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Modeling the Reactions Catalyzed by Coenzyme B_{12}-Dependent Enzymes

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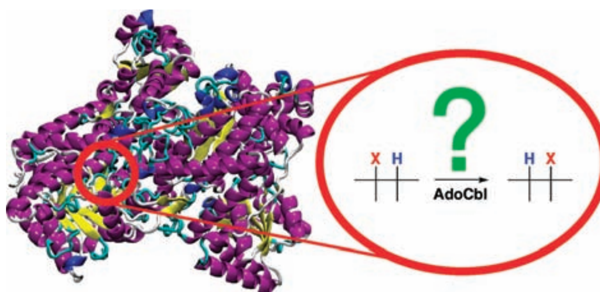

Enzymes accelerate chemical reactions with an exceptional selectivity that makes life itself possible. Understanding the factors responsible for this efficient catalysis is of utmost importance in our quest to harness the tremendous power of enzymes. Computational chemistry has emerged as an important adjunct to experimental chemistry and biochemistry in this regard, because it provides detailed insights into the relationship between structure and function in a systematic and straightforward manner. In this Account, we highlight our recent high-level theoretical investigations toward this end in studying the radical-based reactions catalyzed by enzymes dependent on coenzyme B_{12} (or adenosylcobalamin, AdoCbl). In addition to their fundamental position in biology, the AdoCbl-dependent enzymes represent a valuable framework within which to understand Nature's method of efficiently handling high-energy species to execute very specific reactions.

The AdoCbl-mediated reactions are characterized by the interchange of a hydrogen atom and a functional group on adjacent carbon atoms. Our calculations are consistent with the conclusion that the main role of AdoCbl is to provide a source of radicals, thus moving the 1,2-rearrangements onto the radical potential energy surface. Our studies also show that the radical rearrangement step is facilitated by partial proton transfer involving the substrate. Specifically, we observe that the energy requirements for radical rearrangement are reduced dramatically with appropriate partial protonation or partial deprotonation or sometimes (synergistically) both. Such interactions are particularly relevant to enzyme catalysis, because it is likely that the local amino acid environment in the active site of an enzyme can function in this capacity through hydrogen bonding. Finally, our calculations indicate that the intervention of a very stable radical along the reaction pathway may inactivate the enzyme, demonstrating that sustained catalysis depends on a delicate energy balance.

Radical-based enzyme reactions are often difficult to probe experimentally, so theoretical investigations have a particularly valuable role to play in their study. Our research demonstrates that a small-model approach can provide important and revealing insights into the mechanism of action of AdoCbl-dependent enzymes.

1. Introduction

The existence of life relies on the ability of enzymes to accelerate chemical reactions. Research in the area of harnessing this awesome power has therefore attracted considerable interest. Central to the challenge of exploiting such catalysts, however, is the need to understand, at a fundamental level, the cause-and-effect actions that govern enzyme catalysis. This Account reviews our recent efforts toward this end through the application of high-level theoretical procedures to the radical-based reactions catalyzed by various coenzyme B_{12} (or adenosylcobalamin, AdoCbl)-dependent enzymes.

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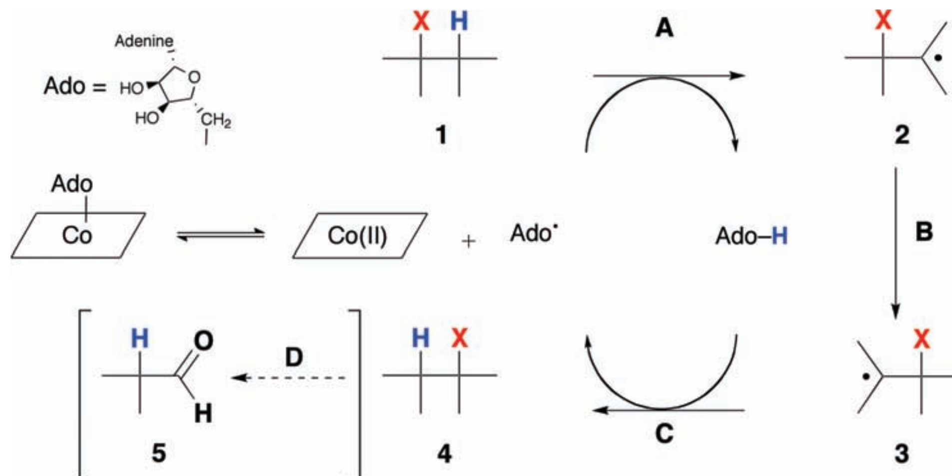
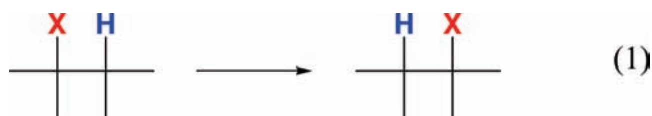
SCHEME 1. Generally Accepted Minimal Mechanism for the Rearrangements Catalyzed by AdoCbl-Dependent Enzymes



The principal role of essentially all AdoCbl-dependent enzymes is to facilitate the interchange of a group **X** and a hydrogen atom (**H**) on adjacent carbon atoms of the substrate:[1]



The identity of the migrating species **X** can be a small carbon-skeleton fragment or a small heteroatom-containing group like OH or NH_2, depending on the enzyme. Scheme 1 outlines the generally accepted mechanism for these reactions[2] and shows how radical intermediates play a crucial mechanistic role. Substrate (**1**) binding induces homolytic cleavage of the Co−C bond of AdoCbl to generate 5′-deoxyadenosyl radical (Ado•) plus cob(II)alamin. Hydrogen abstraction by Ado• from **1** then occurs to form 5′-deoxyadenosine (Ado-H) plus a substrate-derived radical **2** (step A). The rearrangement of **2** gives the product-related radical **3** (step B), which is followed by H-atom transfer from Ado-H to **3** to afford the product **4** and to regenerate Ado• (step C), which is able to recombine with cob(II)alamin to complete the catalytic cycle. In some cases, elimination of H_2O or NH_4^+ from **4** occurs to produce an aldehyde (**5**, step D).

