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Insights into the Hydrogen-Abstraction Reactions of Diol Dehydratase: Relevance to the Catalytic Mechanism and Suicide Inactivation

Gregory M. Sandala,[†,‡] David M. Smith,*[,§] Michelle L. Coote,[‡,⊥] Bernard T. Golding,[||] and Leo Radom*[,†,‡,⊥]

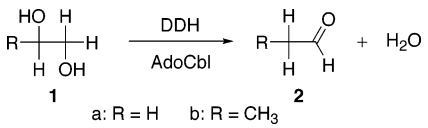
Contribution from the School of Chemistry, University of Sydney, Sydney, NSW 2006, Australia, Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia, Rudjer Boskovic Institute, 10002 Zagreb, Croatia, School of Natural Sciences-Chemistry, University of Newcastle upon Tyne, Bedson Building, Newcastle upon Tyne, NE1 7RU, United Kingdom, and ARC Centre of Excellence in Free Radical Chemistry and Biotechnology

Received November 21, 2005; E-mail: david.smith@irb.hr; radom@chem.usyd.edu.au

Abstract: High-level quantum chemistry calculations have been used to examine the hydrogen-abstraction reactions of diol dehydratase (DDH) in the context of both the catalytic mechanism and the enzyme dysfunction phenomenon termed suicide inactivation. The barriers for the catalytic hydrogen-abstraction reactions of ethane-1,2-diol and propane-1,2-diol are examined in isolation, as well as in the presence of various Brønsted acids and bases. Modest changes in the magnitudes of the initial and final abstraction barriers are seen, depending on the strength of the acid or base, and on whether these effects are considered individually or together. The most significant changes (ca. 20 kJ mol^{-1}) are found for the initial abstraction barrier when the spectator OH group is partially deprotonated. Kinetic isotope effects including Eckart tunneling corrections (KIEs) have also been calculated for these model systems. We find that contributions from tunneling are of a magnitude similar to that of the contributions from semiclassical theory alone, meaning that quantum effects serve to significantly accelerate the rate of hydrogen transfer. The calculated KIEs for the partially deprotonated system are in qualitative agreement with experimentally determined values. In complementary investigations, the ability of DDH to become deactivated by certain substrate analogues is examined. In all cases, the formation of a stable radical intermediate causes the hydrogen re-abstraction step to become an extremely endothermic process. The consequent inability of 5′-deoxyadenosyl radical to be regenerated breaks the catalytic cycle, resulting in the suicide inactivation of DDH.

1. Introduction

Coenzyme B_{12}-dependent enzymes facilitate the interchange of a functional group and a hydrogen atom bound to adjacent carbons.[1] Diol dehydratase (DDH) belongs to this fascinating class of enzymes and catalyzes a 1,2-hydroxyl shift in vicinal diols to generate the related gem-diols, which are then dehydrated to produce an aldehyde plus water.[1b,2]



Like many other coenzyme B_{12}-dependent enzymes, this reaction is believed to occur via a radical mechanism in which 5′-deoxyadenosyl radical, derived from homolytic fission of the Co−C bond of adenosylcobalamin (AdoCbl), participates directly.[3] Scheme 1a depicts the generally accepted minimal mechanism for the reactions catalyzed by DDH.[1b]

The presence of substrate (**1**) activates homolysis of the Co−C bond of AdoCbl bound to its partner enzyme to yield the cob(II)alamin and 5′-deoxyadenosyl (Ado•) radicals. In the next step, the 5′-deoxyadenosyl radical initiates substrate catalysis with a hydrogen-atom abstraction from **1** to form 5′-deoxyadenosine (Ado-H) and a substrate-derived radical **3** (step **A**). The substrate-derived radical **3** then rearranges to the

[†] University of Sydney.
[‡] Australian National University.
[§] Rudjer Boskovic Institute.
[||] University of Newcastle upon Tyne.
[⊥] ARC Centre of Excellence in Free Radical Chemistry and Biotechnology.

(1) See, for example: (a) Banerjee, R. *Chem. Rev.* **2003**, *103*, 2083−2094. (b) Toraya, T. *Chem. Rev.* **2003**, *103*, 2095−2127 and references therein.
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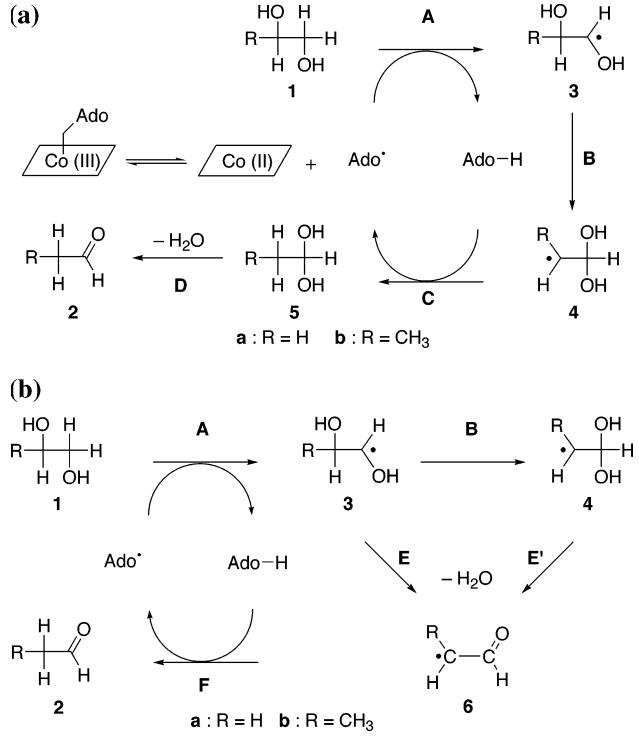
Scheme 1. (a) Generally Accepted Minimal Mechanism and (b) Alternative Pathways for the Reactions Catalyzed by DDH



product-related radical **4** (step **B**), in a process that is facilitated by specific amino acid residues within the active site of DDH.[1b,4] This rearrangement is followed by hydrogen-atom re-abstraction by **4** from Ado-H. This crucial re-abstraction step restores the 5′-deoxyadenosyl radical, thus priming it for another round of catalysis, and produces the closed-shell hydrated product **5** (step **C**). Finally, dehydration of **5** occurs to yield the product aldehyde **2** (step **D**).

Alternative pathways for the reactions carried out by DDH can be envisioned and differ from the mechanism of Scheme 1a with respect to the order of the hydrogen re-abstraction and dehydration steps. Schematic profiles for these alternative pathways are depicted in Scheme 1b.

Like the pathway of Scheme 1a, catalysis proceeds via initial hydrogen-atom abstraction by Ado• from **1** to form a substrate-derived radical **3** and Ado-H (step **A**), which may be followed by rearrangement to the product-related radical **4** (step **B**). Dehydration of **3** or **4** at this stage leads to formation of an allyloxy radical (**6**) plus water (steps **E** or **E′**). For either of these possible pathways to be catalytic, it would need to be feasible for hydrogen-atom re-abstraction from Ado-H by **6** to occur to form the product aldehyde (**2**) and regenerate the 5′-deoxyadenosyl radical (step **F**).

While the pathways of Scheme 1b are generally thought not to be operational in the reactions catalyzed by DDH, it is of interest to consider why this might be so. This is particularly intriguing given the known propensity for α-hydroxy radicals with a leaving group in the β position (such as **3**) to eliminate H_2O in solution.[5] The lability of the fully protonated β-OH group of the substrate-derived radical **3a** has also been demonstrated by theoretical calculations, which indicate spontaneous elimination.[6] In support of this finding, a recent ONIOM study of DDH, in which a quantum-mechanical description of the active site was mechanically embedded into a molecular-mechanical treatment of the protein, has found that H_2O elimination occurs from **3b** when a histidine residue close to the migrating OH group is fully protonated.[7]

Despite the obvious chemical precedent for the elimination of H_2O from **3**, such a pathway is generally disregarded in the reactions of DDH because it has not been considered consistent with the results of experimental isotopic-labeling studies. These studies have demonstrated the presence of a 1,1-dihydroxy species as an obligatory intermediate for the reactions of both ethane-1,2-diol (**1a**)[8] and propane-1,2-diol (**1b**).[9,10] While this is normally interpreted to imply a closed-shell 1,1-diol intermediate, the elimination of H_2O from the product-related radical **4** would also be consistent with the known stereochemical course of the DDH reactions. However, a recent computational exploration of such a proposition concluded that elimination from **4** is not a kinetically viable pathway because a stable aldehyde radical is generated.[11] Given that the principal role of diol dehydratase is to facilitate the elimination of H_2O from diols, it is intriguing that the enzyme has apparently specifically chosen the pathway depicted in Scheme 1a, rather than the favorable dehydration pathways that have been identified in nonenzymatic situations from radical intermediates such as **3**.

