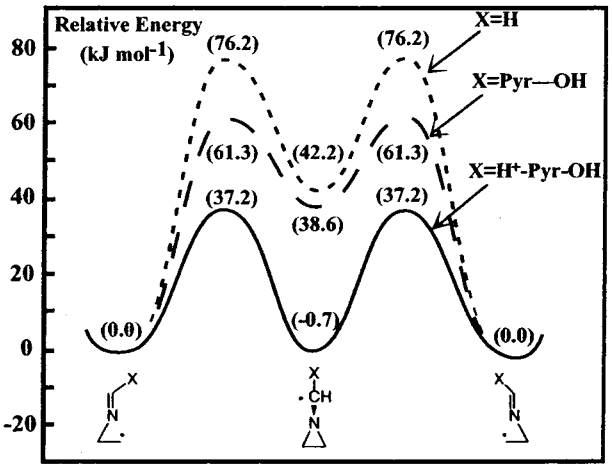


Figure 2. Schematic energy profile for the degenerate rearrangement of substituted 2-(*N*-methylidine)ethyl radicals, where X = H (**4**), Pyr-OH (pyridin-3-ol, **7**), or H$^+$−Pyr-OH (**7**-H$^+$).

Both π-electron withdrawal from the radical center (by PLP) and electron donation to the radical center (by the adjacent nitrogen lone pair in **5** or the Walsh-type orbitals in **8**) are expected to stabilize the cyclic intermediate. The balance between the effects of the increase in electron withdrawal from the radical center (stabilizing) and the decrease in electron donation to the radical center as a result of twisting at the radical center (destabilizing) when the PLP ring is included can perhaps explain the small difference in the relative stabilities of **5** and **8**.

Although the electron-withdrawing ability of the model PLP ring is improved upon protonation, we find little difference between the individual stabilizing effects of the neutral and protonated model PLP rings at an unsubstituted radical center.[47] However, the combined effect of electron withdrawal (by the PLP ring) and electron donation (by the azacyclopropyl C−N bonds) is greater in **8**-H$^+$ than **8**,[48] which results in a greater stabilization for the former with respect to the appropriate ring-opened reactant. This synergistic combination of electron-donor and electron-acceptor substituents has been observed in many other radicals[49,50] and is commonly referred to as captodative stabilization.[50]

The above results (summarized in Figure 2) suggest that the rate of the ring-closing/ring-opening pathway that follows the introduction of a double bond to the migrating group is retarded by the relatively high energy of the three-membered cyclic intermediate. Although effects such as electron donation (by the adjacent nitrogen lone pair in **5** or the azacyclopropyl C−N bonds in **8**) or slight electron withdrawal (by the pyridine ring) can stabilize this intermediate, these effects alone do not yield a reaction barrier low enough to fully explain the observed catalysis. However, when the electron-withdrawing capacity of the ring is improved through protonation, the stability of the cyclic intermediate is greatly enhanced through captodative effects and the reaction barrier is significantly reduced (by 24.1

(47) The RMP2 radical stabilization energies of the PLP-substituted methyl radical and its protonated counterpart are 46.6 and 45.4 kJ mol^{-1}, respectively.

(48) The RMP2 radical stabilization energies for **8** and **8**-H$^+$ are 67.3 and 87.1 kJ mol^{-1}, respectively.

(49) See, for example: (a) Leroy, G.; Sana, M.; Wilante, C. *J. Mol. Struct.* **1991**, *228*, 37−45. (b) Bordwell, F. G.; Zhang, X.-M.; Alnajjar, M. S. *J. Am. Chem. Soc.* **1992**, *114*, 7623−7629. (c) Yu, D.; Rauk, A.; Armstrong, D. A. *J. Am. Chem. Soc.* **1995**, *117*, 1789−1796.

(50) Viehe, H.-G.; Janousek, Z.; Merényi, R.; Stella, L. *Acc. Chem. Res.* **1985**, *18*, 148−154.

Chart 2. Model Systems Showing a Substituent X in Ring-Opened (a) and Cyclic (b) Structures

a **b**

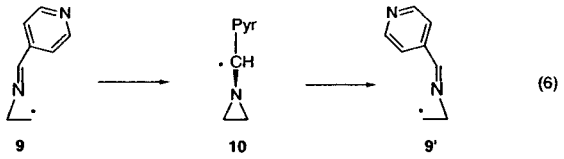
kJ mol^{-1}).[51] From partial-proton-transfer considerations,[18,20] we would expect that full protonation may not be a requirement for a reduced barrier height and that even partial protonation of the pyridine nitrogen by groups in the enzyme will aid catalysis. The implications of these observations will be further discussed in the following section.