The successful characterization of the various steps within Scheme 1 has been made possible through the efforts of many scientists from a wide array of disciplines. The availability of high-performance computers, together with the design and efficient implementation of sophisticated algorithms, has allowed these steps to be explored in depth using computational quantum chemical procedures. This has been a focus of our research in recent years and provides the subject matter of this Account. The theoretical approach to AdoCbl-mediated reactions has also been used by a number of other groups (including particularly the groups of Brown, Brunold, Kozlowski, Morokuma, Paneth, Ryde, Schwarz, Siegbahn, Truhlar, Yoshizawa, and Warshel), whose research is discussed in our original papers but is not always referred to in this Account because of space limitations.

2. Theoretical Approach

2.1. Theoretical Procedures. High-level conventional ab initio and density functional theory (DFT) procedures have been used throughout our investigations. An important aspect has been the selection of reliable theoretical procedures on the basis of assessment studies. In this connection, relative energies have generally been obtained with high-level composite methods that have been found to perform well when assessed against reliable thermochemical data for reactions related to those investigated here.[3] Specifically, the G3(MP2)-RAD method[4] has proven to be a good compromise between accuracy and affordability for the types of systems we explore. In this method, geometries and zero-point vibrational energies (ZPVEs) are generally obtained using the B3-LYP DFT method, the ZPVEs being derived from harmonic vibrational frequencies that are scaled[5] to account for errors associated with the neglect of anharmonicity and the use of a nonexact procedure.

Another high-level method used extensively in our investigations is CBS-RAD.[6] The more economical B3-LYP method has also been employed. In many cases, the performance of B3-LYP with a large basis set is found to be comparable to that of the more expensive procedures, thus providing confidence in its use when the computational cost of higher-level ab initio methods becomes prohibitive.

These three methods all perform well for the specific example of the barrier for the ring opening of the cyclopropylcarbinyl radical, a relevant prototype for the reactions catalyzed by AdoCbl-dependent enzymes. The calculated barriers obtained by CBS-RAD (32.9 kJ mol^{-1}), G3(MP2)-RAD (31.6 kJ mol^{-1}), and B3-LYP (30.7 kJ mol^{-1}) may be compared with the experimental value of 31.2 kJ mol^{-1}.[7]

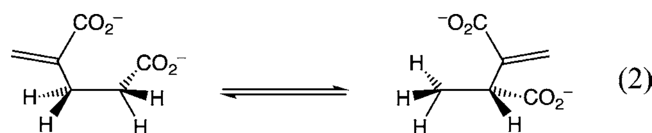
2.2. Choice of Chemical Model. The chemical models that we have used in our studies of the reactions catalyzed by AdoCbl-dependent enzymes typically begin with substrates and reaction intermediates in the absence of bulk enzyme, with an aim being to capture their *intrinsic* reactivity. Such an approach enables us immediately to quantify the extent to which it is necessary for the enzyme to activate its substrate and intermediates.

In the next stage, it is often desirable to also include in the calculations additional species that mimic active site amino acids so as to gain insight into their possible function. The small model approach is particularly useful in this regard because specific interactions that may be responsible for facilitating the reactions can be turned "on" or "off" straightforwardly.

Carboxylates are usually considered as their neutral counterparts. This has the effect of mimicking the likely binding arrangement within the enzyme (e.g., in which a carboxylate anion is associated with an arginine residue),[8] and it also alleviates the potential problems of describing multiply charged anionic species in the gas phase.

3. The Class I Mutases

3.1. 2-Methyleneglutarate Mutase (MGM). 2-Methyleneglutarate mutase catalyzes the reversible conversion of 2-methyleneglutarate to (*R*)-3-methylitaconate in bacterial fermentation:[1]

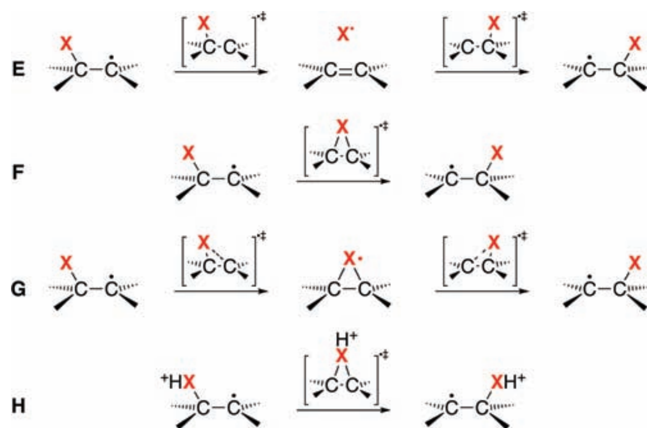


(2)

Though significant effort has been devoted to understanding the mechanism of this rearrangement, a definitive resolution remains elusive.

