Toward a Consistent Mechanism for Diol Dehydratase Catalyzed Reactions: An Application of the Partial-Proton-Transfer Concept

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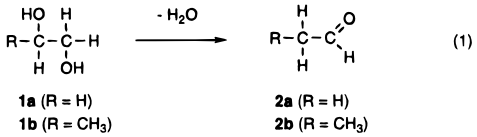
Abstract: Ab initio molecular orbital theory has been used to study the reactions catalyzed by the B_{12}-dependent enzyme diol dehydratase. The calculations show that a pathway involving the 1,2-shift of a hydroxyl group is greatly facilitated by *partial proton transfer* to the migrating oxygen. These results suggest a conceptually simple mechanism for the rearrangement whose reaction rate is consistent with experiment. The inclusion of a *gem*-diol intermediate in the proposed pathway is in accordance with ^{18}O-labeling experiments and thus overcomes important shortcomings in previously proposed mechanisms.

Introduction

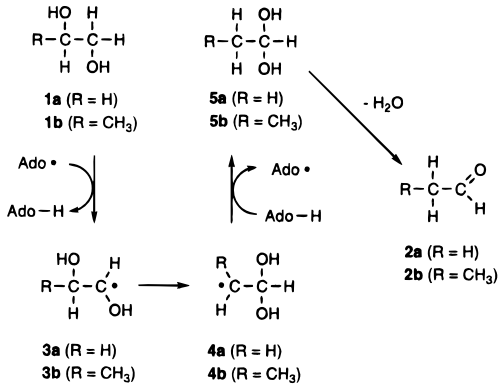
Diol dehydratase is a coenzyme B_{12}-dependent enzyme that catalyzes the transformation of 1,2-ethanediol (**1a**) into acetaldehyde (**2a**) plus water.[1] The same protein also catalyzes an



analogous reaction involving the dehydration of either enantiomer of 1,2-propanediol (**1b**) to give propionaldehyde (**2b**) plus water. These apparently simple dehydration reactions seem to proceed via an unusual mechanism involving protein-bound free-radical intermediates.[2]

As with most coenzyme B_{12}-dependent reactions, it is thought that a substrate hydrogen atom is initially abstracted by the 5′-deoxyadenosyl radical (Ado•, supplied by the coenzyme;[3] see Scheme 1), resulting in a substrate-derived radical (**3**). This species could conceivably eliminate water and abstract a hydrogen atom to form the product aldehyde (**2**).[4] However, ^{18}O-labeling experiments indicate that a *gem*-diol (i.e., 1,1-diol) intermediate (such as **5**) needs to be present on the reaction pathway in order to explain the observed isotope distributions.[5,6] Thus, experiments carried out with ethane(1-^{18}O)-1,2-diol show that approximately half the label is retained in the product aldehyde.[5] Furthermore, experiments carried out with enantiomeric propane(1-^{18}O)-1,2-diols show that most of the label is retained in the propanal product starting from the *R*-isomer, whereas most of the label is lost starting from the *S*-isomer.[6] These data are consistent with the involvement of a 1,1-diol intermediate which undergoes enzyme-mediated stereospecific dehydration (preferential loss of the pro-*S* oxygen).[3]

Scheme 1. Proposed Mechanism for the Diol Dehydratase Catalyzed Reactions[a]



[a] Ado• denotes the 5′-deoxyadenosyl radical supplied by the coenzyme. See ref 3.

Given the likely intermediacy of the 1,2-diol-derived radical (**3**) and the 1,1-diol (**5**), it would seem necessary to include the 1,1-diol-related radical (**4**) on the reaction pathway. Such a species could possibly be formed via the rearrangement of the 1,2-diol-derived radical (**3**), and it is this intrinsically difficult step that is the focus of the present study. Once formed, the

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(1) See, for example: (a) Zagalak, B.; Frey, P. A.; Karabatsos, G. L.; Abeles, R. H. *J. Biol. Chem.* **1966**, *241*, 3028. (b) Babior, B. M. *Acc. Chem. Res.* **1975**, *8*, 376−384. (c) Golding, B. T. In *Comprehensive Organic Chemistry*; Barton, D., Ollis, W. D., Eds.; Permagon Press: Oxford, 1979; Vol. 5, p 580. (d) Toraya, T.; Fukui, S. In *B_{12}*; Dolphin, D., Ed.; J Wiley & Sons: New York, 1982; Vol. 2, p 233.