Understanding the precise nature of this refined selectivity may be assisted by reflecting upon the results of the numerous studies devoted to revealing the mechanism of action of coenzyme B_{12}-dependent enzymes in general, and DDH in particular. In this connection, valuable insights have emerged from examples where modifications of the coenzyme,[12,13] or the enzyme itself,[14] have resulted in varying degrees of altered enzyme activity. Perhaps the most direct procedure for obtaining mechanistic details concerning coenzyme B_{12}-dependent en-

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zymes involves the use of substrate analogues that result in mechanism-based inactivation. For diol dehydratase, such studies date back nearly 40 years,[15,16] although only recently have the inactivation products been characterized experimentally.[17]

Recently, we have employed theoretical techniques to explore the inactivation by selected substrate analogues of DDH and ethanolamine ammonia-lyase, another coenzyme B_{12}-dependent enzyme,[1b] and found that the essence of this inactivation was the inability of the hydrogen-atom re-abstraction step to occur.[18] In such circumstances, the 5′-deoxyadenosyl radical cannot be regenerated to continue further catalysis. This devastating effect is of clear significance and has thus prompted us to further explore the hydrogen-abstraction reactions of DDH to see if a similar phenomenon may contribute to nature's choice of the catalytic mechanism. Hence, in the present Article, we have expanded our preliminary account,[18a] with the aim of examining alternative pathways for the hydrogen-abstraction reactions of DDH and the reasons for the failure of some of these reactions to take place. This has been accomplished by carrying out high-level quantum chemistry calculations on small model systems of the substrate and a selected number of its analogues. In some cases, Brønsted acids and bases have been used to partially protonate the migrating OH group and/or deprotonate the spectator OH group of the substrate to determine their effects on the hydrogen-transfer reactions. In other investigations, tritium kinetic isotope effects coupled with Eckart tunneling corrections have been calculated for various models of catalytic substrates to illuminate how the rate of hydrogen transfer may be influenced.

The energy requirements for the hydrogen-transfer steps of DDH with the catalytic substrates and potassium ion have previously been examined by Toraya and co-workers.[11] While a portion of the present study overlaps with some of this earlier work, the combined research efforts can be viewed as providing useful complementarity. The present work focuses specifically on the hydrogen-transfer reactions of DDH and factors that influence them. At the same time, insight gained from these investigations is projected toward a deeper understanding of the concept of suicide inactivation for DDH, and several examples of suicide inactivation are examined in detail.

2. Theoretical Methodology

Standard ab initio[19] and density functional theory[20] calculations have been used for this study. Geometries and scaled (by 0.9806)[21] vibrational frequencies have been obtained at the B3-LYP/6-31G(d,p) level of theory. Polarization functions on the hydrogen atoms have been included with the aim of providing a more accurate description of the hydrogen-abstraction reactions and hydrogen-bonded species of this study. Relative energies were obtained with the high-level composite method G3(MP2)-RAD.[22] This approach approximates the URCCSD(T)/G3MP2Large level of theory by performing single-point energy calculations with URCCSD(T)/6-31G(d), RMP2/6-31G(d), and RMP2/G3MP2Large on the B3-LYP/6-31G(d,p) geometries. In addition, a higher-level correction is included in the final energy expression.[22b]

For most of the hydrogen-abstraction steps of the DDH-catalyzed reactions, ethanol is used as a model for 5′-deoxyadenosine (Ado-H). This choice is based on the results of a previous study that demonstrated ethanol to provide an adequate model for the hydrogen-abstraction steps of a related coenzyme B_{12}-dependent enzyme, methylmalonyl-CoA mutase.[23] However, for some of the reactions in sections 3.1.2 and 3.1.3, in which the effects of push−pull catalysis on the hydrogen-abstraction reactions are explored, ethane is used as a model for Ado-H. This was done to minimize collapse of our small model systems, which sometimes occurred with ethanol due to strong intermolecular hydrogen bonding.[24]

Because quantum mechanical tunneling of hydrogen has been implicated to play an important role in biological systems,[25] we have obtained corrections for tunneling using the Eckart method[26] for the hydrogen-transfer reactions of DDH. Normally, the influence of quantum mechanical tunneling on the rate constant for a reaction is incorporated via a multiplicative factor κ;[27,28] that is, the semiclassical rate constant including tunneling ($k_{tun}(T)$) is a multiple of the semiclassical rate constant in the absence of tunneling ($k_{SC}(T)$):

$$k_{tun}(T) = \kappa \times k_{SC}(T) \qquad (1)$$

where κ is the tunneling correction for motion along the reaction coordinate. Defined in this way, a κ value greater than unity means that tunneling has increased the reaction rate.

In the present work, the minimum energy path for the reaction is approximated using an Eckart function, for which the one-dimensional Schrödinger equation has an analytical solution.[26] We have fitted the Eckart function to the reaction path curvature at the transition structure, as measured using the imaginary frequency.[29] Preliminary indications reveal that this method provides substantially more accurate tunneling corrections than other popular methods such as the Wigner and Bell corrections.[30]

Kinetic isotope effects (KIEs) were calculated using the Redlich−Teller product rule.[31] This approach reduces the rotational, translational, and vibrational partition functions of the kinetic formulas to products

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(24) We note that the transition structures obtained for the hydrogen-abstraction steps are not necessarily those of lowest energy. The latter lead in some cases to unrealistically small hydrogen-abstraction barriers as a result of strong intermolecular hydrogen bonding between the substrate and the model for Ado-H. We aimed to avoid or minimize the impact of such artificially introduced intermolecular interactions in a consistent manner. For the initial hydrogen-abstraction reaction, Ado• was always positioned orthogonal to the plane of the C−C bond at C1 of the substrate and then optimized, while for the hydrogen re-abstraction reaction, Ado-H was always positioned collinear to the plane of the C−C bond at C2 of the substrate and then optimized.

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(27) See, for example: Laidler, K. J. *Chemical Kinetics*, 3rd ed.; HarperCollins Publishers, Inc.: New York, 1987.

(28) Nonclassical reflection along the reaction coordinate is often also incorporated into κ. However, treatment of such effects is beyond the scope of the present Article.

(29) For more details on this procedure, see: Coote, M. L.; Collins, M. A.; Radom, L. *Mol. Phys.* **2003**, *101*, 1329−1338.

of vibrational terms only. Previous ab initio studies have shown this method to be more robust than the explicit determination of partition functions in handling random errors associated with the calculation of the force constants and frequencies that are required for the calculation of KIEs.[32]

Relative energies were obtained within each reaction scheme by maintaining a constant stoichiometry throughout. The sum of the energies of isolated, individual species prior to any reaction is taken as the zero level, and subsequent relative energies along the reaction coordinate are determined relative to this energy. Unless otherwise noted, all relative energies in this paper refer to G3(MP2)-RAD values at 0 K. All calculations were performed with the MOLPRO 2002.6[33] or Gaussian 03[34] software packages.

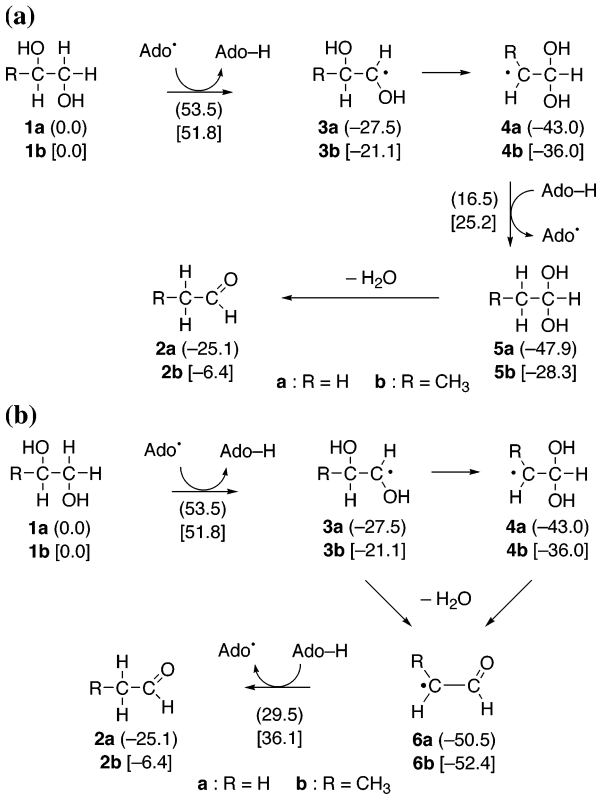
3. Results and Discussion

3.1. Hydrogen-Abstraction Reactions for Catalytic Substrates. 3.1.1. Ethane-1,2-diol and Propane-1,2-diol. Diol dehydratase (DDH) catalyzes the conversion of ethane-1,2-diol (**1a**) and propane-1,2-diol (**1b**) into water plus acetaldehyde (**2a**) and propionaldehyde (**2b**), respectively.[1b] To examine the hydrogen-abstraction reactions of DDH and the essential features that are inherent in a catalytic mechanism, we have determined selected barriers and reaction enthalpies for the reactions of **1a** and **1b** using ethanol as the model for Ado-H (Scheme 2a).