The participation of radical intermediates allows for a variety of ways in which the rearrangement might proceed. Scheme 2 highlights four radical-based pathways describing the migration of a group **X** between adjacent carbon centers. Pathway E, termed fragmentation−recombination (F/R), involves cleavage of the bond from carbon to **X** to produce

SCHEME 2. Possible Mechanisms for Free-Radical-Based 1,2-Migrations



two distinct species that can recombine at the adjacent carbon to give the rearranged product-related radical. The remaining pathways involve the (intramolecular) migration of **X** without its formal detachment from the two-carbon unit.

Pathway F depicts a concerted rearrangement pathway in which a bridged transition structure (TS) connects the substrate-derived and product-related radicals. Pathway G is a variant of F in which the rearrangement proceeds via a cyclopropyl-type species as an intermediate rather than a TS. This addition−elimination (A/E) mechanism, which requires appropriate unsaturation within **X**, can be described as an addition of the unpaired electron to the π system of the migrating moiety to form the cyclopropyl-type species, which can then ring open via homolytic cleavage of the adjacent bond (see also section 2.1). Pathway H involves protonation of the migrating moiety and is of interest because early investigations predicted that protonation in this manner lowers the energy requirements for rearrangement.[9]

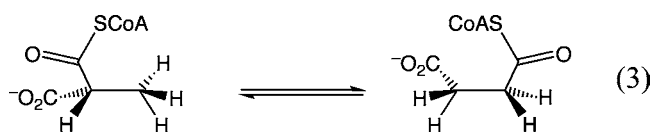
If the degenerate rearrangement of the but-3-enyl radical is taken as an initial model for the MGM reaction, the barrier involving a F/R mechanism (**X** = $CH{=}CH_2$, pathway E of Scheme 2) is found to require almost 150 kJ mol^{-1}.[10] In contrast, the barriers for ring closing and ring opening in the A/E mechanism (pathway G) are calculated to be only ca. 40 kJ mol^{-1}. Full protonation of the terminal methylene group in the but-3-enyl radical (pathway H) leads to the rearrangement of a partially ring-opened methyl cyclopropane radical cation with a barrier of only ca. 10 kJ mol^{-1}.

Improving the model from but-3-enyl radical to the substrate-derived radical of 2-methyleneglutaric acid is found to modify the reaction profile slightly, partly on account of preferential stabilization of the radical center by the adjacent CO_2H group. Despite this difference, the A/E pathway is found to remain considerably lower in energy than the F/R pathway. In

addition, a reduction in the rearrangement barrier is again observed upon protonation of the migrating group in this model.

The theoretical calculations clearly identify the A/E pathway as being of low energy, especially when combined with protonation of the migrating group.[10] However, a F/R pathway has been suggested on the basis of isotopic labeling studies and conformational arguments.[11] Clearly, further research is desirable in order to definitively establish the mechanism of action of MGM.

3.2. Methylmalonyl-CoA Mutase (MCM). MCM is the only AdoCbl-dependent enzyme in humans and catalyzes the reversible rearrangement of methylmalonyl-CoA to succinyl-CoA in the catabolism of odd-chain fatty acids, branched-chain amino acids, and cholesterol:[1]



The presence of appropriate unsaturation in the migrating thioformyl-CoA moiety in these substrates allows the possibility of both A/E and F/R rearrangement pathways.

Using 3-propanal radical as a model for the substrate-derived radical of methylmalonyl-CoA, we find the barrier for the F/R pathway to be quite high (93.2 kJ mol^{-1}).[12,13] The intramolecular A/E pathway, on the other hand, is predicted to have a considerably smaller barrier of 46.9 kJ mol^{-1}. Full protonation of the carbonyl oxygen yields a rearrangement barrier of just 10.0 kJ mol^{-1}.

While these findings are attractive from an energetic viewpoint, the weakly acidic groups within the active site of MCM are unlikely to lead to extensive protonation of the relatively weak carbonyl base of the substrate. A possible solution to this predicament is *partial* protonation, as potentially provided by hydrogen bonding with the enzyme, which we have modeled with a series of small gas-phase molecules (Figure 1).[12,13]

When the weak gas-phase acid hydrogen fluoride (HF) is used to partially protonate the migrating moiety, there is only a small reduction of 5.5 kJ mol^{-1} in the barrier. The use of the strong gas-phase acid H_3O^+ leads to a barrier (10.3 kJ mol^{-1}) similar to that with full protonation. When ammonium cation NH_4^+ is used as the catalyst, a reduction of 22.4 kJ mol^{-1} is observed, bringing the overall rearrangement barrier to an intermediate value of 24.5 kJ mol^{-1}.

This systematic lowering of the rearrangement barrier reflects the effect of partial proton transfer between the acid catalyst and the migrating carbonyl oxygen, which is stronger in the transition structure than in the reactant. This is exemplified in Figure 2 where the N−H bond of NH_4^+ extends from 1.102 Å in the reactant radical to 1.123 Å in the TS. The type of qualitative behavior seen with NH_4^+ may be regarded as ideal in the context of enzyme catalysis, since a significant lowering of the barrier can be achieved without full proton transfer by the enzyme.