C. Role of B_6. From our results, we propose that PLP has two important roles. The first is to introduce unsaturation to the migrating group. This step is essential for the intramolecular pathway involving a cyclic intermediate, which is a much lower energy pathway than the pathways considered that have a saturated migrating group (see also section D). The second important role for PLP is to stabilize the high-energy intermediate through the cooperation of electron donation to the radical center (by the azacyclopropyl C−N bonds) and π-electron withdrawal from the radical center (in particular by the protonated pyridoxal ring).[52]

Our hypothesis is simply that a group X (Chart 2) with π-electron-withdrawing capabilities is likely to stabilize the cyclic intermediate **b**, and, therefore, the transition structure for ring closing, to a greater extent than the ring-opened structure **a**. The degree to which the cyclic intermediate is stabilized, and the rearrangement barrier reduced, will depend on the electron-withdrawing ability of the functional group X.

The captodative stabilizing role of PLP supports hypotheses that the pyridoxyl functionality acts as an "electron sink".[7,10] On the other hand, recent theoretical studies of decarboxylation reactions indicate that the PLP ring plays a minor role relative to imine formation.[53] Thus, although dispersal of the developing negative charge by PLP does not increase the reaction rate of decarboxylation, dispersal of electron density by PLP does reduce the reaction barrier for 1,2-amino shifts by stabilizing the radical intermediate. This suggests that the responsibilities of the PLP ring may vary with the nature of the reaction.[54] The hypothesis that PLP provides captodative stabilization of the intermediates in aminomutase-catalyzed reactions can be tested through consideration of a range of protonated and nonprotonated functional groups.

(1) Role of the PLP Hydroxyl Group. PLP-dependent decarboxylases have been previously shown to benefit from the Coulombic stabilization provided by the ring hydroxyl group.[53a] We have examined the effects of the hydroxyl group on the reactions catalyzed by aminomutases by replacing X = 3-hydroxypyridinyl (Chart 2 and eq 4) with X = pyridinyl (eq 6).



9 **10** **9'** (6)

The barrier for ring closing of **9** (Table 3) is only slightly smaller (by 13.1 kJ mol^{-1} at RMP2) than the model that includes the hydroxyl group (**7**, Table 2). However, the cyclic intermediate **10** is stabilized substantially more than **8** with respect to the appropriate ring-opened reactant. The stabilization of **10** may be attributed to its preferred structure (Figure 3), which allows maximum overlap between the nitrogen lone pair, the π-system of the pyridine ring, and the singly occupied orbital and results in enhanced spin delocalization[55] and captodative effects. In contrast, intramolecular hydrogen bonding between the hydroxyl group and the imine nitrogen in **8** (and also present in **7**) leads to a structure with less effective overlap between the Walsh-type orbitals, the pyridine ring, and the singly occupied orbital (Figure 1).

The changes in geometry and spin distribution when the hydroxyl group is removed from the model PLP ring result in a substantial relative stabilization of the cyclic intermediate (**10,** Table 3). This stabilization is even more pronounced when the pyridine model is protonated: the cyclic intermediate (**10**-H$^+$) is now found to lie nearly 50 kJ mol^{-1} *below* the reactant (**9**-H$^+$) with the consequence that the intermediate lies in a potential well 79 kJ mol^{-1} deep.[56] Considering the ease of redistribution of internal energy in the enzymatic environment, a well of this depth could conceivably result in the trapping of the intermediate and inactivation of the enzyme.[57,58] This is clearly an undesirable state of affairs in the reaction mechanism.

Thus, although the effect on the barrier height of removal of the hydroxyl group is relatively small, the stabilization effect in the cyclic intermediate is large and results in an apparent overstabilization of this intermediate. This is particularly true when the pyridine ring is protonated. We see here an example of the fact that an optimum reaction profile must not only avoid large barriers but also lack deep valleys. That is, the overstabilization of intermediates, such as **10**-H$^+$, is likely to be counterproductive in a biological context and should be avoided if possible. Our calculations suggest that the B_6 hydroxyl group serves this role in the aminomutase-catalyzed reactions.

The substantial stabilization of the cyclic intermediate in our truncated model may have other important consequences. In particular, experimental studies have yet to identify the reaction intermediate in the reactions catalyzed by B_6, B_{12}, and aminomutases.[12] Our results indicate that if a PLP model system without the hydroxyl substituent is used, then the cyclic

(51) Although the presence of a net charge can lower a reaction barrier relative to that for the related neutral reaction, preliminary calculations indicate that an innocuous counterion has only a small effect on the relative energies of the species involved in reaction 5 and the barrier indeed decreases upon protonation of the PLP ring.

(52) In a previous communication,[22] we suggested that the captodative stabilization comes from the nitrogen lone pair. Clearly this contribution is not possible in the favored orientation of **8** and **8**-H$^+$ (Figure 1). Sufficient stabilization comes instead from the orbitals within the azacyclopropyl group.

(53) (a) Bach, R. D.; Canepa, C.; Glukhovtsev, M. N. *J. Am. Chem. Soc.* **1999**, *121*, 6542−6555. (b) Toney, M. D. *Biochemistry* **2001**, *40*, 1378−1384.

(54) We note that PLP-dependent decarboxylation and transamination reactions involve carbanionic intermediates, while PLP-dependent aminomutases catalyze radical reactions.