Substrate catalysis is initiated when the homolytic cleavage product of adenosylcobalamin, 5′-deoxyadenosyl radical (Ado•), abstracts a hydrogen atom from **1a** or **1b**. The products of this abstraction reaction are the substrate-derived radicals **3a** or **3b** plus 5′-deoxyadenosine, Ado-H. We calculate the barrier for this initial hydrogen-abstraction from ethane-1,2-diol (**1a**) to form **3a** to be 53.5 kJ mol^{-1} and the process to be exothermic by 27.5 kJ mol^{-1}. Similar energy requirements are found for propane-1,2-diol (**1b**); specifically, there is an abstraction barrier of 51.8 kJ mol^{-1} and an associated exothermicity of 21.1 kJ mol^{-1}. The substrate-derived radicals **3a** or **3b** then rearrange to the corresponding product-related radicals **4a** or **4b** in processes calculated to be exothermic by 15.5 or 14.9 kJ mol^{-1}, respectively.[35]

This latter result is curious as it implies that an equilibrium established between **3** and **4** would favor the product-related radical **4**. Recent experiments based on EPR spectra derived from isotopically labeled propane-1,2-diol (**1b**) identified coupling between the cobalt of cob(II)alamin and the C1-centered substrate-derived radical of **1b**.[36] This suggests that if an equilibrium were established between the radical intermediates, it would favor the substrate-derived radical **3b**. Indeed, on the basis of this assumption, it was speculated that the energy

Scheme 2. (a) Energy Requirements for the Generally Accepted Mechanism and (b) Alternative Pathways for the DDH-Catalyzed Reaction of Ethane-1,2-diol (**1a**) and Propane-1,2-diol (**1b**) (Relative Energies in Parentheses for **1a** and Square Brackets for **1b**, kJ mol^{-1})



difference between **3b** and **4b** is >6 kJ mol^{-1} in favor of **3b** at 37 °C.[36] This apparent discrepancy could be a result of differential binding of the radical intermediates by the protein. The recent ONIOM QM/MM study on DDH with approximately 13 500 atoms indeed supports this conclusion.[7] In a model with protonated His143, it was found that the substrate-derived radical **3b** is approximately 5 kJ mol^{-1} lower in energy than the product-related radical **4b**. In contrast, when an unprotonated His143 model was employed, the relative energy of **3b** is approximately 8 kJ mol^{-1} higher in energy than **4b**. On the basis of these results alone, it appears as though a protonated His143 is more consistent with the EPR data.[37]

In any event, following the formation of the product-related radical **4a** or **4b**, hydrogen-atom re-abstraction from 5′-deoxyadenosine is proposed to occur to form the gem-diol product **5a** or **5b**. This significant step regenerates the 5′-deoxyadenosyl radical (Ado•), enabling further catalysis by holoenzyme DDH. For this hydrogen re-abstraction, we calculate a barrier of 59.5 kJ mol^{-1} when **4a** abstracts an H-atom from Ado-H, and a barrier of 61.2 kJ mol^{-1} for the same reaction with **4b** as the abstracting species.

When the product-related radical is derived from ethane-1,2-diol (**4a**), we find the re-abstraction step to be exothermic by

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(34) Frisch, M. J.; et al. *Gaussian 03*; Gaussian, Inc.: Pittsburgh, PA, 2003.
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(36) Yamanishi, M.; Ide, H.; Murakami, T.; Toraya, T. *Biochemistry* **2005**, *44*, 2113−2118.
(37) It should be noted that, while the relative energies of **3b** and **4b** from the ONIOM QM/MM calculations of ref 7 for the protonated His143 model are in qualitative agreement with the EPR data of ref 36, this does not say anything about the rearrangement mechanism to convert **3b** to **4b**. Indeed, with the protonated His143 model the rearrangement step led to dissociation of **3b** to form an aldehyde radical plus water. On the basis of this result, the authors of ref 7 have argued against the dominant involvement of protonated His143 in the facilitation of the 1,2-OH shift.

4.9 kJ mol^{-1}. However, when the product-related radical is derived from propane-1,2-diol (**4b**), the re-abstraction step is calculated to be slightly endothermic (by 7.7 kJ mol^{-1}). This difference can be understood in terms of an enhanced radical stabilization provided to **4b** (relative to **4a**) by the adjacent methyl substituent.[38] Having formed the hydrated gem-diol product **5a** or **5b**, dehydration from each occurs in endothermic steps (by 22.8 and 21.9 kJ mol^{-1}, respectively) to form acetaldehyde (**2a**) or propionaldehyde (**2b**).

In the context of the suicide inactivation of DDH, it is instructive to point out alternative pathways for the reactions catalyzed by DDH. These latter pathways proceed in a fashion identical to that depicted in Scheme 2a until the formation of the substrate-derived radical species **3a** or **3b** (Scheme 2b).[39] At this stage, an alternative possible pathway for continued catalysis involves elimination of H_2O from **3** to form an allyloxy radical (**6**). Dehydration from the ethane-1,2-diol-derived substrate radical (**3a**) and the propane-1,2-diol-derived substrate radical (**3b**) is calculated to be a favorable exothermic process (by 23.0 and 31.3 kJ mol^{-1}, respectively), in agreement with the previously mentioned chemical precedent.[5]

For continued catalysis within this mechanism, hydrogen re-abstraction must occur from Ado-H by **6a** or **6b**. Such a step would generate the product aldehyde (acetaldehyde (**2a**) or propionaldehyde (**2b**)) directly and would simultaneously regenerate the Ado• cofactor. However, the kinetics and thermodynamics for this re-abstraction reaction appear to be unfavorable. That is, the allyloxy radicals **6a** or **6b** are resonance-stabilized species and, as such, release only 393.8 or 373.2 kJ mol^{-1}, respectively, upon capture of a hydrogen atom, as determined from their calculated bond dissociation energies. On this basis, the transfer of a hydrogen atom from Ado-H to **6a** or **6b**, to produce a relatively unstabilized radical like Ado• (with an estimated BDE of 419.2 kJ mol^{-1}), would be expected to be energetically demanding. Indeed, we calculate hydrogen re-abstraction by **6a** from Ado-H to be associated with a barrier of 80.0 kJ mol^{-1} and an endothermicity of 25.4 kJ mol^{-1}. The analogous transfer involving **6b** is predicted to be even more difficult, with a barrier of 88.5 kJ mol^{-1} and an endothermicity of 46.0 kJ mol^{-1}.

These data may well provide a partial explanation for the known stereochemical course of the reactions catalyzed by DDH.[8−10] Specifically, despite the possibility of facile elimination of H_2O from **3a** or **3b**, the resulting unreactive intermediates (**6a** and **6b**) are unlikely to accomplish the high-barrier hydrogen re-abstraction step that is essential for catalysis to proceed. Nevertheless, such proposals have been advanced in recent years. For example, elimination of H_2O was proposed from the fully protonated variant of the substrate-derived radical **3a**.[6] To account for the experimental observation of racemization at C2 of the product aldehyde (**2a**) derived from (*R*)- and (*S*)-[1-^{2}H,1-^{3}H]-ethane-1,2-diol,[8] it was postulated that C−C rotation of the aldehyde radical (**6a**) occurred.[6c] While this is conceptually plausible, we find the barrier for this rotation to be a sizable 40.5 kJ mol^{-1}. This result contrasts sharply with the facile C−C rotation of the product-related radical **4a**, for which we calculate the barrier to be 6.3 kJ mol^{-1}. Moreover, the low barrier in the latter case supports Arigoni's original rationalization for racemization occurring via C−C bond rotation in the product-related radical **4a**.[8]

Before proceeding further, it is important to note that the intermediacy of **4b** is required to explain the observed retention of isotopic label in experiments carried out with racemic propane-(2-^{18}O)-1,2-diol.[9] We have, therefore, calculated the energy for dehydration of **4a** and **4b**, for which we find exothermicities of 7.5 and 16.4 kJ mol^{-1}, respectively. However, as discussed above, continued catalysis requires hydrogen re-abstraction from Ado-H by **6a** or **6b**, which we have shown to be associated with relatively high barriers (80.0 and 88.5 kJ mol^{-1}, respectively).

In summary, we find that the initial hydrogen-abstraction reaction is an exothermic process when either ethane-1,2-diol (**1a**) or propane-1,2-diol (**1b**) is the substrate. Upon formation of the product-related radicals **4a** or **4b**, two pathways can be envisioned for ultimate product formation, which remain consistent with experimental labeling findings. In the generally accepted mechanism of DDH catalysis, hydrogen re-abstraction from 5′-deoxyadenosine (Ado-H) by **4a** or **4b** occurs before dehydration, with barriers of 59.5 and 61.2 kJ mol^{-1}, and reaction enthalpies of −4.9 and +7.7 kJ mol^{-1}, respectively. The alternative pathway for product formation entails elimination of H_2O from the product-related radicals **4a** or **4b** occurring before re-abstraction, generating the corresponding allyloxy radicals **6a** or **6b**. However, hydrogen-atom re-abstraction from Ado-H by **6a** or **6b** is calculated to be associated with relatively high barriers (80.0 and 88.5 kJ mol^{-1}) and endothermicities (25.4 and 46.0 kJ mol^{-1}). These results support the notion that competent DDH catalysis occurs as depicted in Scheme 2a, where hydrogen-atom re-abstraction precedes dehydration. Additional support for the fact that the hydrogen re-abstraction step can have an important, and even dominating, effect upon the mechanism will emerge from later sections that pertain to the suicide inactivation of DDH by compounds closely resembling the catalytic substrates.

Finally, it is interesting to note that, regardless of whether **1a** or **1b** is the substrate, the barrier for the initial hydrogen-abstraction reaction is found to be smaller than the corresponding barrier for the hydrogen re-abstraction reaction. These results are in qualitative agreement with the findings that the hydrogen re-abstraction step is rate limiting for the reactions catalyzed by DDH.[40] Nonetheless, given the success of the partial-proton-transfer concept for catalysis,[35d,41] we have determined the barriers for hydrogen-abstraction and re-abstraction with slightly expanded models of the substrate to incorporate such interactions.