The crystal structure of MCM reveals that a histidine residue (His244) is within H-bonding distance of the migrating carbonyl oxygen of the substrate (Figure 3),[14] thus providing an attractive H-bond donor to mediate substrate rearrange-

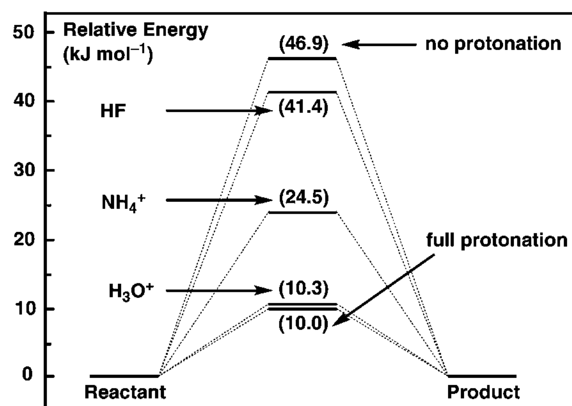


FIGURE 1. Schematic energy profiles for the rearrangement of the 3-propanal radical, showing barriers (CBS-RAD(p), kJ mol^{-1}) associated with various degrees of protonation.[12]

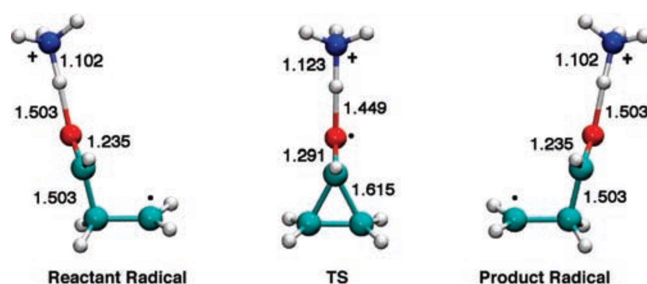


FIGURE 2. B3-LYP/6-31G(d,p) structures and selected bond lengths (in Å) of the complexes between NH_4^+ and the propanal radical for the 1,2 shift relevant to the MCM-catalyzed reaction.[13]

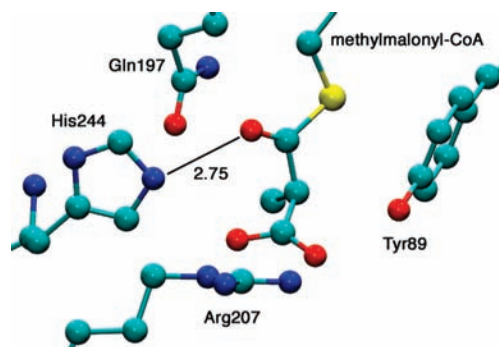
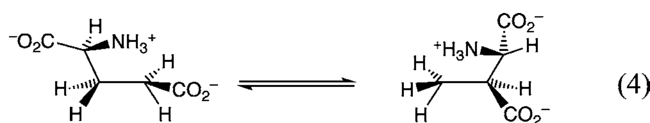


FIGURE 3. Selected region of the active site of MCM (PDB code 4REQ) showing the close proximity (in Å) of His244 to the carbonyl oxygen of the migrating group.

ment via partial proton transfer. Mutagenesis experiments performed by Banerjee and co-workers showed a 300-fold decrease in catalytic efficiency when His244 was replaced by glycine.[15] Other experiments with His244Ala and His244Gln mutants found similar decreases in catalytic efficiency (100−1000-fold).[16] There is thus strong experimental support for the involvement of His244 in providing partial proton transfer to assist the carbon-skeleton rearrangement in the MCM-catalyzed reaction.

3.3. Glutamate Mutase (GM). Glutamate mutase catalyzes the reversible isomerization of (*S*)-glutamate to (2*S*,3*S*)-3-methylaspartate, which is the first step in the bacterial fermentation of glutamate to ammonia, acetate, butyrate, CO_2, and H_2:[1]



(4)

The absence of appropriate unsaturation in the migrating group in this case means that an A/E mechanism is not likely to be operative.[17] Support for a dissociative F/R pathway comes from the laboratory of Marsh and co-workers, who have shown that glycyl radical is a kinetically competent intermediate in this reaction.[18]

We have demonstrated theoretically the importance of the protonation state of the participating species in this reaction.[17] For example, with protonated 4-glutamyl radical, the intermediate fragments are found to lie 182.5 kJ mol^{-1} higher in energy than the reactant, and the overall reaction is endothermic by 41.7 kJ mol^{-1}. In contrast, the energy requirements for the F/R pathway of neutral 4-glutamyl radical are much lower. The barrier to fragmentation is only 59.9 kJ mol^{-1}, the intermediate fragments lie just 34.7 kJ mol^{-1} higher than the reactant, and the reaction endothermicity is just 20.3 kJ mol^{-1}. The low energy of the separated intermediate fragments is the result of captodative stabilization of the glycyl radical, involving the strong π-donor (amino) and strong π-acceptor (carboxylic acid) substituents adjacent to the unpaired electron.[19] Protonation of the amino group disrupts this stabilization.

The crystal structure of GM reveals an active site that is highly ordered (Figure 4), with Glu171 well placed to partially deprotonate the amino group of the substrate.[20] Mutagenesis experiments with Glu171Gln demonstrate a 50-fold reduction in the catalytic activity of GM,[21] suggesting that Glu171 does indeed act as a proton acceptor in these reactions.