(55) The exocyclic C−N bond length decreases (from 1.419 to 1.385 Å) when the hydroxyl group is removed (i.e., **8** → **10**). As a result of this increased interaction with the radical center and the slight changes in the electron-withdrawing abilities of X (Chart 2) in **10** compared with **8**, the spin density at the radical center is slightly reduced (from 0.74 in **8** to 0.70 in **10**).

(56) The spin density at the primary radical center in **10**-H$^+$ is small (0.40 at RMP2), which reflects an improvement in the electron-withdrawing ability of X and the electron-donating ability of the aziridine ring (Chart 2) in **10**-H$^+$ compared with **10**. The latter is reflected in the shortened exocyclic C−N bond (1.342 Å) in **10**-H$^+$. These effects lead to a large captodative stabilization in **10**-H$^+$.

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

(58) This is analogous to the state of affairs leading to the (suicide) inactivation of diol dehydratase and ethanolamine ammonia lyase by glycolaldehyde (Abend, A.; Bandarian, V.; Reed, G. H.; Frey, P. A. *Biochemistry*, **2000**, *39*, 6250−6257) and the inactivation of ethanolamine ammonia lyase by hydroxyethylhydrazine (Bandarian, V.; Reed, G. H., *Biochemistry*, **1999**, *38*, 12394−12402).

Table 3. Relative Energies (kJ mol^{-1})[a] for Additional Models of B_6-Assisted 1,2-Amino Shifts (See Eqs 6, 7, and 9)[b]

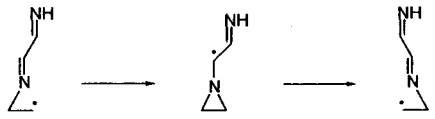
	G3(MP2)-RAD(p)	RMP2/ G3MP2large
9		0.0
TS:**9**→**10**		48.2
10		16.2
9-H+		0.0
TS:**9**-H$^+$→**10**-H$^+$		30.5
10-H$^+$		−48.5
11	0.0	0.0
TS:**11**→**12**	59.0	55.1
12	19.8	7.8
11-H$^+$	0.0	0.0
TS:**11**-H$^+$→**12**-H$^+$	2.2	2.7
12-H$^+$	−81.0	−105.2
13	0.0	0.0
TS:**13**→**14**	55.6	49.2
14	9.8	−0.5
13-H$^+$	0.0	0.0
TS:**13**-H$^+$→**14**-H$^+$	24.2	20.2
14-H$^+$	−12.5	−28.7

[a] Relative energies with respect to **9**, **9**-H$^+$, **11**, **11**-H$^+$, **13**, or **13**-H$^+$. [b] Calculations performed on B3-LYP/6-31G(d,p) geometries.

intermediate will be greatly stabilized and should be more readily trapped and identified.[59]

The preclusion of overstabilization of the cyclic intermediate must not come at the expense of too great a diminution in the lowering of the rearrangement barrier. In the following examples, we shall see further illustrations of this delicate balance between sufficient and excessive stabilization of the reaction intermediate.

(2) Small Model System for PLP. Investigation of smaller model systems is attractive, partly because high-level molecular orbital calculations can be performed and used to assess the accuracy of the RMP2 results presented for the larger models. A useful computational model for pyridine is an imine functional group. Therefore, we consider the following rearrangement:

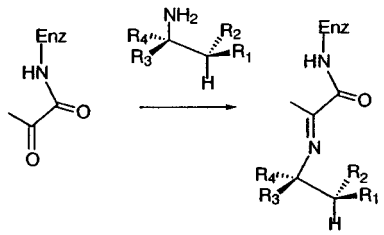


11 **12** **11'** (7)

As noted for the other small models investigated in the present work, there is good agreement between the G3(MP2)-RAD(p) and RMP2 rearrangement barrier for eq 7 (Table 3).[43] The RMP2 barrier for the rearrangement of **11** is only slightly smaller (by 6.2 kJ mol^{-1}) than the rearrangement barrier of **7**. However, the cyclic intermediate **12** is much more stable (by 30.8 kJ mol^{-1} at RMP2) than **8** with respect to the appropriate ring-opened radical.[60] It is interesting that the barrier to ring closure changes only slightly, despite the greater stabilization of the reaction intermediate, for the rearrangement of **11** compared with **7**.

As noted for models **8** and **10**, substantial stabilization of the reaction intermediate is observed upon protonation.[61] This results in a greatly reduced cyclization barrier (2 kJ mol^{-1}) and a very deep well (83 kJ mol^{-1}) along the reaction coordinate. Although the reaction barrier is greatly reduced, the significant stability of the intermediate would be expected to prevent formation of the desired product within the enzymatic environment. Nature must ensure a balance between reducing the barrier to cyclization and overstabilizing the cyclic intermediate in aminomutase-catalzyed rearrangements. PLP may provide the delicate requirement of a suitable protonation site within an imine π-system to achieve the appropriate balance.