3.1.2. Influence of Push−Pull Catalysis on the Hydrogen-Abstraction Reactions. The partial-proton transfer concept for

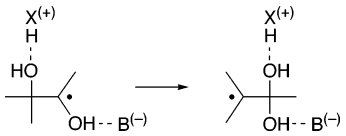
(38) Henry, D. J.; Parkinson, C. J.; Mayer, P. M., Radom, L. *J. Phys. Chem. A* **2001**, *105*, 6750−6756.
(39) Note that Scheme 2b represents a quantification of Scheme 1b; see Introduction.
(40) (a) Frey, P. A.; Karabatsos, G. L.; Abeles, R. H. *Biochem. Biophys. Res. Commun.* **1965**, *18*, 551−556. (b) Essenberg, M. K.; Frey, R. A.; Abeles, R. H. *J. Am. Chem. Soc.* **1971**, *93*, 1242−1251. (c) Eagar, R. G., Jr.; Bachovchin, W. W.; Richards, J. H. *Biochemistry* **1975**, *14*, 5523−5528.
(41) See, for example: (a) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 1037−1044. (b) Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 1383−1384. (c) Wetmore, S. D.; Smith, D. M.; Radom, L. *J. Am. Chem. Soc.* **2000**, *122*, 10208−10209. (d) Wetmore, S. D.; Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 7963−7972. (e) Wetmore, S. D.; Smith, D. M.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 8678−8689. (f) Semialjac, M.; Schwarz, H. *J. Am. Chem. Soc.* **2002**, *124*, 8974−8983. (g) Wetmore, S. D.; Smith, D. M.; Bennett, J. T.; Radom, L. *J. Am. Chem. Soc.* **2002**, *124*, 14054−14065. (h) Semialjac, M.; Schwarz, H. *J. Org. Chem.* **2003**, *68*, 6967−6983.

Table 1. Barriers and Reaction Enthalpies for Model Systems Relevant to the Hydrogen-Abstraction Reactions of DDH[a]

	hydrogen-abstraction		hydrogen re-abstraction[d]	
substrate[b]	barrier	enthalpy	barrier	enthalpy
propane-1,2-diol (**1b**)	51.8	−21.1	61.2	7.7
ethane-1,2-diol (**1a**)	53.5	−27.5	59.5	−4.9
1a with K^+	55.4	−15.2	54.5	−5.7
FH···**1a**	53.9	−22.7	59.5	−6.5
NH_4^+···**1a**	54.5	−22.8	54.2	−8.9
1a···NH_3	47.8	−30.7	58.2	−4.3
1a···$OCHO^-$ [c]	34.4	−39.3	54.5	−9.2
FH···**1a**···$OCHO^-$ [c]	36.9	−39.6	48.5	−12.4
NH_4^+···**1a**···NH_3	52.1	−31.9	55.6	−7.1

[a] Calculated with G3(MP2)-RAD at 0 K, kJ mol^{-1}. [b] The notation FH···**1a** indicates partial protonation of the migrating OH group, while **1a**···NH_3 indicates partial deprotonation of the spectator OH group. [c] Ethane was used as a model for 5′-deoxyadenosine; see Theoretical Methodology. [d] From the product-related radical **4** (see Scheme 2a).

catalysis involves partially protonating the migrating moiety and/or partially de-protonating an adjacent spectator moiety to mediate otherwise difficult chemical transformations by lowering the energy requirements.[35d] Computationally, this concept is realized with the use of Brønsted acids ($XH^{(+)}$, which may be cationic or neutral) and bases ($B^{(-)}$, which may be anionic or neutral) to "push" and "pull" the migrating and spectator OH groups, respectively.[42]



Such models have been successfully applied previously to the rearrangement step of a number of coenzyme B_{12}-dependent enzymes.[35d,41] Accordingly, it is sensible to consider the hydrogen-abstraction reactions under similar conditions to determine what effects, if any, emerge.

With ethane-1,2-diol (**1a**) as the substrate, the entries of Table 1 consist of the barriers and enthalpies of reaction for the initial hydrogen-abstraction reaction from **1a** and the re-abstraction reaction from the product-related radical **4a** (see Scheme 2a for pictorial representations of **1a** and **4a**). For completeness, results obtained with propane-1,2-diol (**1b**) have also been included, although push−pull effects were not considered for this system due to increased model size.

To provide a baseline, the first two rows of Table 1 give results for the models of uncomplexed propane-1,2-diol (**1b**) and ethane-1,2-diol (**1a**), respectively. As previously seen within Scheme 1a, the barriers for the re-abstraction reactions (61.2 kJ mol^{-1} for **1b** and 59.5 kJ mol^{-1} for **1a**) are slightly larger than those for the initial abstraction reactions (51.8 kJ mol^{-1} for **1b** and 53.5 kJ mol^{-1} for **1a**). We also point out the relatively large exothermicity for the initial abstraction when compared to the re-abstraction reaction for either **1a** or **1b**.

Because potassium ion, K^+, is known to be essential for efficient catalytic activity of DDH,[43] and has been found in its crystal structure,[44] we have determined the effects of coordination of K^+ to ethane-1,2-diol (**1a**) on the hydrogen-abstraction barriers. Table 1 shows us that with K^+ bound to **1a**,[45] the abstraction barriers lie close to one another, with 55.4 kJ mol^{-1} for the initial abstraction barrier and 54.5 kJ mol^{-1} for the re-abstraction barrier. At the same time, we see that the exothermicity for the initial abstraction is reduced by 12.3 kJ mol^{-1}, while the re-abstraction reaction becomes slightly more exothermic (by 0.8 kJ mol^{-1}) relative to the uncomplexed model **1a**.

Moving on to the concept of partial protonation, the fourth row of Table 1 displays the results of using hydrogen fluoride, FH, to partially protonate the migrating OH group of ethane-1,2-diol (**1a**).[46] It can be seen that interaction with FH does not significantly change the energy profile relative to the uncomplexed model system **1a**. Again, the re-abstraction barrier is slightly larger than the initial abstraction barrier, with the latter reaction much more exothermic. These results contrast with the scenario when ammonium cation, NH_4^+, is used to partially protonate the migrating OH group. In this instance, we see that the re-abstraction barrier is reduced (by 5.3 kJ mol^{-1}) while the initial abstraction barrier is essentially unchanged. Regarding the exothermicity of the hydrogen-transfer reactions for partially protonated substrates, it can be seen that the initial abstraction becomes slightly less favored, while the re-abstraction reaction becomes slightly more favored.

An opposite, and probably more profound, effect is observed when partial deprotonation of the spectator OH group of ethane-1,2-diol (**1a**) takes place. In this case, the initial abstraction barrier is considerably reduced relative to the uncomplexed and partially protonated models of **1a** in a manner similar to that observed previously in the context of the ribonucleotide-reductase-catalyzed reaction.[47] For example, with the weak neutral base ammonia, the abstraction barrier is reduced by 5.7 kJ mol^{-1} relative to the uncomplexed model **1a**, while the re-abstraction barrier changes by only 1.3 kJ mol^{-1}. If formate anion, $OCHO^-$, is used instead to effect partial deprotonation of the spectator OH group of **1a**, the abstraction and re-abstraction barriers are reduced by 19.1 and 5.0 kJ mol^{-1}, respectively, relative to those for the isolated model **1a**. At the same time, we observe that the exothermicities for both the abstraction and the re-abstraction reactions tend to increase with increasing base strength. This arises because the partial deprotonation of the spectator OH leads to better electron donation to the incipient radical center from a group in the α (abstraction) or β (re-abstraction) position. One may consider the correlation of barriers with exothermicities as a manifestation of the Evans−Polanyi principle.[48] On the whole, our results imply that partial deprotonation of the spectator OH group has the effect of significantly diminishing the effective barrier for the initial hydrogen-abstraction while leaving the re-abstraction barrier effectively unchanged.

When the influences of partial protonation and deprotonation are combined, we observe results that depend on whether a weak acid is used in conjunction with a strong base, for example, FH

(42) The terms "push" and "pull" relate to the location of the catalytic moiety with respect to the migrating group. If the catalytic moiety is located adjacent to the origin of the migration it is deemed to be "pushing", whereas if it is located adjacent to the endpoint of the migration it is deemed to be "pulling".

(43) Lee, H. A., Jr.; Abeles, R. H. *J. Biol. Chem.* **1963**, *238*, 2367−2373.

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

(45) Potassium ion coordination is in a bridging arrangement analogous to that found in the crystal structure.[44]

(46) Although FH has no direct counterpart in biological systems, it is used here to model the effect of a weak neutral (gas-phase) acid.

(47) Mohr, M.; Zipse, H. *Chem.-Eur. J.* **1999**, *5*, 3046−3054.

(48) See, for example: Fischer, H.; Radom, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 1340−1371.