(2) See, for example: (a) Finke, R. G.; Schiraldi, D. A.; Mayer, B. J. *Coord. Chem. Rev.* **1984**, *54*, 1−22. (b) Hartmanis, M. G. N.; Stadtman, T. C. *Proc. Natl. Acad. Sci.* **1987**, *84*, 76−79. (c) Golding, B. T. *Chem. Br.* **1990**, *26*, 950−954.

(3) For a recent review, see: Golding, B. T.; Buckel, W. In *Comprehensive Biological Catalysis*; Sinnott, M. L., Ed.; Academic Press: London, 1997; Vol. III, pp 239−259.

(4) (a) It has been established that, in solution, α-hydroxy radicals, such as **3a,** rapidly undergo an acid- or base-catalyzed fragmentation to •CH_2-CHO + H_2O.[2a,4b] (b) See, for example: Livingstone, R.; Zeldes, H. *J. Am. Chem. Soc.* **1966**, *88*, 4333−4336.

(5) Arigoni, D. In *Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor*; Zagalak, B., Friedrich, W., Eds.; W de Gruyter: Berlin, 1979; pp 389−410.

(6) (a) Rétey, J.; Umani-Ronchi, A.; Seibl, J.; Arigoni, D. *Experientia* **1966**, *22*, 502. (b) Rétey, J.; Robinson, J. A. In *Stereospecificity in Organic Chemistry and Enzymology*; Ebel, H. F., Ed.; Verlag Chemie: Weinheim, 1982; pp 185−207.

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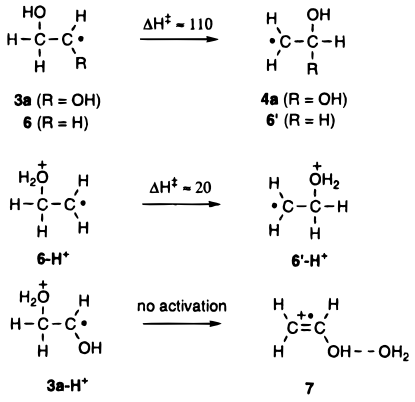
Scheme 2. Results of Previous Calculations[7,9] on Rearrangements of Putative Radical and Protonated Radical Intermediates in the Diol Dehydratase Catalyzed Reactions[a]



[a] Reaction barriers ($\Delta H^{\ddagger}$) are given in kJ mol^{-1}.

1,1-diol-related radical (**4**) would need to reabstract a hydrogen atom from 5′-deoxyadenosine (Ado-H, Scheme 1) in order to give the closed-shell species (**5**). Subsequent elimination of water would afford the product aldehyde (**2**) in a manner consistent with the isotope substitution experiments.[5,6]

Using the experimental rate constant of $k_{cat} = 150$ s^{-1} and a sensible entropy range, George et al. suggested that the barrier for the rate-limiting step (which could be either the hydrogen abstraction (**1** → **3**) or the radical rearrangement (**3** → **4**); see Scheme 1) should lie between approximately 55 and 70 kJ mol^{-1}.[7b] The barrier for the rearrangement should therefore have a value within or below this range. However, simple 1,2-migrations in free radicals (e.g., **3** → **4**) are known to be associated with quite high activation energies.[8,9] For example, ab initio calculations on the 2-hydroxyethyl radical (**6**)[7,9] and the 1,2-dihydroxyethyl radical (**3a**)[7] show that the 1,2-shift of an OH group (whether by dissociation/recombination or by a concerted shift) requires on the order of 95−120 kJ mol^{-1} (see Scheme 2). Given the estimated upper limit to the barrier mentioned above, and the high probability for the involvement of such a 1,2-shift, it becomes necessary to consider ways in which this rearrangement could be facilitated.