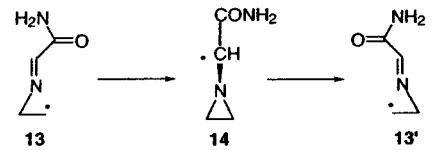
(3) PLP versus Pyruvate. Previous theoretical studies have concluded that the decrease in the barrier height for decarboxylation of glycine does not change significantly when a model imine system is extended to include a model for either PLP or pyruvate.[53a,62,63] Although pyruvate is a covalently bound catalytic group required by some enzymes, such as amino acid decarboxylases and reductases, it is not believed to play a direct role in any 1,2-amino shifts catalyzed by B_{12}-dependent aminomutases.[64] Pyruvate is linked to enzymes through an amide group, and in a manner similar to PLP-dependent enzymes, a Schiff base is formed with the substrate (eq 8). The main role



(8)

of the pyruvoyl group is believed to be associated with the electron-attracting properties of the carbonyl group, analogous to the "electron sink" concept discussed for PLP.[7] On the basis of our hypothesis that one of the major roles of B_6 in aminomutase-catalyzed reactions is to captodatively stabilize the cyclic intermediate, thereby lowering the reaction barrier, we might expect similar behavior for pyruvoyl- and PLP-assisted 1,2-amino shifts. It is therefore of interest to examine a model for a pyruvoyl-dependent 1,2-shift in the hope of understanding why in fact no B_{12}-dependent aminomutases utilize this coenzyme rather than B_6.

We employ a model similar to that used by Bach et al. (**13**, eq 9).[43,53a,63] The G3(MP2)-RAD(p) barrier for cyclization of



13 **14** **13'** (9)

(59) The additional stabilization of **10** over **8** is in part due to geometrical differences, which may not be possible within the enzyme. If we remove such differences, for example by imposing C_s symmetry on **10** and **10**-H$^+$, then we find that these species lie, respectively, 29.0 kJ mol^{-1} above and 16.3 kJ mol^{-1} below the relevant ring-opened reactant. This translates into well depths similar to those calculated for the PLP model that includes the hydroxyl group. Therefore, if reorganization is not permitted within the active site of the enzyme, then removal of the hydroxyl group alone may not be sufficient to stabilize the reaction intermediate for identification.

(60) Geometrical differences between **8** and **12**, in part, lead to greater captodative stabilization of the latter. The structure of **12** allows maximum overlap between the π-system of X (Chart 2), the nitrogen lone pair in **12**, and the orbital containing the unpaired electron (as also discussed for models **5** and **10**).[43]

(61) Stabilization of **12**-H$^+$ compared with **12** results from the improved electron-withdrawing ability of the imine group following protonation and the increased donation by the nitrogen lone pair (as reflected in the shortening of the exocyclic C−N bond from 1.383 Å in **12** to 1.333 Å in **12**-H$^+$). The combination of these effects leads to a large decrease in the spin density at the primary radical center (from 0.65 in **12** to 0.31 in **12-H$^+$**).

(62) Bach, R. D.; Canepa, C. *J. Org. Chem.* **1996**, *61*, 6346−6353.

(63) Bach, R. D.; Canepa, C. *J. Am. Chem. Soc.* **1997**, *119*, 11725−11733.

(64) Pyruvate has, however, been shown to stimulate the activity of β-lysine aminomutase.[6]