Table 2. Calculated Tritium Kinetic Isotope Effects Excluding Tunneling (KIE_{SC}), Eckart Tunneling Coefficients ($^1\kappa/^3\kappa$), and Tritium Kinetic Isotope Effects Including Tunneling (KIE_{tun}) for Various Models Relevant to the Hydrogen-Transfer Reactions of DDH[a]

	hydrogen-abstraction			hydrogen re-abstraction[d]		
substrate[b]	KIE_{SC}	$^1\kappa/^3\kappa$	KIE_{tun}	KIE_{SC}	$^1\kappa/^3\kappa$	KIE_{tun}
propane-1,2-diol (**1b**)	10.9	8.9	97	10.0	9.7	97
ethane-1,2-diol (**1a**)	11.1	9.1	101	10.7	10.9	116
FH···**1a**	11.1	10.0	110	10.8	10.8	117
NH_4^+···**1a**	11.9	11.8	140	10.9	7.9	86
1a···NH_3	10.4	5.7	59	10.7	10.5	113
1a···$OCHO^-$ [c]	9.4	4.3	40	10.5	10.6	111
FH···**1a**···$OCHO^-$ [c]	9.7	5.0	49	10.2	8.7	89
NH_4^+···**1a**···NH_3	11.3	9.7	110	10.9	8.3	90

[a] Calculated at 298 K. [b] The notation FH···**1a** indicates partial protonation of the migrating OH group, while **1a**···NH_3 indicates partial deprotonation of the spectator OH group. [c] Ethane was used as a model for 5′-deoxyadenosine; see Theoretical Methodology. [d] From the product-related radical **4** (see Scheme 2a).

and $OCHO^-$, or a strong acid is used in conjunction with a weak base, for example, NH_4^+ and NH_3. With the FH/$OCHO^-$ combination, trends are observed that are similar to those seen for the model only employing $OCHO^-$. That is, the initial abstraction barrier is again reduced considerably (by 16.6 kJ mol^{-1}), although in this instance the re-abstraction barrier is reduced noticeably as well (by 11.0 kJ mol^{-1}). Moreover, the trend of increased exothermicities for the hydrogen transfers in the abstraction and re-abstraction steps is observed. On the other hand, using the NH_4^+/NH_3 model, we observe little influence on either hydrogen-transfer barrier relative to the uncomplexed model **1a**, and the increase in exothermicities is also not as dramatic.

To summarize this portion of the Article, it seems as though Brønsted bases (but not Brønsted acids) have the ability to substantially alter the magnitudes of the abstraction and re-abstraction barriers and exothermicities for the hydrogen-transfer steps relevant to DDH. Thus, we find that partial protonation of the migrating OH group has little effect on the barrier for the hydrogen-abstraction reactions, whereas partial deprotonation of the spectator OH group tends to reduce the initial H-atom barrier significantly while leaving the re-abstraction barrier effectively unchanged. In addition, partial deprotonation of the spectator OH group causes the abstraction and re-abstraction steps to become more exothermic.

It is noteworthy that large kinetic isotope effects (KIEs) have been observed for the hydrogen-transfer reactions of DDH.[40b,49] These are typically understood to result from nonclassical barrier penetration, or quantum mechanical tunneling.[50] Consequently, our calculated barriers for hydrogen transfer may not give a true reflection of the kinetics that take place in the enzyme system. To examine these effects computationally, we have evaluated tritium kinetic isotope effects, including corrections for tunneling, for the hydrogen transfer from substrate to Ado• and from Ado-H to the product-related radical **4**.

3.1.3. Kinetic Isotope Effects and Quantum Mechanical Tunneling in the DDH Hydrogen-Transfer Reaction. Large kinetic isotope effects (KIEs) have been observed for the initial and final hydrogen-abstraction reactions of DDH, implying that QM tunneling takes place.[40b] With propane-1,2-diol (**1b**) as substrate, the tritium isotope effects (k_H/k_T) of 20 for the initial abstraction reaction[40b] and 83 for the re-abstraction reaction[40b,49] are unusually large and certainly suggest nonclassical behavior. To provide some insight into these observations, we have calculated tritium kinetic isotope effects with and without tunneling corrections for some of the models examined in section 3.1.2, with the aim of revealing conditions under which the rate of hydrogen transfer may be accelerated or attenuated. It should be noted that, although a quantitative description of quantum mechanical tunneling in enzyme systems may require a multidimensional treatment,[51] our focus at present is a more modest one, namely to identify the qualitative trends that may influence such behavior.

Table 2 highlights some of the components pertaining to the calculation of the KIEs with tunneling corrections for the various model systems examined. The semiclassical KIE, which excludes tunneling corrections, is denoted KIE_{SC}. The protium over tritium tunneling correction is given by the ratio $^1\kappa/^3\kappa$, and the KIE that includes tunneling corrections is denoted KIE_{tun}. We note that KIE_{tun} is simply the product of KIE_{SC} and $^1\kappa/^3\kappa$ (see eq 1 in Theoretical Methodology).[52]

As in Table 1 of section 3.1.2, the first two rows of Table 2 correspond to the models of uncomplexed propane-1,2-diol (**1b**) and ethane-1,2-diol (**1a**), respectively. For uncomplexed **1b**, we make the following observations. First, the KIE_{tun} values for both the abstraction and the re-abstraction steps are identical at 97. We can de-convolute these KIEs by examining the relative contributions from the semiclassical KIE component, KIE_{SC}, and the tunneling corrections, $^1\kappa/^3\kappa$, separately. As seen in Table 2, for the hydrogen-abstraction or the hydrogen re-abstraction step, the contribution from hydrogen tunneling is generally comparable to the contribution from the semiclassical KIE alone. Moreover, although the re-abstraction step has a larger classical barrier than the initial abstraction step (Table 1), the propensity for hydrogen-atom tunneling to occur in the re-abstraction step is greater, as evidenced by the larger $^1\kappa/^3\kappa$ ratio. When we examine results for uncomplexed ethane-1,2-diol (**1a**), similar behavior is observed. Once again, contributions from tunneling to the overall KIEs of the abstraction and re-abstraction reactions are of magnitude similar to the contributions from KIE_{SC} alone. Also, we again notice the larger $^1\kappa/^3\kappa$ value for the re-abstraction reaction, despite the slightly larger classical barrier (Table 1).

(49) For a revised value of the tritium isotope effect for DDH for the transfer of tritium from 5′-deoxyadenosine to product, see: Chih, H.-W.; Marsh, E. N. G. *Biochemistry* **2001**, *40*, 13060−13067.
(50) Bell, R. P. *The Tunnel Effect in Chemistry*; London: Chapman and Hall, 1980.
(51) See, for example: Alhambra, C.; Gao, J.; Corchado, J. C.; Villà, J.; Truhlar, D. G. *J. Am. Chem. Soc.* **1999**, *121*, 2253−2258.
(52) Because of numerical rounding, the result obtained by multiplying the KIE_{SC} and $^1\kappa/^3\kappa$ values provided in Table 2 does not always reproduce the KIE_{tun} values to the number of digits quoted.

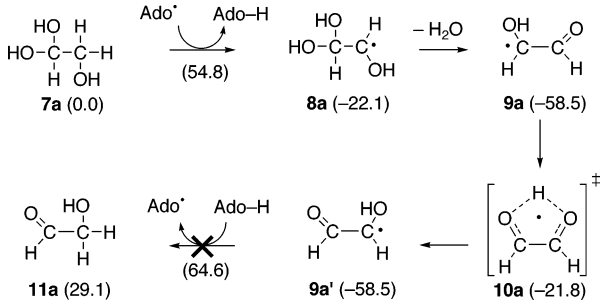
We now examine the influence of partially protonating the migrating OH group and partially deprotonating the spectator OH group of ethane-1,2-diol (**1a**) to determine how such interactions may affect the calculated kinetic isotope effects. The third row of Table 2 shows the results of using hydrogen fluoride, FH, to partially protonate the migrating OH group of ethane-1,2-diol (**1a**). The data for this system are not too dissimilar from those obtained with the uncomplexed model **1a**, with the largest proportional changes (of 0.9) occurring for the tunneling factor and KIE_{tun} for the initial hydrogen-abstraction. Interestingly, when the ammonium cation, NH_4^+, is used to partially protonate the migrating OH group, we observe the ${}^1\kappa/{}^3\kappa$ ratio to increase by 2.7 for the initial hydrogen-abstraction relative to the uncomplexed model **1a**, but to decrease by 3.0 for the hydrogen re-abstraction step. These data, along with the corresponding KIE_{SC} values, translate into a KIE_{tun} of 140 for the initial hydrogen-abstraction and 86 for the hydrogen re-abstraction.

When the spectator OH group of ethane-1,2-diol (**1a**) is partially deprotonated, an opposite trend emerges with respect to the magnitudes of KIE_{tun}. For instance, with ammonia effecting partial deprotonation of the spectator OH group of **1a**, we observe minimal changes in KIE_{SC} for the abstraction and re-abstraction reactions, whereas a large change (of 3.4) occurs for the tunneling component for the initial hydrogen-abstraction relative to the uncomplexed model **1a**. These data lead to tunneling-corrected isotope effects of 59 for the initial hydrogen-abstraction and 113 for the hydrogen re-abstraction reaction. The attenuation of the KIE_{tun} for the initial hydrogen-abstraction becomes even more pronounced when formate anion, $OCHO^-$, is used to effect partial deprotonation of **1a**. In this case, KIE_{tun} diminishes to 40 for hydrogen-abstraction, while the hydrogen re-abstraction KIE_{tun} becomes 111. If we consider the individual components of KIE_{tun} for the models that include a Brønsted base at the spectator OH group, we observe that the leading contribution to the diminished KIE_{tun} for initial hydrogen-abstraction is the decrease in the ${}^1\kappa/{}^3\kappa$ ratio, implying that partially deprotonating the spectator OH group has the effect of reducing the propensity for tunneling to occur. This phenomenon is a direct result of the changed barrier heights and reaction enthalpies observed during partial deprotonation (Table 1). That is, in the systems examined here, the barrier width (which is a principal ingredient in the calculation of tunneling factors) is found to increase with decreasing barrier height. As a result, the range of energies over which the tunneling distance is comparable to the wavelength of the hydrogen atom diminishes, thereby reducing the extent of tunneling.