Further evidence for GM utilizing hydrogen bonding for both binding *and* catalysis is provided by its observed *inactivation* with (*S*)-2-thiolglutarate. This appears to involve a sulfur-stabilized thioglycolyl radical.[22] However, the calculated energy profile of neutral (*S*)-2-thiolglutaric acid is found to be not very different from that of other known catalytically active substrates and thus not consistent with the observed inactivation.[23] Instead, we find that the F/R products of the reaction involving the S-centered *anion* of (*S*)-2-thiolglutaric acid are especially low in energy because of effective captodative stabilization, which *would* account for the ability of this analogue to inactivate GM. The involvement of the S-centered anion of the thioglycolyl radical is also consistent with the role of Glu171 as a proton acceptor in the GM-catalyzed reactions.[21]

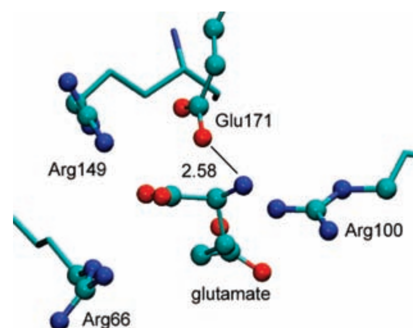
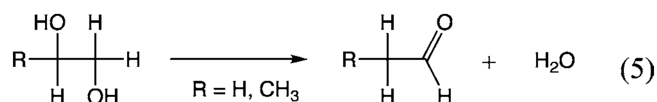


FIGURE 4. Selected region of the active site of GM (PDB code 1I9C) showing the close proximity (in Å) of Glu171 to the nitrogen atom of glutamate.

4. The Class II Eliminases

4.1. Diol Dehydratase (DDH). Diol dehydratase catalyzes the irreversible dehydration of ethane-1,2-diol (R = H) and propane-1,2-diol (R = CH_3) to acetaldehyde and propionaldehyde, respectively:[1]



(5)

Early isotopic-labeling studies demonstrated the presence of a 1,1-dihydroxy species as an obligatory intermediate for the reactions of both substrates, implying that the reactions involve an energetically difficult 1,2-OH shift. Inspired by earlier work,[9,12,13] we examined the interactions with the migrating OH group of a series of Brønsted acids and found that the resulting *partial* protonation substantially reduces the rearrangement barrier.[24]

In a subsequent study, the partial-proton-transfer concept was extended to include partial *deprotonation* of the spectator OH group.[25] Figure 5 displays the influence of acidic and basic catalysis on the rearrangement barrier for the 1,2-dihydroxyethyl radical. Relative to the uncatalyzed case (110.4 kJ mol^{-1}), the barrier is reduced to 49.0 kJ mol^{-1} through par-

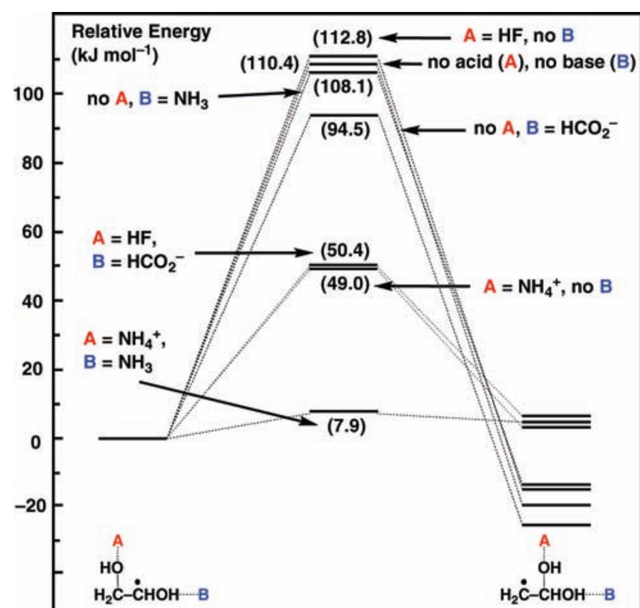


FIGURE 5. Schematic energy profiles for the rearrangement of the 1,2-dihydroxyethyl radical resulting from the interaction of acids (**A**) with the migrating OH together with the interaction of various bases (**B**) with the spectator OH. Relative energies (G3(MP2)-RAD, kJ mol^{-1}) are given in parentheses.

tial protonation of the migrating OH by NH_4^+, while HF is found to increase the rearrangement barrier to 112.8 kJ mol^{-1}. Partial deprotonation of the spectator OH group is found to reduce the barrier to rearrangement with either NH_3 (108.1 kJ mol^{-1}) or HCO_2^- (94.5 kJ mol^{-1}).

In a powerful display of synergism, we observe a rearrangement barrier of just 7.9 kJ mol^{-1} when NH_4^+ and NH_3 are used simultaneously as the acidic and basic catalysts, respectively. If these effects were simply additive, then one would expect a rearrangement barrier of 46.7 kJ mol^{-1}. This has been referred to as "push—pull" catalysis, signifying that the acidic catalyst "pushes" the migrating OH group through the 1,2-rearrangement, while the basic catalyst "pulls" it. Similar synergism is observed with the push—pull combination of HF and formate (Figure 5). These substantial synergistic barrier reductions provide insight into how AdoCbl-dependent enzymes might use active-site residues to facilitate the demanding radical rearrangement step.