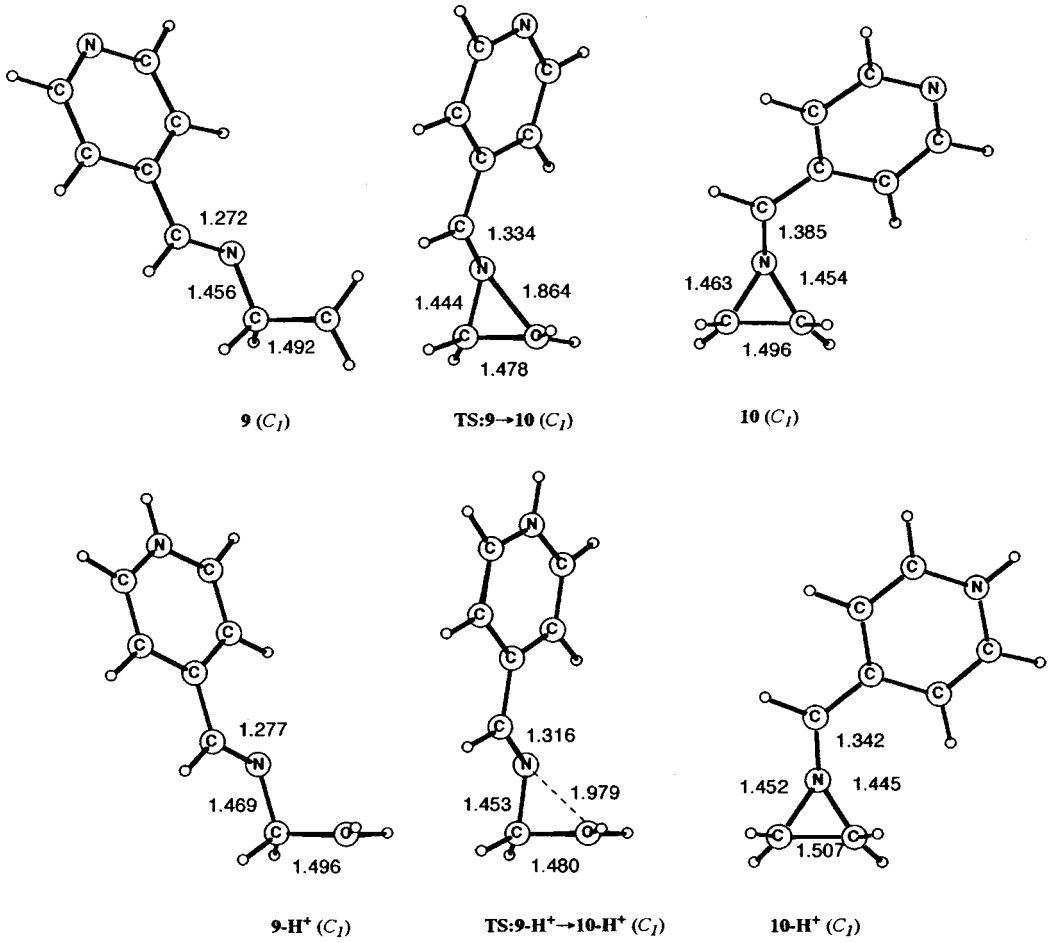


Figure 3. B3-LYP optimized structures and selected bond lengths (Å) for the species involved in the degenerate rearrangement of **9** and **9**-H$^+$. See also eq 6.

13 is 55.6 kJ mol^{-1} (Table 3). The cyclic intermediate (**14**) is predicted to lie only 9.8 kJ mol^{-1} above the ring-opened reactant (**13**). Upon protonation at oxygen (**13**-H$^+$), the rearrangement barrier is reduced by 31.4 kJ mol^{-1} and the cyclic intermediate is stabilized by ~20 kJ mol^{-1}. The RMP2 energetics are consistent with these results.

We find a similar reduction in the rearrangement barrier height upon protonation of the pyruvoyl (**13**) and PLP (**7**) groups. However, a notable difference between these systems is the stabilization of the cyclic intermediates, the intermediate being stabilized by ~40 kJ mol^{-1} more in the former model relative to the open-chain reactants.[65]

The calculated relative energies for the pyruvoyl- and PLP-dependent rearrangements verify that the major role of both coenzymes would be the same. More specifically, both provide captodative stabilization of the cyclic intermediate and lower the reaction barrier height, effects that are enhanced upon protonation. This supports speculation that pyruvate and PLP aid enzymatic reactions by acting as an "electron sink". However, although pyruvate can lower the reaction barrier to an acceptable level, the resulting cyclic intermediate could be considered too stable, especially upon protonation. This result could explain why B_{12}-dependent aminomutases do not use pyruvate to introduce unsaturation to the migrating group and to stabilize the intermediates.[66]

(65) Once again, the greater stability of **14** compared with **8** may be attributed to greater captodative effects, which are associated in part with geometrical differences (i.e., a change in orientation at the radical center as discussed for models **5**, **10**, and **12**).[43]

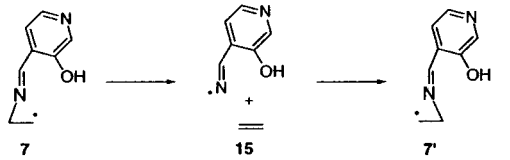
The investigations of the pyridine, small imine, and pyruvate models provide support for our hypothesis that one of the roles of PLP is to captodatively stabilize the cyclic intermediate, thereby reducing the reaction barrier height. Furthermore, the high-level calculations performed on the small models provide support for our choice of a simpler computational level for the larger systems. Overall, the barriers calculated with RMP2 are in reasonable agreement with G3(MP2)-RAD(p), while RMP2 generally somewhat overestimates the stability of the cyclic intermediate. With a better understanding of the role of PLP, we now refocus our attention on the radicals involved in the rearrangement mechanism.

D. Support for the Intramolecular Radical Rearrangement Mechanism in Aminomutase-Catalyzed Reactions. (1) An Alternative Rearrangement Pathway. We have focused so far on the addition−elimination-type mechanism involving a cyclic intermediate for the reactions catalyzed by aminomutases. Our calculations show how this mechanism can help explain why and how B_6 aids these difficult rearrangements. However, a route involving detachment of the migrating group followed by readdition to a neighboring site (the fragmentation−recombination mechanism) has been proposed in the literature as a possible pathway for 1,2-amino shifts catalyzed by B_{12}-

(66) We cannot discount the possibility that differences in the gas-phase geometries of the PLP and pyruvate models could change in the enzymatic environment and result in similar reaction energetics for the two models. If **14**-H$^+$ is considered to have a C_s structure similar to **8**-H$^+$ (see Figure 1), it lies higher in energy than the optimized C_1 form by 18.2 kJ mol^{-1} and has one imaginary frequency. The well depth for **14**-H$^+$ in such circumstances would be reduced from 48.9 to 30.7 kJ mol^{-1} (with RMP2).

dependent aminomutases[4] and therefore warrants examination. Previous theoretical work on B_{12}-assisted migrations has shown that when the migrating group is unsaturated, an intramolecular pathway (i.e., addition−elimination) is energetically favored over a route involving detachment of the migrating group (i.e., fragmentation−recombination).[19,20,27] However, the importance of the latter mechanism increases when the migrating group contains stabilizing substituents, which can reduce the combined energy of the separated products and therefore the reaction barrier.[27] We briefly examine the fragmentation−recombination mechanism to determine whether it might be important for the aminomutase reactions.