Combination of the effects of partial protonation and deprotonation with $FH/OCHO^-$ reveals trends that are similar, although not as large, to those observed for the isolated model with only $OCHO^-$ (Table 2). When the NH_4^+/NH_3 model is employed, the general trends are intermediate between those observed for NH_4^+ and NH_3 acting individually.

At this point, it is informative to reflect upon the experimental isotope effects for the DDH system with propane-1,2-diol as substrate.[40b,49] Recall that initial hydrogen-abstraction is associated with a KIE of 20, while the hydrogen re-abstraction is associated with a KIE of 83.[40b,49] When formate anion, acting alone or in conjunction with FH, is used to partially deprotonate the spectator OH group of ethane-1,2-diol (**1a**), we find the

Scheme 3. Proposed Mechanism for the Suicide Inactivation of DDH by Ethane-1,1,2-triol (**7a**) (Relative Energies in Parentheses, kJ mol^{-1})



KIE_{tun} values to be qualitatively in agreement with those observed experimentally for propane-1,2-diol. In contrast, when a cationic acid is used to partially protonate the migrating OH of **1a**, the qualitative nature of the calculated KIE_{tun} disagrees with experiment. Hence, the results presented here imply that partial deprotonation of the spectator OH group is likely to provide the leading contribution to the observed isotope effect for the hydrogen-transfer steps that differ by a factor of 4.

Having examined the hydrogen-abstraction reactions for a catalytic DDH mechanism, we now turn our attention to instances where these reactions are inhibited by a substrate analogue, resulting in suicide inactivation. In section 3.2, inactivation mechanisms brought about by the substrate analogues glycolaldehyde (3.2.1), propane-1,2,2-triol (3.2.3), chloroacetaldehyde (3.2.4), and glyoxal (3.2.5) are discussed.

3.2. Hydrogen-Abstraction Reactions for Substrate Analogues. 3.2.1. Inactivation Mechanism for Glycolaldehyde. The ability of diol dehydratase (DDH) to become inactivated in the presence of a variety of vicinal diols has been known for some time.[15] We begin our discussion here with the reaction between glycolaldehyde, DDH, and adenosylcobalamin.[16a] Originally, the aerobic inactivation products were identified as glyoxal and 5′-deoxyadenosine, although at that time a mechanism for the inactivation process was not suggested.[16a] Recently, however, a mechanism formulated on the basis of EPR spectroscopic results has been proposed, and the causative agent for inactivation has been identified as *cis*-ethanesemidione radical.[17a] To explore this further, we have calculated relevant barriers and reaction enthalpies for the reaction of glycolaldehyde (**11a**) and 5′-deoxyadenosyl radical (Ado•).

Scheme 3 illustrates the proposed inactivation mechanism for DDH when the hydrate of glycolaldehyde (**11a**), ethane-1,1,2-triol (**7a**), is the substrate. Initial hydrogen abstraction by Ado• from C2 of **7a** to generate the substrate-derived radical **8a** is calculated to be exothermic by 22.1 kJ mol^{-1}, with an associated barrier of 54.8 kJ mol^{-1}. These results are comparable to those of the normal substrates (cf., ethane-1,2-diol (**1a**) and propane-1,2-diol (**1b**) of Scheme 2a). We note that if initial hydrogen abstraction were to occur from C1 of **7a**, the resulting radical (not shown) is 4.3 kJ mol^{-1} higher in energy than **8a**.

In the next step of the catalytic reaction of DDH, a 1,2-OH shift of the substrate-derived radical occurs to generate the product-related radical (step **B**, Scheme 1a). However, in the present situation, migration of an OH group within **8a** would merely generate an equivalent structure. Thus, it is proposed that dehydration from **8a** (or its equivalent rearranged structure) occurs to form the glycolaldehyde radical **9a** (or **9a′**). We

calculate this dehydration step to be exothermic by 36.4 kJ mol^{-1}. Interestingly, we find *cis*-ethanesemidione radical **10a** to be a transition structure on the potential energy surface corresponding to the degenerate interconversion of **9a** and **9a′** and that **10a** lies 36.7 kJ mol^{-1} higher in energy than **9a**. We will address this point further below. For the moment, we note that for a catalytic mechanism to continue, hydrogen-atom re-abstraction from Ado-H by **9a** (or **9a′**) must occur to complete a catalytic cycle. We calculate the barrier and endothermicity for this process to be 123.1 and 87.6 kJ mol^{-1}, respectively! These values contrast strongly with the analogous re-abstraction reaction for the catalytic substrates ethane-1,2-diol (**1a**) and propane-1,2-diol (**1b**), for which the re-abstraction barriers are 59.5 and 61.2 kJ mol^{-1}, respectively, and the reactions are mildly exo- or endothermic (Scheme 2a). The effect of tunneling of the hydrogen is not of much assistance in this situation because it must occur at energies higher than or equal to the overall reaction enthalpy (87.6 kJ mol^{-1}). Thus, it appears that the high endothermicity for the hydrogen re-abstraction reaction is the foundation for the inactivation of DDH by ethane-1,1,2-triol (**7a**), with the resulting inactivated complex remaining stable under anaerobic conditions for several days.[17a]

Clearly, the glycolaldehyde radical (**9a**) is a stable species, a result that can be attributed to the captodative stabilization provided to the radical center by the adjacent π-electron-withdrawing (CHO) and π-electron-donating (OH) groups.[53] It is this enhanced stabilization that prevents the necessary hydrogen-atom transfer from Ado-H from taking place. The results provided in Scheme 3 also suggest that glycolaldehyde (**11a**) need not be hydrated as ethane-1,1,2-triol (**7a**) for DDH to become inactivated. That is, assuming **11a** can indeed bind within holoenzyme DDH, the barrier and exothermicity of the resulting hydrogen-abstraction reaction, calculated as the reverse of the final step in Scheme 3 (i.e., **11a** → **9a′**), are 35.5 and 87.6 kJ mol^{-1}, respectively, yielding the inactivating species **9a′**. Thus, for either substrate analogue **7a** or **11a**, a deep energy well is encountered and embodied in the form of the glycolaldehyde radical (**9a**).[54]

3.2.2. On the Nature of *cis*-Ethanesemidione Radical. The original interpretations of the EPR spectra obtained from the glycolaldehyde-induced suicide inactivation of diol dehydratase (DDH) implicated the *cis*-ethanesemidione radical (**10a**) as the species responsible for the inactivation process.[17a] This identification was based on structural changes observed in the spectra upon isotopic labeling of solvent and substrate. These data provided solid evidence concerning the inactivating radical: (i) it is derived from glycolaldehyde (**11a**), (ii) it contains one solvent exchangeable proton, and (iii) it has a highly delocalized electronic structure. On the basis of these results, and with the aid of comparisons with literature hyperfine splitting constants for semidiones, it was concluded that *cis*-ethanesemidione radical (**10a**) was the species responsible for the inactivation of DDH.[17a]

However, in a preliminary Communication in this journal, we reported that the *cis*-ethanesemidione radical (**10a**) is not actually a minimum on the potential energy surface.[18a] Rather, it serves as a transition structure for the degenerate rearrangement of **9a**, with an associated barrier of 36.7 kJ mol^{-1}. To address this apparent discrepancy, we have examined the role of the active site of DDH.

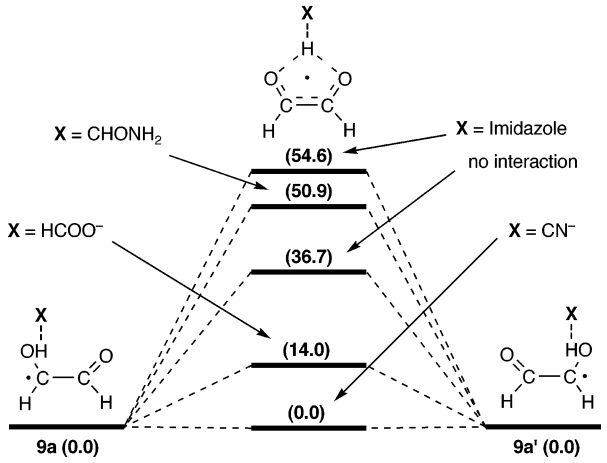


Figure 1. Effect of interaction of a base with the OH group on the barrier for degenerate rearrangement of glycolaldehyde radical (**9a**, relative energies in parentheses, kJ mol^{-1}).