An early suggestion regarding the catalytic mechanism was that protonation of the migrating hydroxyl group might lead to a reduction in the barrier and hence facilitate the rearrangement.[9] This was indeed found to be the case for the 2-hydroxyethyl radical (**6**), the 1,2-shift in the protonated 2-hydroxyethyl radical (**6**-**H**$^+$) being found to have a barrier of just 18 kJ mol^{-1} (Scheme 2).[7,9] However, in the case of the 1,2-dihydroxy systems, the protonated 1,2-dihydroxyethyl radical (**3a**-**H**$^+$) appears to be inherently unstable[4,7] and, at least in the gas phase, rearranges without activation to a hydrogen-bonded hydrate of the vinyl alcohol radical cation (**7**).[7] On the basis of this observation, George et al. rejected a simple protonation mechanism in favor of a "predissociation" mechanism whereby, after various proton- and H-atom-transfer steps, the complex (**7**) dissociates to give acetaldehyde plus water. However, their postulated mechanism appears *not* to be consistent with the ^{18}O-labeling results. That is, according to their mechanism, the oxygen atom originally attached to the radical center in **3** will always be incorporated into the carbonyl group of the product aldehyde. This prediction is seemingly at odds with the available experimental evidence.[5,6] Furthermore, the hydrogen-bonding site taken up by water in **7** is almost certainly used by the enzyme to bind the substrate and would therefore be unlikely to be available for participation in such complexes.

In the present paper, we report the results of ab initio molecular orbital calculations that show how the radical rearrangement step of Scheme 1 (**3** → **4**) is greatly assisted by *partial* protonation of the migrating hydroxyl group. Not only is such a pathway consistent with the available experimental information, but application of the *partial-proton-transfer* concept makes a significant contribution to the understanding of the mechanism of action of diol dehydratase. Our results provide strong support for previous suggestions[1c,2a] that the enzyme is likely to use a suitable hydrogen-bond donor to bind the migrating hydroxyl group of the substrate.

Theoretical Procedures

Standard ab initio molecular orbital calculations[10] were performed with GAUSSIAN 94,[11] GAUSSIAN 98,[12] and MOLPRO 97.[13] We have previously found the CBS-RAD[14] method to perform well for radical rearrangement reactions similar to those investigated in the present study.[15] However, the size of some of the systems presented herein serves to make the CBS-RAD calculations impractical. Instead, we have evaluated the energies in the current work using a model chemistry that we will refer to as G2(MP2,SVP)-RAD(p). This technique employs the B3-LYP/6-31G(d,p) method for the prediction of equilibrium geometries and vibrational frequencies. Our previous work[15a,b] has successfully employed the 6-31G(d) basis set in the evaluation of these properties, but in the present work, we have included polarization functions on hydrogen atoms to provide a better description of those species containing hydrogen bonds. Using the B3-LYP/6-31G(d,p) geometries, improved relative energies were obtained with a variation

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(13) MOLPRO 97 is a package of ab initio programs written by H.-J. Werner and P. J. Knowles with contributions from J. Almlof, R. D. Amos, A. Berning, D. L. Cooper, M. J. O. Deegan, A. J. Dobbyn, F. Eckert, S. T. Elbert, C. Hampel, R. Lindh, W. A. Lloyd, W. Meyer, A. Nickless, K. Peterson, R. Pitzer, A. J. Stone, P. R. Taylor, M. E. Mura, P Pulay, M. Schütz, H. Stoll, and T. Thorsteinsson.

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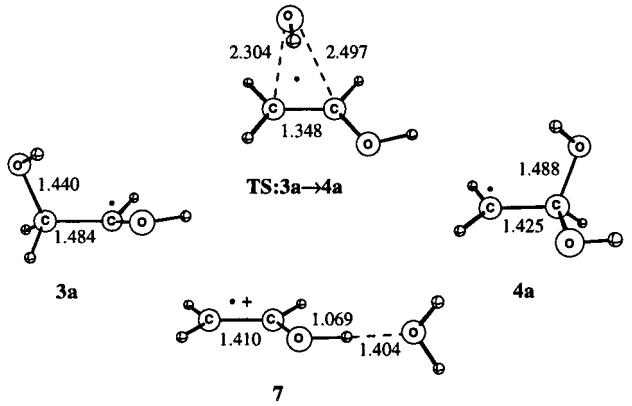