We use a model that accounts for the effects of the PLP ring (**7**) to examine the significance of the fragmentation−recombination mechanism:[43]

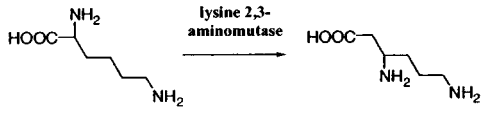


(10)

As for many B_{12}-mediated reactions,[19,20,27] we find fragmentation−recombination to be associated with a barrier (119.8 kJ mol^{-1} at RMP2) much higher than the route involving a cyclic intermediate (61.3 kJ mol^{-1}).[67] In fact, the separated products in the fragmentation−recombination mechanism (**15**) lie 87.4 kJ mol^{-1} above the reactant (Table 2). Protonation of the PLP ring does not improve the energetics, with even the separated products now more than 100 kJ mol^{-1} higher in energy than the reactant.

These results indicate that the fragmentation−recombination route is a high-energy alternative for 1,2-amino shifts assisted by a cooperative effort of B_6 and B_{12}. Additionally, it appears that B_6 would not facilitate such a pathway compared with the simple migration of an amino group (**1** → **1′**). Thus, our present calculations provide additional support that addition−elimination, coupled with (partial) protonation by the enzyme, is strongly favored over fragmentation−recombination.

(2) EPR Parameters. The key experimental evidence for the involvement of radicals in the reactions catalyzed by aminomutases originates from a series of careful studies performed by Frey, Reed, and co-workers.[9,11−15] These EPR studies investigate lysine 2,3-aminomutase, which catalyzes the transformation of α-lysine to *β*-lysine (eq 11). This enzyme differs



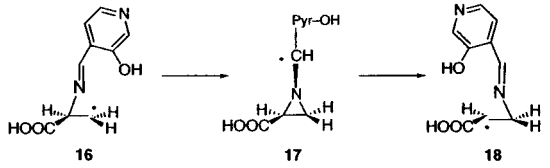
(11)

from other aminomutases (Scheme 1) since it utilizes an iron-sulfur cluster and *S*-adenosylmethionine ("poor man's coenzyme B_{12}") to abstract the migrating hydrogen rather than coenzyme B_{12}. Lysine 2,3-aminomutase is known, however, to require PLP. It would be expected that the rearrangement mechanism for the aminomutases is independent of how the radical is generated.

Electron paramagnetic resonance experiments have characterized both the product[11,12,25] (**E**, Scheme 2) and the substrate[15,16] (**D**, Scheme 2) radicals for the rearrangement catalyzed by lysine 2,3-aminomutase, although the latter was only identified through examination of either sulfur[15] or allylic[16] analogues due to high reactivity. Sophisticated EPR experiments have also verified the inclusion of PLP in the product radical.[13,68] The intermediate radical has not been observed in any EPR studies to date.[12]

Comparison of couplings calculated with quantum chemical methods and experimental results has become an important tool for identifying the radicals involved in biochemical reactions.[69] The most important detail to remember when comparing theory and experiment is that the anisotropic coupling (T_{ii}) can be calculated with a wide variety of quantum chemical techniques to a high degree of accuracy, while the isotropic component (A_{iso}) is more difficult to calculate precisely. Therefore, we use the anisotropic contributions to the HFCCs rather than the principal components (A_{ii}) to help identify the radicals.[70]

Since the HFCC is an electronic property, substituents adjacent to the radical site with significant electron-donating or -withdrawing capabilities can have large effects on the numerical value. Therefore, we employ a model for the radical intermediates involved in the reaction catalyzed by lysine 2,3-aminomutase that includes the carboxylic acid functionality, but in which the amino acid side chain ($CH_2CH_2CH_2CH_2NH_2$) is replaced by methyl (eq 12).[71] Selected couplings calculated for



(12)

the product (**18**), reactant (**16**), and cyclic intermediate (**17**), as well as the radical component of the possible fragmentation products (**15**), are displayed in Table 4.[43] In addition to the couplings presented in Table 4, significant isotropic HFCCs are also calculated for each atom in the model PLP ring, which reflects the delocalization of spin throughout the molecule and supports the hypothesis of radical stabilization by the PLP ring (for a full list of couplings see Supporting Information).

Reliable experimental couplings have previously been extracted through the use of computer simulations and assigned to the product radical.[12] The agreement between the experimental data and the HFCCs calculated in the present work for the three radicals believed to be involved in the 1,2-amino shift in α-lysine is somewhat disappointing. The experimental anisotropic H_α, H_β, and C_α couplings are in best agreement with those calculated for the product radical (**18**). However, the

(67) Despite the fact that the intrinsic reaction coordinate (IRC) leads from TS:**7** → **15** to a C_s ring-opened isomer of **7**, the relative energy is reported with respect to the C_1 ring-opened structure for **7** (Figure 1). This would seem the appropriate choice since it is expected that if the ring-opened structure is formed, then the system will have sufficient energy to rearrange to the lowest energy structure.