The crystal structure of DDH shows a complex substrate-binding site. In particular, four amino acid residues, His143, Glu170, Gln296, and Asp335, are found to be in close proximity to the bound substrate.[44] Given the nature of the various EPR spectra and the fact that *cis*-ethanesemidione radical (**10a**) is not stable in its own right, these amino acid residues are likely to have an influence on the observed EPR spectra. To explore this in greater detail, we have determined the effects that interaction of various bases in the enzyme with the OH of glycolaldehyde radical (**9a**) might have on the barrier for the degenerate rearrangement of **9a**.

Figure 1 demonstrates how the strength of an enzymatic base clearly influences the barrier for interconversion of equivalent forms of glycolaldehyde radical (**9a**). The strongest base modeled, that is, cyanide ion, forms an effectively stable, symmetrical species with **9a**.[55] However, for formate anion, which can be viewed as a good model for Glu170 or Asp335, an unsymmetrical structure is preferred, but with a barrier to interconversion between equivalent unsymmetrical structures of just 14.0 kJ mol^{-1}. When formamide and imidazole are used as models for Gln296 and His143, respectively, we find that the barrier for the degenerate rearrangement of **9a** is actually higher than that for the uncomplexed model. Thus, with formamide, the barrier is increased from 36.7 to 50.9 kJ mol^{-1}, while with imidazole the barrier is increased to 54.6 kJ mol^{-1}.

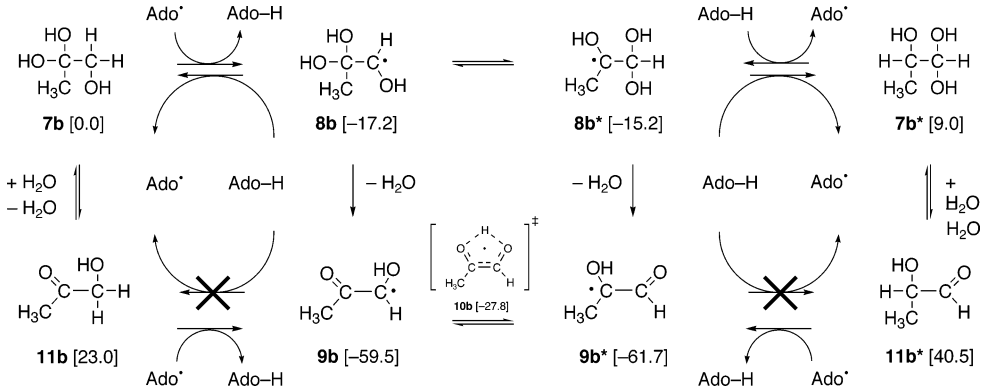
These results suggest that the apparent symmetrical nature of species assigned previously on the basis of EPR spectra[17a] may partly be the result of an interaction between glycolaldehyde radical (**9a**) and an anionic active site residue such as Asp335 or Glu170. Spectra obtained from isotopic substitution of glycolaldehyde (**11a**) likely reflect the overlap of individual

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

(54) While the complex of DDH, cob(II)alamin, and **9a** is stable for several days under anaerobic conditions, exposure to oxygen produces glyoxal. Consistent with this finding, we calculate the dissociation energy of the O−H bond of **9a**, which would result in the formation of glyoxal, to be only 192.8 kJ mol^{-1}.

(55) The inclusion of zero-point vibrational energy (ZPVE) in the calculations on the complex with CN^- makes the symmetrical species 0.04 kJ mol^{-1} more stable than the unsymmetrical species. If ZPVE is not included, the symmetrical species is 1.4 kJ mol^{-1} higher in energy than the unsymmetrical species. Despite the presence of a 239 cm^{-1} imaginary frequency for the symmetrical species, one may therefore consider that under thermal equilibrium conditions the cyano complex is effectively symmetrical.

Scheme 4. Proposed Mechanisms for the Suicide Inactivation of DDH by Propane-1,2,2-triol (**7b**) and Propane-1,1,2-triol (**7b***) (Relative Energies in Square Brackets, kJ mol^{-1})



signals from distinct isotopomers of **9a** that developed during a rapid equilibration within the room-temperature incubation period.

Regardless of whether the symmetrical *cis*-ethanesemidione radical (**10a**) represents a stable equilibrium structure (such as when interacting with a strong base) or a transition structure for the rapid interconversion of two asymmetric structures (such as **9a** and **9a′**), the force driving the inactivation is the same. In either scenario, a radical is formed that is too stable to enable the crucial re-abstraction step to occur and turnover is terminated. These observations clearly demonstrate the detrimental consequences when an unavoidable re-abstraction step is too energetically demanding. In contrast, the difficult re-abstraction step involving the allyloxy radicals **6a** or **6b** that would arise following dehydration of **3a** or **3b**, originally seen in Scheme 2b for the alternative dehydration mechanisms of DDH, *is* avoidable. Indeed, it is conceivable that it is the avoidance of this step that is responsible for the mechanism shown in Schemes 1a and 2a.

3.2.3. Inactivation Mechanisms for Propane-1,2,2-triol and Propane-1,1,2-triol. Given that DDH can bind and process both ethane-1,2-diol and propane-1,2-diol, whereas ethane-1,1,2-triol (**7a**) has been implicated as initiating suicide inactivation of DDH (see section 3.2.1), we have examined corresponding reactions of the methyl-substituted derivatives of **7a**, propane-1,2,2-triol (**7b**) and propane-1,1,2-triol (**7b***). Not surprisingly, energy profiles similar to that shown in Scheme 3 for **7a** are obtained with **7b** and **7b*** as substrates (Scheme 4).

Initial hydrogen-abstraction from propane-1,2,2-triol (**7b**) by 5′-deoxyadenosyl radical (Ado•) generates a substrate-derived radical **8b** plus 5′-deoxyadenosine (Ado-H) in a reaction that is exothermic by 17.2 kJ mol^{-1}. At this stage, one possibility is that **8b** eliminates H_2O in a reaction that is exothermic by 42.3 kJ mol^{-1} to produce **9b**. Alternatively, a slightly (by 2.0 kJ mol^{-1}) endothermic 1,2-OH migration may occur from **8b** to form **8b***. If we first follow the dehydration pathway that produces **9b**, a catalytic cycle requires that hydrogen re-abstraction from Ado-H by **9b** takes place. We calculate this reaction to be endothermic by 82.5 kJ mol^{-1}. This reaction enthalpy is similar to that calculated for hydrogen re-abstraction from Ado-H by glycolaldehyde radical (**9a**, 87.6 kJ mol^{-1}, Scheme 3). Similarly, if **8b*** is produced via a 1,2-OH migration, a 46.5 kJ mol^{-1} exothermic dehydration step produces an isomer of **9b**, that is, **9b***. In the same way that **9b** is unlikely to allow H-atom transfer from Ado-H, we calculate H-atom transfer from Ado-H to **9b*** to produce **11b*** to be associated with an endothermicity of 102.2 kJ mol^{-1}. Thus, the formation of either **9b** or **9b*** from propane-1,2,2-triol (**7b**) is predicted to result in the inactivation of DDH on account of the inability in either instance to regenerate Ado•.

It is clear from Scheme 4 that identical inactivation pathways can be envisioned if we start from the propane-1,1,2-triol (**7b***) isomer. We note also that **9b** and **9b*** are related via the intermediacy of a propanesemidione-type transition structure (**10b**).

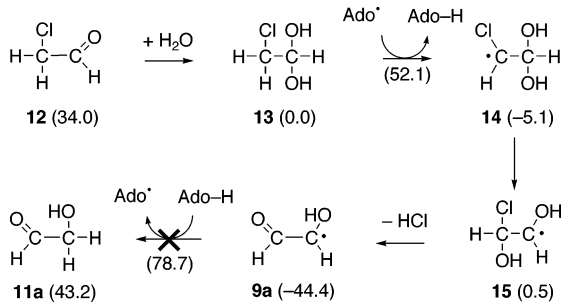
Finally, our proposed mechanisms for the suicide inactivation of DDH by propane-1,2,2-triol (**7b**) and propane-1,1,2-triol (**7b***) show that the nonhydrated forms of **7b** and **7b***, that is, **11b** and **11b***, should also be able to serve as suicide inactivators of DDH. Initial hydrogen-abstraction by Ado• from **11b** and **11b*** will produce the putative inactivating radical species **9b** and **9b*** directly via reactions that are exothermic by 82.5 and 102.2 kJ mol^{-1}, respectively.

Overall, provided that these methyl-substituted derivatives of glycolaldehyde and ethane-1,1,2-triol bind to DDH, the inactivation mechanisms are similar to those proposed for the parent systems (Scheme 3). That is, the presence of a stable radical intermediate elicits the suicide inactivation of DDH by inhibiting the regeneration of Ado•.

3.2.4. Inactivation Mechanism for Chloroacetaldehyde. Among the various substrate analogues that have been shown to result in mechanistic-based suicide inactivation of DDH, chloroacetaldehyde (**12**) is a notable illustration.[16b] Recent EPR investigations have identified the inactivation product to be the *cis*-ethanesemidione radical (**10a**),[17b] the same radical species implicated in the suicide inactivation of DDH by glycolaldehyde. At the same time, an inactivation mechanism was proposed to account for the EPR indications of **10a**.[17b] Given the results obtained when glycolaldehyde (or its hydrate) is the substrate, such a mechanism is indeed plausible. We have therefore explored a mechanism for inactivation of DDH with chloroacetaldehyde (**12**).