The crystal structure of DDH reveals a close association of the substrate with histidine (His143) and glutamate (Glu170) residues (Figure 6).[26] It has been argued that these residues are likely candidates to act as acidic and basic catalysts, respectively.[25] Mutagenesis experiments have shown that His143 is indeed an important contributor to the 1,2-shift in DDH,[27] while associated QM/MM computational studies have demonstrated the importance of both His143 and Glu170 in effecting efficient substrate rearrangement.[28]

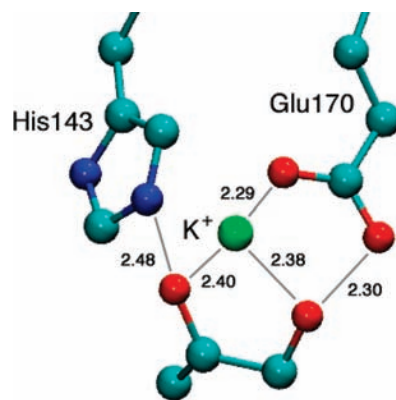
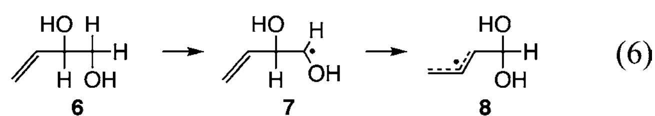


FIGURE 6. Portion of the active site of DDH (PDB code 1DIO) showing the substrate (*S*)-propane-1,2-diol, His143, Glu170, and K^+. Internuclear separations given in Å.

In addition to the ability of active site residues to mediate the stability of the participating radical species, a recurrent theme within the eliminases is their susceptibility toward mechanism-based inactivation. For example, glycolaldehyde has long been known to inactivate DDH. Recent experiments attributed this inactivation to the involvement of a very stable *cis*-ethanesemidione radical.[29] Our calculations support the notion that the inactivation is the result of the presence of a very stable radical.[30] However, we find that the relevant species is the captodatively stabilized glycolaldehyde radical and that the *cis*-ethanesemidione radical is instead a TS that connects equivalent forms of the glycolaldehyde radical. The barrier and reaction enthalpy for the H-atom reabstraction step involving the glycolaldehyde radical (step C of Scheme 1) are calculated to be too great to allow this step to proceed. This means that Ado• would not be regenerated and the catalytic cycle would be prevented from continuing.

Subsequent investigations have found several additional instances of similar inactivation mechanisms in the reactions catalyzed by DDH with other substrate analogues.[31] In all cases, the presence of a very stable radical intermediate appears to disrupt the catalytic cycle by making the H-atom reabstraction from Ado-H energetically prohibitive.

In recent studies, the substrate analogue but-3-ene-1,2-diol (**6**) was found to inactivate both DDH[32] and the closely related enzyme glycerol dehydratase (GDH).[33] It has been suggested that a very stable radical contributes to the inactivation of DDH.[32] In this connection, we find that rearrangement of the substrate-derived radical (**7**) to the stabilized radical **8** is exothermic by 83.4 kJ mol^{-1}:[34]



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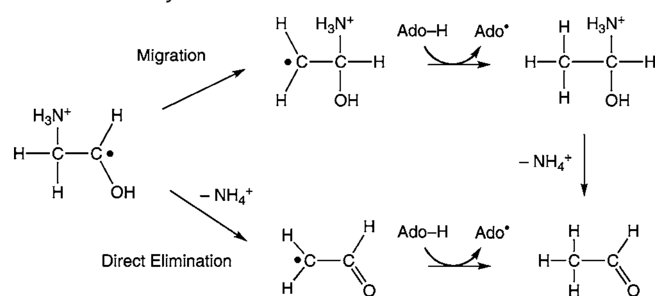
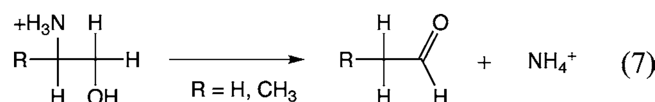
SCHEME 3. Proposed Migration and Direct Elimination Pathways for the EAL-Catalyzed Reaction



Curiously, however, isotopic labeling studies involving GDH and **6** suggest the presence of **7** and not **8**.[33] Further experiments are desirable in order to help understand why the transformation of **7** to **8** appears not to take place, particularly given that both DDH and GDH exhibit similar inactivation outcomes.[32,33]

4.2. Ethanolamine Ammonia Lyase (EAL). EAL catalyzes the irreversible deamination of (protonated) 2-aminoethanol (R = H) to give acetaldehyde (and 2-aminopropanol (R = CH_3) to give propionaldehyde):[1]



Two mechanisms have been advanced for the EAL-catalyzed reaction (Scheme 3). The migration pathway involves a 1,2-shift of the (protonated) amino group in the substrate-derived radical to form a product-related radical, which, after H-atom reabstraction, can eliminate NH_4^+ (cf. Scheme 1, step D). The alternative direct elimination route involves loss of NH_4^+ from the substrate-derived radical to give an allyloxy radical, which can generate acetaldehyde directly via reabstraction of an H atom from Ado-H.

Our calculations indicate the migration pathway to be more likely than the direct elimination route, because the latter would require the energetically difficult reabstraction of an unactivated hydrogen atom from Ado-H by the stabilized allyloxy radical.[35] Similar results were obtained by Semialjac and Schwarz.[36]

The barrier for the intramolecular 1,2-shift (cf. pathway F, Scheme 2) in protonated 2-amino-1-hydroxyethyl radical is found to be 65.8 kJ mol^{-1},[35] a value that is consistent with the experimental reaction rate. However, full deprotonation of the (protonated) amino group leads to a rearrangement barrier 30.7 kJ mol^{-1} higher than its protonated counterpart. Even partial deprotonation of the (protonated) amino group is found to be anticatalytic. Since binding of (protonated) aminoethanol to the enzyme through hydrogen bonding should result in at least partial deprotonation of its (protonated) amino group, the consequent increased barrier is likely to no longer be consistent with the experimental reaction rate. It would seem that EAL must then utilize another strategy to lower the barrier.