(68) From the EPR studies of Ballinger et al., the distance between C_α in the product radical and the hydrogen at C4′ in PLP (see Chart 1) was estimated to be smaller than 3.5 Å.[13] The B3-LYP optimized parameters in our model Schiff bases (**7**, **7**-H$^+$, **18**) are slightly larger than this value, ranging between 3.633 and 3.716 Å.

(69) (a) Himo, F.; Graslund, A.; Eriksson, L. A. *Biophys. J.* **1997**, *72*, 1556−1567. (b) Wetmore, S. D.; Himo, F.; Boyd, R. J.; Eriksson, L. A. *J. Phys. Chem. B* **1998**, *102*, 7484−7491. (c) Himo, F.; Babcock, G. T.; Eriksson, L. A. *J. Phys. Chem. A* **1999**, *103*, 3745−3749. (d) Lassmann, G.; Eriksson, L. A.; Himo, F.; Lendzian, F.; Lubitz, W. *J. Phys. Chem. A* **1999**, *103*, 1283−1290.

(70) The experimental isotropic and anisotropic components were derived from the principal components of the couplings obtained from simulations of the experimental EPR spectra using the relations $A_{ii} = A_{iso} + T_{ii}$ and $A_{iso} = {}^1/_3\Sigma_i A_{ii}$.

(71) Although carboxylic acid groups can stabilize the radical center, and therefore potentially affect the reaction energetics, calculations on a small model system, which replaces the model PLP ring in **16**, **17**, and **18** with hydrogen, that accounts for this functionality indicate that the forward and reverse barriers are changed by only +5.8 and −10.3 kJ mol^{-1}, respectively, at the G3(MP2)-RAD(p) level. Our calculations also indicate that our small model for reaction 12 is exothermic by 34.3 kJ mol^{-1}.

Table 4. Experimental and B3-LYP HFCCs (MHz) for the Radicals Involved in the 1,2-Shift Catalyzed by Lysine 2,3-Aminomutase

radical	atom	A_{iso}	T_{XX}	T_{YY}	T_{ZZ}
16	H_α	−65.9	−39.4	−1.1	40.5
	H_α	−63.3	−39.2	−1.3	40.6
	H_β	85.9	−5.9	−3.9	9.7
	C_α	79.8	−78.7	−78.2	156.9
	N_β	19.9	−2.0	−1.1	3.1
17	H_α	−43.2	−25.1	−1.3	26.4
	C_α	57.2	−54.0	−52.7	106.6
	N_β	2.1	−1.5	0.1	1.4
18	H_α	−55.2	−33.3	−2.1	35.4
	H_β	122.0	−5.3	−2.5	7.8
	H_β	51.3	−4.9	−3.1	8.0
	C_α	65.8	−67.6	−67.1	134.7
	N_β	4.9	−0.9	−0.1	1.0
18-10°	H_α	−54.9	−33.6	−2.3	36.0
	H_β	10.0	−4.7	−3.6	8.3
	H_β	36.4	−4.6	−3.9	8.5
	C_α	66.9	−68.5	−67.8	136.3
	N_β	20.0	−1.9	−1.6	3.5
18-7°	H_α	−55.0	−33.7	−2.3	36.0
	H_β	13.4	−4.7	−3.6	8.3
	H_β	31.3	−4.5	−4.0	8.5
	C_α	67.0	−68.6	−67.9	136.5
	N_β	20.4	−1.9	−1.6	3.6
15	H_α	205.8	−5.7	−4.6	10.3
	C_α	−60.5	−18.2	2.3	15.9
	C_β	68.7	−9.7	−5.5	15.1
	N_β	18.6	−44.9	−32.4	77.3
exptl[a]	H_α	−60.8	−30.8	−2.3	32.5
	H_β	14.9	−4.8	−4.8	9.8
	C_α	86.9	−62.8	−62.8	126.1
	N_β	23.2			

[a] Reference 12. See also footnote 70.

isotropic H_β and N_β couplings calculated for **18**, and the other potential radical intermediates examined, differ significantly from the experimental results.