The proposed mechanism for suicide inactivation of DDH by chloroacetaldehyde (**12**) is depicted in Scheme 5. The first step involves the 34.0 kJ mol^{-1} exothermic hydration of **12** to yield 2-chloroethane-1,1-diol (**13**). Presumably, it is **13** that actually binds to the active site of DDH as it shows the greater similarity to catalytic substrates, especially propane-1,2-diol (**1b**, Scheme 2a). Initial hydrogen-abstraction occurs from C2 of **13** to generate the substrate-derived radical **14**. We calculate this

Scheme 5. Proposed Mechanism for Suicide Inactivation of DDH by Chloroacetaldehyde (**12**) (Relative Energies in Parentheses, kJ mol^{-1})



reaction to be exothermic by 5.1 kJ mol^{-1}, with an associated barrier of 52.1 kJ mol^{-1}. Interestingly, we calculate the C1-generated radical from **13** (not shown) to be 12.5 kJ mol^{-1} lower in energy than the C2 substrate-derived radical **14**.

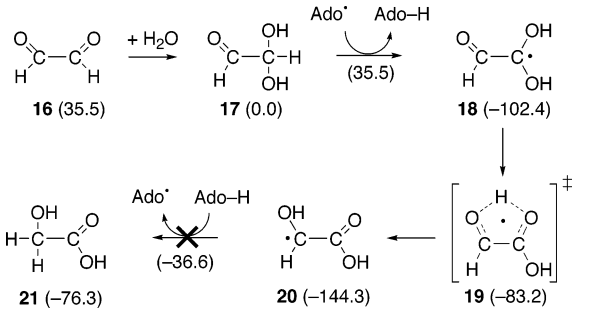
In a manner similar to a catalytic substrate, a 1,2-OH shift of the substrate-derived radical **14** is proposed to occur to form the product-related radical **15**. This rearrangement is calculated to be slightly endothermic (by 5.6 kJ mol^{-1}). The next step in the inactivation reaction of DDH by chloroacetaldehyde is elimination of HCl from **15** to form the glycolaldehyde radical (**9a**). This step is calculated to be exothermic by 44.9 kJ mol^{-1}. As seen previously with the inactivation of DDH by glycolaldehyde (**11a**) or ethane-1,1,2-triol (**7a**) (Scheme 3), the formation of **9a** leads to a deep energy-well, and **9a** becomes unable to execute the catalytically necessary hydrogen re-abstraction from Ado-H.

Another possibility exists for the product-related radical **15**. Instead of HCl loss as shown in Scheme 5, **15** could conceivably re-abstract a hydrogen atom from Ado-H, thus generating 1-chloroethane-1,2-diol and regenerating Ado• (not shown). This would then allow further catalysis to continue because Ado• would become regenerated. We have explored this possibility and find the barrier and endothermicity of the reaction to be 78.1 and 20.0 kJ mol^{-1}, respectively. As compared to the facile 44.9 kJ mol^{-1} exothermic loss of HCl, it is difficult to imagine why DDH would prefer the higher energy pathway. Besides, even if hydrogen-atom re-abstraction did occur to regenerate Ado• and form 1-chloroethane-1,2-diol, HCl loss would presumably then occur (as H_2O loss occurs for catalytic substrates), to form glycolaldehyde (**11a**), which, as shown as the reverse of the last step in Scheme 3, can lead directly to the suicide-inactivating glycolaldehyde radical (**9a**) via a highly exothermic (87.6 kJ mol^{-1}) hydrogen-atom transfer.

Thus, it appears that the mechanism depicted in Scheme 5 for the suicide inactivation of DDH by chloroacetaldehyde is indeed viable. The formation of **9a**, as previously seen for ethane-1,1,2-triol (**7a**, Scheme 3), results in a deep energy well from which hydrogen re-abstraction from the unactivated methyl group of Ado-H cannot occur. The end result is that the 5′-deoxyadenosyl radical cannot be regenerated and the catalytic cycle is broken.

3.2.5. Inactivation Mechanism for Glyoxal. At the same time that it was found that the incubation of DDH with glycolaldehyde (**11a**) resulted in the complete inactivation of DDH, it was also found that glyoxal (**16**) could perform the same function.[16a] Interestingly, **16** is also the product derived from glycolaldehyde (**11a**) upon aerobic protein denaturation

Scheme 6. Proposed Mechanism for the Suicide Inactivation of DDH by Glyoxal (**16**) (Relative Energies in Parentheses, kJ mol^{-1})



of the inactivated complex of DDH and **11a**.[54] Given that the inactivation mechanisms of DDH with **11a** and chloroacetaldehyde (**12**) lead to a highly stabilized radical that cannot perform the hydrogen-atom re-abstraction step, it seems likely for a similar mechanism to be operational when glyoxal is the substrate. To determine whether this may be the case, we have applied techniques similar to those already outlined to explore the situation in detail.

The proposed inactivation mechanism of DDH by glyoxal (**16**) is depicted in Scheme 6 and begins with a 35.5 kJ mol^{-1} exothermic hydration of **16** to form 2,2-dihydroxyacetaldehyde (**17**). Notice that **17** may simply be regarded as an OH-substituted derivative of glycolaldehyde (**11a**). The calculated barrier for the initial hydrogen-atom abstraction from 2,2-dihydroxyacetaldehyde (**17**) by Ado• to form the substrate-derived radical **18** plus Ado-H is calculated to be 35.5 kJ mol^{-1}. This energy requirement is less than the corresponding values for the catalytic substrates ethane-1,2-diol and propane-1,2-diol (53.5 and 51.8 kJ mol^{-1}, respectively, Scheme 2a). In addition, the associated exothermicity for this initial hydrogen-atom abstraction is a large 102.4 kJ mol^{-1}! The generated substrate-derived radical **18** can then rearrange via the intermediacy of transition structure **19** to the product-related radical **20**, which lies 41.9 kJ mol^{-1} lower in energy than **18**. Both the substrate-derived radical **18** and the product-related radical **20** are captodatively stabilized.[53]

Like the glycolaldehyde radical (**9a**), the radical **20** represents a stable radical species. This is evident in the computed barrier and endothermicity for the hydrogen re-abstraction reaction between **20** and Ado-H, which are calculated to be 107.7 and 68.0 kJ mol^{-1}, respectively. Such energy requirements are apparently too demanding for the reaction to proceed. Thus, it is quite likely that **20** is the final destination under anaerobic conditions for the reaction between DDH and glyoxal. If this is indeed the case, we see yet again that substrate-analogue-induced suicide inactivation results from the inability of 5′-deoxyadenosyl radical to become regenerated due to the presence of a stable radical species.

4. Concluding Remarks

The results presented in this study on the hydrogen-abstraction steps relevant to the reactions catalyzed by diol dehydratase (DDH) reinforce the notion that enzymes operating via radical mechanisms must maintain a delicate balance of activity for sustained catalysis. The calculated energy requirements for mechanisms of catalytic rearrangements with ethane-1,2-diol (**1a**) and propane-1.2-diol (**1b**) as substrates establish approximate boundaries for the functional capacity of DDH, which

prove useful in considerations of the phenomenon of suicide inactivation. Our calculations suggest that pathways in which elimination of H_2O occurs from either the substrate-derived radical **3** or the product-related radical **4** are not viable for the reactions catalyzed by DDH because a relatively stabilized allyloxy radical (**6**) is generated, which is unable to re-abstract an H atom from Ado-H for the purpose of continuing the catalytic cycle. It is intriguing to wonder whether these relatively demanding pathways reflect a lost generation of suicide inactivation mechanisms prior to the conception of the re-abstraction step preceding H_2O elimination, or even whether such pathways are available to DDH currently, albeit to a much lesser extent, since DDH is known to exhibit time-dependent inactivation with many substrate analogues.[15b]

The partial-proton-transfer concept for catalysis has been examined in relation to its influence on the hydrogen-abstraction barriers and kinetic isotope effects for the catalytic reaction of DDH with ethane-1,2-diol (**1a**). We find that partial protonation of the migrating OH group has a minimal effect on the barriers for the hydrogen-abstraction reactions, whereas partial deprotonation of the spectator OH group can substantially lower the initial abstraction barrier. Examination of the components of the tunneling-corrected KIE values reveals that contributions from tunneling are of a magnitude similar to that of the contributions from semiclassical theory alone. In addition, comparison of the calculated KIEs with the experimentally observed values supports a mechanism involving partial deprotonation of the spectator OH group.

Finally, suicide inactivation has been examined for the reactions of DDH with the substrate analogues glycolaldehyde, propane-1,2,2-triol, propane-1,1,2-triol, chloroacetaldehyde, and glyoxal. For each analogue examined, stable radicals have been identified along the reaction pathway that lead to the potential hydrogen re-abstraction steps being exceedingly endothermic. The net result is that 5′-deoxyadenosyl radical cannot be regenerated so as to continue the catalytic cycle, causing the suicide inactivation of DDH.

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Supporting Information Available: Gaussian archive entries of the B3-LYP/6-31G(d,p) geometries (Table S1), G3(MP2)-RAD total energies (Table S2), and complete citations for refs 33 and 34. This material is available free of charge via the Internet at http://pubs.acs.org.

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