Figure 1. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for the dihydroxyethyl radicals (**3a** and **4a**), the transition structure (**TS:3a→4a**) for their interconversion, and the fully protonated dihydroxyethyl radical (**7**).

of the G2(MP2,SVP) method[16] that employs restricted-open-shell methodology in an effort to provide improved thermochemistry for free radicals (G2(MP2,SVP)-RAD[17]). The relative energies (ΔE) for species in the present work are therefore obtained from the following expression:

$$\Delta E(\text{G2(MP2,SVP)-RAD(p)}) = \Delta E(\text{RCCSD(T)/6-31G(d)}) + \Delta E(\text{RMP2/6-311+G(3df,2p)}) - \Delta E(\text{RMP2/6-31G(d)}) + \Delta E(\text{ZPVE}) \quad (2)$$

As we only present relative energies for species on a single electronic surface, the empirical higher level correction of G2(MP2,SVP) cancels and the resulting energy differences are purely ab initio. We have consequently employed the optimum ZPVE scaling factor (0.9806)[18] in obtaining zero-point vibrational energies (ZPVEs).

The parent method (G2(MP2,SVP)-RAD(B3-LYP)) (which uses the 6-31G(d) basis set for geometries and frequencies) has recently been shown to reliably predict the radical stabilization energies of a range of organic free radicals.[17] In addition, results for those systems in the current work whose size was conducive to CBS-RAD calculations showed the G2(MP2,SVP)-RAD(p) method to be in good agreement with its computationally more expensive counterpart (CBS-RAD(p); see below). Calculated G2(MP2,SVP)-RAD(p) total energies and GAUSSIAN 94 and GAUSSIAN 98 archive entries for the RMP2/6-31G(d)//B3-LYP/6-31G(d,p) calculations for all relevant structures are presented in Tables S1 and S2 of the Supporting Information.

Results and Discussion

Figure 1 shows the B3-LYP/6-31G(d,p) optimized structures and selected bond lengths for the 1,2-dihydroxyethyl radical (**3a**), the 2,2-dihydroxyethyl radical (**4a**), and the transition structure connecting the two (**TS:3a→4a**). The G2(MP2,SVP)-RAD(p) methodology predicts this rearrangement to require 113.1 kJ mol^{-1}, marginally less than the combined energy of the separated fragments (vinyl alcohol plus the hydroxyl radical at 115.8 kJ mol^{-1}). This result provides further confirmation that an unassisted radical shift is unlikely to be operative in the biological system. The thermodynamics of the rearrangement (**3a** → **4a**), on the other hand, are predicted to be favorable, with a calculated exothermicity of 20.3 kJ mol^{-1}.

The present calculations verify that, although protonation assists the hydroxyl migration in the hydroxyethyl radical ($\Delta H^{\ddagger}$ = 26.8 kJ mol^{-1} for the **6-H**$^+$ → **6′-H**$^+$ rearrangement), the protonated 1,2-dihydroxyethyl radical is indeed unstable and spontaneously rearranges to **7** (also included in Figure 1), as predicted earlier by George et al.[7] However, as mentioned above, it is important to recognize that any mechanism involving an intermediate such as **7**, in which the "migrating" oxygen is always displaced in the dehydration step, *cannot* be an accurate representation of the true pathway due to inconsistencies with the isotope substitution experiments.

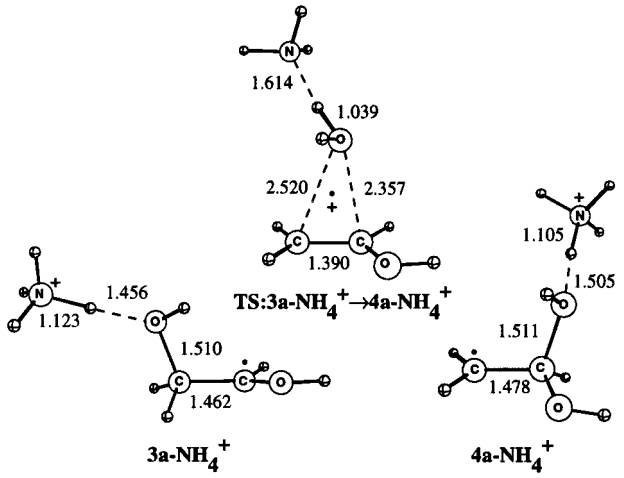

Figure 2. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in the catalysis by NH_4^+ of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical.