The disagreement between theory and experiment can be rationalized by noting that isotropic β-HFCCs are very sensitive to the geometrical configuration. More specifically, isotropic β-HFCCs are sensitively dependent on the dihedral angle between the axis of the singly occupied p-orbital and the β-bond of interest, where maximum overlap leads to a value near zero. The dihedral angle between the axis of the singly occupied p-orbital and the C_β−N bond is nearly 90° in the optimized gas-phase structure of the product radical (**18**). Analysis of the experimental nitrogen splitting, however, indicates that this dihedral angle lies between approximately 7° and 10° in the enzymatic environment.[12]

The difference between the gas-phase geometry and that exhibited experimentally may be caused by hydrogen-bonding interactions between the carboxylate group in the substrate and other functional groups within the enzyme and corresponds to a relatively low-energy motion. We have therefore reevaluated the HFCCs in the product radical at two geometries in which the dihedral angle between the axis of the singly occupied p-orbital and the C_β−N bond is fixed to either 7° or 10° (the corresponding dihedral angles for the C_β−H bond being 65.7° and 58.7°, respectively).[72] The resulting isotropic β-HFCCs (for **18**-7° and **18**-10°, Table 4) are consistent with the experimentally observed radical being the product radical. More specifically, one of the calculated isotropic H_β couplings when the C_β−N bond is 7° off the axis defined by the singly occupied orbital (13.4 MHz) is in good agreement with the experimental result (14.9 MHz).[73] Additionally, the calculated N_β coupling (20.4 MHz) is in much better agreement with the experimental value (23.2 MHz) at this degree of rotation. The changes in the remaining couplings due to this rotation are small.

The agreement between theoretical and experimental HFCCs observed for the product radical (**18**-7°) is encouraging. It is noteworthy that the HFCCs calculated for the radical intermediate formed in the fragmentation−recombination mechanism (**15**, eq 10) are in poor agreement with the experimental results (Table 4). A full list of the computed HFCCs is included in the Supporting Information. We hope that these values might prove useful in future experimental studies.

Conclusions

Experimental electron paramagnetic resonance experiments provide evidence that 1,2-amino shifts catalyzed by aminomutases occur through radical intermediates. Support for the identity of one of the radicals involved in these rearrangements is provided in the present study through comparison of experimental and theoretical EPR parameters. However, no experimental data are yet available that yield detailed information about the rearrangement mechanism, which is known to involve a coenzyme form of both B_{12} and B_6. Therefore, theory has a potentially significant role to play in evaluating the importance of different mechanisms and understanding the roles of the required participants.

Our calculations predict that 1,2-amino shifts involving radical intermediates are relatively high-energy processes in the absence of pyridoxal 5′-phophate. Our calculations suggest that the first important role of PLP is to introduce unsaturation to the migrating group. This modification permits a low-energy, intramolecular reaction mechanism involving a cyclic intermediate. An alternative pathway involving fragmentation of the reactant radical, and recombination of the separated products, is found to be a higher energy route.

The present work also identifies a second important role of PLP. We conclude that PLP captodatively stabilizes the cyclic intermediate, and lowers the reaction barrier, by providing a delicate balance between stabilizing and overstabilizing effects. Through examination of several model systems that exhibit a range of ability to (captodatively) stabilize the reaction intermediate, we reveal the true delicate nature of this balance. More specifically, we find that in several other systems (including pyruvate) the captodative stabilization is able to reduce the barrier to ring closure, but that there is an overstabilization of the reaction intermediate, particularly upon protonation. The generation of such an intermediate could potentially inactivate the enzyme. Our calculations suggest that this overstabilization provides a possible explanation why there are no naturally occurring B_{12}-dependent aminomutases that utilize pyruvate in place of B_6, despite the anticipated similar roles of these two coenzymes.

From our calculations, we are able to propose two important roles for the hydroxyl group of B_6. First, it may play a part in controlling the protonation site of the migrating group. Second,

(72) We note that these rotated structures are higher in energy than the optimized structure previously discussed by only 6.8 and 7.2 kJ mol^{-1}, as determined from B3-LYP/6-311G(2d, p) single-point calculations.

(73) Only one H_β coupling is reported experimentally, but there are two calculated couplings since the model system replaces the amino acid side chain with methyl. The hydrogen with a coupling in best agreement with experiment has a dihedral angle between the axis of the singly occupied p-orbital and the C_β−H bond of 65.7° in **18**−7° and 58.7° in **18**−10°. Replacing the remaining hydrogen with the amino acid side chain results in the correct stereochemistry for lysine.

it prevents overstabilization of the cyclic intermediate, which could lead to enzyme inactivation.

Although the 1,2-shift of an amino group appears to be a demanding task, our calculations show that the rearrangement may be efficiently accomplished as a result of an intricate relationship between the enzyme and its cofactors. Thus, we believe that coenzyme B_{12} is responsible for activating the substrate to rearrangement by removal of a hydrogen atom. Vitamin B_6 (PLP) introduces a seemingly essential double bond into the migrating group, as well as imparting the potential for the cyclic intermediate radical to be captodatively stabilized through protonation of the pyridine nitrogen. The hydroxyl group of PLP ensures that the reaction intermediate is not overstabilized in order to prevent inactivation of the enzyme. The enzyme itself holds all the components in place and provides an environment in which the pyridine nitrogen can be (fully or partially) protonated. This cooperative action of the enzyme and cofactors is able to mediate an otherwise extremely difficult reaction.

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Supporting Information Available: RMP2/G3MP2large total energies (Table S1), B3-LYP EPR hyperfine coupling constants (Table S2 and Scheme S1) and GAUSSIAN 98 archive entries for the RMP2/6-31G(d)//B3-LYP/6-31G(d,p) calculations for all relevant structures (Table S3) (pdf). This information is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

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