In this connection, we note that the spectator OH group of aminoethanol appears to play a crucial role in lowering the rearrangement barrier. Full deprotonation of the spectator OH group of neutral 2-amino-1-hydroxyethyl radical leads to a substantial decrease in the barrier to fragmentation from 96.5 to 29.5 kJ mol^{-1}.[35] Although partial deprotonation also leads to a lowering of the barrier, the effect is markedly reduced, even for strong bases such as OH^-, where the barrier is 62.7 kJ mol^{-1}.

The key conclusion is that a *combination* of a protonated migrating group (bound by a hydrogen-bond acceptor) with a basic catalyst interacting with the spectator hydroxyl group constitutes the most plausible mechanism for the EAL-catalyzed rearrangements. The synergistic benefits of such interactions at the migrating and spectator substituents are well illustrated by our results for neutral 2-amino-1-hydroxyethyl radical.[35] In the absence of any catalysis the rearrangement barrier is 96.5 kJ mol^{-1}. Interaction of NH_4^+ at the migrating NH_2 group (tantamount to NH_3 interacting with a protonated migrating group) is found to increase the barrier by 2.6 kJ mol^{-1}. Only a small lowering in the barrier (of −1.8 kJ mol^{-1}) is observed if NH_3 interacts with the spectator OH group. However, if both of these interactions occur together, then the rearrangement barrier is reduced to 66.0 kJ mol^{-1}, which is consistent with the experimental rate. Synergistic benefits of this type have also been observed elsewhere for this reaction.[36]

The ability of EAL to tolerate substrate modifications has led to a set of interesting and diverse side reactions. For instance, 2-hydroxyethylhydrazine (HEH) has been found to cause the irreversible inactivation of EAL.[37] We have characterized a low-energy direct elimination pathway that leads to the hydrazinium radical cation.[38] This is a very stable radical that inhibits the necessary H-atom reabstraction step (step C of Scheme 1).

5. The Class III Aminomutases

The third class of AdoCbl-dependent enzymes to be examined is the aminomutases, including lysine 5,6-aminomutase (5,6-LAM), which are responsible for effecting a 1,2-NH_2 shift in certain amino acids for their subsequent cleavage into easily metabolized intermediates.[1] These enzymes also require pyridoxal 5′-phosphate (PLP) for activity, the PLP being joined

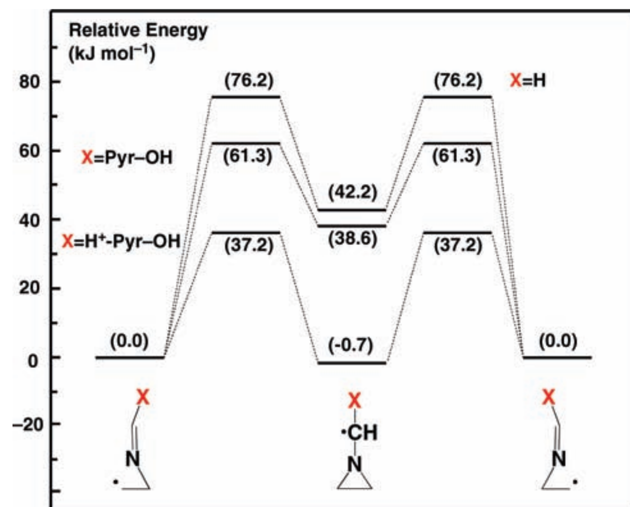
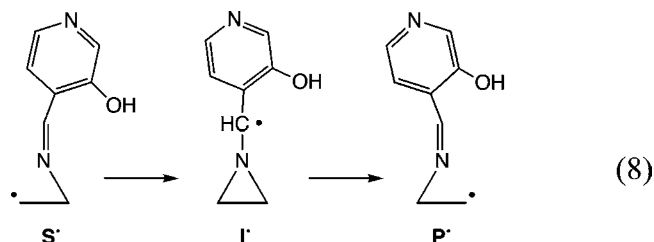


FIGURE 7. Schematic energy profiles (RMP2/G3MP2Large, kJ mol^{-1}) for the degenerate rearrangement of substituted 2-(*N*-methylidine)ethyl radicals.[39]

via an aldimine linkage to the migrating amino group of the substrate. The rearrangement is thought to proceed via an A/E pathway involving an azacyclopropylcarbinyl radical (**I•**) intermediate (see also pathway G, Scheme 2):



(8)

Uncertainty concerning the rearrangement mechanism for these enzymes prompted us to investigate the role of PLP and how it might facilitate these difficult 1,2-shifts. In the absence of PLP, the unassisted 1,2-NH_2 shift in 2-aminoethyl radical is calculated to require 90.5 kJ mol^{-1}, while protonation of the amino group actually increases the barrier to 104.8 kJ mol^{-1}.[39]

In principle, PLP can assist the 1,2-NH_2 shift by introducing unsaturation into the migrating group to permit an intramolecular rearrangement pathway. Using the 2-(*N*-methylidine)ethyl radical as a model, we find that the barrier for ring closure to generate the cyclic 1-aziridinylcarbinyl radical is indeed lowered to 76.2 kJ mol^{-1}, while the reaction is endothermic by 42.2 kJ mol^{-1} (Figure 7). The added functionality of the pyridoxal moiety (as in **S•**, eq 8) is found to decrease the barrier to ring closure to 61.3 kJ mol^{-1}. Protonation at the pyridine nitrogen further reduces this barrier to 37.2 kJ mol^{-1}, and the cyclic intermediate is substantially stabilized (Figure 7).