Apart from the fact that it is difficult to reconcile the protonation/predissociation mechanism (**3a-H**$^+$ → **7** → products) with experimental labeling data, a protonation-based mechanism could possibly be ruled out simply because the substrate is too weak a base. That is, it is unlikely that the enzyme possesses a sufficiently acidic group (p$K_a \approx -3$)[19] to generate an appreciable concentration of protonated substrate. An alternative scenario is suggested by our recent demonstration that partial proton transfer can provide some of the benefit that could be derived from full protonation, without resorting to the extreme.[15c] We show here how the partial-proton-transfer concept overcomes the shortcomings in previously proposed mechanisms for the reactions catalyzed by diol dehydratase.

Perhaps the simplest conceptual demonstration of the effectiveness of partial protonation comes from an examination of the catalysis of the rearrangement of the 1,2-dihydroxyethyl radical by the ammonium ion (NH_4^+). The complex between the 1,2-dihydroxyethyl radical and NH_4^+ (**3a-NH**$_4^+$; see Figure 2) is found to be quite stable, in stark contrast to the fully protonated analogue (**7**). The N−H distance in the hydrogen bond of **3a-NH**$_4^+$ is 1.123 Å as compared with 1.027 Å in isolated ammonium, an elongation that is indicative of partial transfer of the proton. The most significant geometric consequence of this partial proton transfer is the notable lengthening of the adjacent C−O bond from 1.440 Å in the isolated radical (**3a**, Figure 1) to 1.510 Å in the partially protonated structure (**3a-NH**$_4^+$, Figure 2). This lengthening implies a more weakly bound, and hence a more labile, hydroxyl group. In accordance with this observation, we find a transition structure (**TS:3a-NH**$_4^+$**→4a-NH**$_4^+$) for the 1,2-shift of the partially protonated hydroxyl group lying just 49.6 kJ mol^{-1} higher in energy than the reactant complex. The product of the rearrangement, the partially protonated 2,2-dihydroxyethyl radical (**4a-NH**$_4^+$), is also stable, and is found to lie only slightly higher in energy

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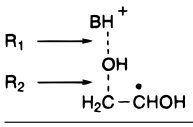
Table 1. Selected Geometric Parameters for the Complexes of Protonated Bases (Acids, B−H$^+$) with the 1,2-Dihydroxyethyl Radical (**3a**) and Thermochemical Quantities Relevant to the Associated 1,2-Hydroxyl Shift

	CH_3NH_2[a]	NH_3[a]	CH_3OCH_3[a]	CH_3OH[a]	H_2O[a]
R_1 (Å)[b]	1.556	1.456	1.119	1.020	1.006
R_2 (Å)[b]	1.499	1.510	1.607	1.962	2.028
$\Delta H^{\ddagger}$ (kJ mol^{-1})[c]	76.1	49.6[d]	18.5	2.8	0.0[d,e]
ΔH (kJ mol^{-1})[c]	1.1	3.1[d]	16.8	1.2	−0.9[d]
PA (kJ mol^{-1})[f]	895.0	847.4	787.3	749.1	682.3

[a] Conjugate base (B) of interacting acid (B−H$^+$). [b] Bond lengths (B3-LYP/6-31G(d,p)) indicating the extent and effect of partial proton transfer. [c] Calculated at the G2(MP2,SVP)-RAD(p) level. [d] The corresponding CBS-RAD(p) predictions are $\Delta H^{\ddagger} = 53.1$ kJ mol^{-1} and $\Delta H = 5.2$ kJ mol^{-1} for B = NH_3 and $\Delta H^{\ddagger} = 4.2$ kJ mol^{-1} and $\Delta H = 2.3$ kJ mol^{-1} for B = H_2O. [e] A finite barrier (3.4 kJ mol^{-1}) is found to disappear ($\Delta H^{\ddagger} = 0.0$ kJ mol^{-1}) upon inclusion of ZPVE, indicating little or no activation for rearrangement. [f] Proton affinity of the base (B) calculated by G2 theory. See ref 20.

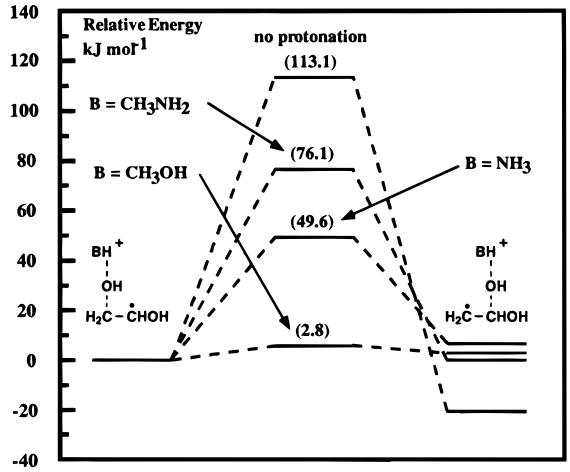


Figure 3. Schematic energy profile for the catalysis of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical by various acids (B−H$^+$). Relative energies (G2(MP2,SVP)-RAD(p) values in parentheses) are given in kJ mol^{-1}.

than the reactant, the overall rearrangement being endothermic by 3.1 kJ mol^{-1}.

The motion of the assisting proton during the rearrangement is an interesting one. Although the proton is largely localized on the nitrogen in both the reactant and product complexes, the greater affinity of the proton for the migrating oxygen in the transition structure can be clearly seen, the N−H bond having stretched to 1.614 Å (Figure 2). Thus, in a situation less extreme than full protonation, the obvious stabilizing influence imparted by the proton upon the transition structure is able to be exploited without significantly disrupting the reactant or product radicals. Put another way, the neutral ammonia serves to "hold back" the proton from the otherwise unstable protonated dihydroxyethyl radicals while the more basic transition structure is able to draw the proton closer in an energetically beneficial manner.[15c] Whichever way the effect is interpreted, it is clear that the interaction of ammonium with the dihydroxyethyl radicals softens the extreme of full protonation in such a way that the reactant and product radicals are now stable entities. At the same time, however, the partially transferred proton preferentially stabilizes the transition structure relative to the reactant and product radicals, causing the migration barrier to be reduced from more than 100 to about 50 kJ mol^{-1}.

Our calculations reveal that partial protonation by NH_4^+ represents only one point on the spectrum between full protonation (**3a-H$^+$** → **7**) and no protonation (**3a** → **4a**) (Scheme 2). While the two extremes do not appear mechanistically significant in this case, our calculations reveal a rich field of examples in between. Table 1 presents the conjugate bases (B) of a variety of acids (B−H$^+$) in order of decreasing base strength (proton affinities or PAs[20]). For each base (B), we have listed the calculated H−O (R_1) and C−O (R_2) bond lengths of the relevant complex with the 1,2-dihydroxyethyl radical (**3a**) (Table 1 graphic). In addition, Table 1 shows the relevant thermochemical parameters ($\Delta H^{\ddagger}$ and ΔH) for the 1,2-migration. The decreasing H−O bond length is the most direct measure of the extent of proton transfer to the migrating hydroxyl group. It can be seen that R_1 decreases monotonically as the strength of the acid increases. As a consequence of the increasing degree of proton transfer, the C−O bond is seen to lengthen and the migration of the hydroxyl group becomes progressively more facile. A subset of the results from Table 1 is presented graphically in Figure 3, showing the energetic effect of the varying degrees of proton transfer.