Our calculations reveal two important roles for PLP in these reactions. First, the presence of unsaturation lowers the energy of the intermediates by enabling an intramolecular pathway. Second, PLP stabilizes high-energy intermediates, like **I•**, through a captodative interaction provided by the cooperation of electron donation (by the nitrogen lone pair) to the radical center and π-electron withdrawal from the radical center. The latter is assisted by protonation of the pyridoxal ring.

Most of the data implicating radicals in these reactions has been obtained through experiments with the related enzyme lysine 2,3-aminomutase.[40] Although EPR spectroscopy has identified **P•** and **S•** for this reaction,[40] observation of the cyclic intermediate (**I•**) remains elusive. We find that substitution with strong π-acceptors at the exocyclic carbon stabilizes **I•** to the extent that experimental verification, especially in the reactions catalyzed by 5,6-LAM, may be possible.[41] For example, ethynyl substitution lowers the energy of **I•** relative to **S•** and **P•** by ca. 20 kJ mol^{-1}. Whether these enzymes can tolerate such changes in the substrate analogues is presently unknown, though strategies like these may enable the first experimental characterization of an **I•**-like structure in the aminomutase-catalyzed reactions.

6. Summary

Understanding the details of enzyme catalysis is an important goal that represents a significant challenge to theory. Encouragingly, continuing advances in technology are making computational quantum chemistry investigations of enzyme catalysis more accessible.

Our approach in this regard has been to apply high-level ab initio methods to small model systems. We have particularly focused on reactions catalyzed by various AdoCbl-dependent enzymes, since these reactions are radical-based and are generally difficult to probe experimentally. Theory therefore has a useful role to play. Our primary aim is to identify the key features responsible for catalysis in the AdoCbl-mediated reactions, which represent a universal paradigm from which to understand how enzymes control and manipulate high-energy intermediates.

Our calculations support the thesis that the primary function of AdoCbl is to provide a source of radicals so that the reactions, characterized by the interchange of a functional group and a hydrogen on adjacent carbon atoms, can be moved to the radical potential energy surface.

A key role of the enzyme is to facilitate the otherwise difficult radical rearrangement step. We find that partial protonation and partial deprotonation at appropriate locations in the substrates, as potentially provided by hydrogen bonding with the enzyme, can substantially lower the barrier for rearrange-

ment in these reactions. Observations that these individual effects can be *synergistically* amplified when combined provide an intriguing backdrop from which to ponder the evolution of tailored active sites in enzyme catalysis. The realization that complete protonation is not necessary to capture a significant barrier reduction for the rearrangement steps suggests that the H-bonding capacity of an enzyme might perform the dual role of *both* binding and catalysis.

The importance of the relative stabilities of the radical intermediates in determining the course of the AdoCbl-mediated reactions has also been demonstrated in various contexts, the efficiency of the catalysis being found to depend on a delicate energy balance. This balance appears to be achieved for the native substrates, with the radical intermediates being neither too stable nor too unstable. In some cases, alternative substrates do not shift this balance significantly, and only modest rate attenuations are observed. In more extreme cases, however, highly stable radical intermediates are found to disrupt the catalytic cycle and lead to irreversible suicide inactivation. This latter phenomenon can, nevertheless, be used to constructively design appropriate substrate analogues in order to enable experimental characterization of otherwise high-energy radical intermediates.

We conclude that the application of a small model approach to enzyme catalysis offers a straightforward, useful, and revealing adjunct to experiment.

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BIOGRAPHICAL INFORMATION

Gregory M. Sandala was born in Oakville, Canada, in 1977. In 2006, he received a Ph.D. from the Australian National University (ANU) under the guidance of Professor Leo Radom. Since then, Greg has enjoyed postdoctoral training at the University of Sydney (USyd) with Professor Radom and, more recently, as an NZZ postdoctoral fellow in Dr. David Smith's research group at the Ruđer Bošković Institute (RBI). Greg is currently an Honorary Associate of USyd and a Research Associate in the group of Professor Lou Noodleman at the Scripps Research Institute. His research interests lie under the broad umbrella of enzyme catalysis and the relationship between structure and function.

David M. Smith was born in Dubbo, Australia, in 1973. He completed his Ph.D. at the ANU in 1999 under the supervision of Professor Leo Radom. After a brief stint as a postdoctoral fellow in 2000 with Zvonimir Maksić at the RBI, he moved to the group of Hendrik Zipse at the LMU in Munich as a fellow of the AvH foundation. In 2004, he accepted a permanent position at the RBI, where he is now a senior research associate, leader of the Group for Quantum Organic Chemistry, and Assistant Director for International Collaboration and Projects. His research interests include the application of quantum and classical techniques to problems of biological significance.

Leo Radom was born in Shanghai, China, in 1944. He completed a Ph.D. at USyd in 1969 and spent 1969−1972 as a postdoctoral fellow with John Pople at Carnegie-Mellon University, before returning to Australia to the ANU in 1972. In 2003, he moved to USyd where he is currently a Professor of Chemistry. Leo is a Fellow of the Australian Academy of Science and has been elected to the International Academy of Quantum Molecular Science. His research interests are concerned with the application of computational quantum chemical procedures to problems of structure and mechanism.

FOOTNOTES

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