Contrary to the conclusions drawn in previous reports,[7] we find that it is quite feasible for the diol dehydratase catalyzed reaction to proceed with actual transfer of an OH group from one carbon to the other. Such a mechanism, made possible by partial proton transfer, is (potentially) consistent with the important ^{18}O-labeling experiments[5,6] since it includes the vital *gem*-diol intermediate.

The process of binding the substrate diol by the enzyme is highly likely to make use of the polar hydroxyl groups. The results presented herein indicate that the binding of the migrating hydroxyl group by a hydrogen-bond donor would not only serve to anchor the substrate but also facilitate the rearrangement reaction. Given the barrier range estimated from the enzymatic reaction rate (less than approximately 70 kJ mol^{-1}), any of the examples presented in Table 1 are possible candidates. However, only species with pK_a values similar to those of methylamine and ammonia would be expected to be protonated at biological pH. We would therefore expect any possible partial-proton-transfer agents to exhibit hydrogen-bonding properties similar to those of these two effective catalysts.

The second "spectator" hydroxyl group is also likely to be utilized in the process of binding by the protein. The most likely mode of binding at this site is that of a hydrogen-bond acceptor on the protein. For example, the recent suggestion of the involvement of "ketyl" radical anion intermediates[21] would require a basic group on the enzyme to enable the proposed proton abstraction from the spectator hydroxyl group to take place. This radical anion mechanism, in its current form,[21] proposes a fragmentation/recombination pathway for the migrating hydroxyl group. While we believe that it might not be necessary to have complete deprotonation of the spectator hydroxyl, or an intermolecular pathway, there are indications that a hydrogen-bond acceptor at this site can nevertheless be mechanistically useful.[22] The similarities between the reaction currently under investigation and the reaction catalyzed by the related ribonucleotide reductase[23] also lend weight to the proposed involvement of a hydrogen-bond acceptor for the

(20) G2 calculated proton affinities at 0 K are taken from Smith, B. J.; Radom, L. *J. Am. Chem. Soc.* **1993**, *115*, 4885−4888.

(21) Buckel, W.; Golding, B. T. *FEMS Microb. Rev.* **1998**, *22*, 523−541.

(22) Smith, D. M.; Golding, B. T.; Radom, L. Work in progress.

spectator hydroxyl group. If such a hydrogen bond were to exist, it would seem to preclude the formation of intermediates such as **7**.

Concluding Remarks

The participation of protein-bound free radicals (e.g., **1**) in the reactions catalyzed by diol dehydratase is now generally accepted. Similarly, the presence of a 1,1-diol intermediate (such as **5**) on the reaction pathway is considered necessary to explain important isotope labeling experiments. However, the combination of these two mechanistic aspects requires the inclusion of an energetically difficult step involving the 1,2-shift in a radical of a hydroxyl group (**3** → **4**). The investigation some time ago of a simplified model system led to the suggestion of acceleration of this step by protonation. This proposal was later rejected on the basis of calculations on a more complete model system. However, the suggested alternative did not include the requisite 1,1-diol intermediate.

Our calculations show that partial proton transfer provides an ideal solution to this dilemma. That is, even in a situation where the extreme of full protonation does not lend itself to a consistent mechanism, the idea of proton assistance need not be rejected. Indeed, the biologically more realistic situation involving catalysis by a hydrogen-bond donor provides a conceptually simple mechanism that is consistent with experiment. Partial transfer of the proton can be seen to be responsible for a significant lowering of the rearrangement barrier, without substantial distortion of the reactant and product radicals. Furthermore, the inclusion of the 1,1-diol intermediate on the reaction pathway allows consistency with the ^{18}O-labeling experiments.

While we do not claim that our calculations give a quantitatively accurate representation of a biological macromolecule, we do believe that the results presented herein constitute a useful and workable conceptual model of the enzymatic process. We believe that computational investigations of the substrate chemistry, of the type presented here, can provide valuable insights as to the nature and purpose of important interactions in the biological system.

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Supporting Information Available: G2(MP2,SVP)-RAD-(p) total energies (Table S1) and GAUSSIAN 94 and GAUSSIAN 98 archive entries for the RMP2/6-31G(d)//B3-LYP(6-31G(d,p) calculations for all relevant structures (Table S2) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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