An *ab Initio* Computational Molecular Orbital Study of Radical, Protonated Radical (Radical Cation), and Carbocation Species That Have Been Proposed in Mechanisms for the Transfer Process in the Enzyme−Coenzyme B_{12}-Catalyzed Dehydration of 1,2-Dihydroxyethane

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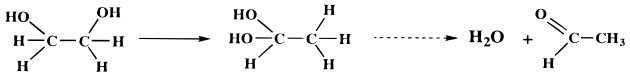
Received October 1, 1996. Revised Manuscript Received May 16, 1997[⊗]

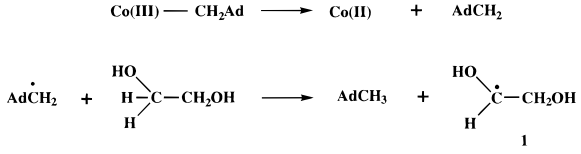
Abstract: The mechanism of action of the enzyme diol dehydrase has variously been suggested to involve a radical, a protonated radical (radical cation), or a carbocation in the apparent transfer of HO from one carbon atom to the other to give acetaldehyde and water. This adenosylcobalamin-requiring B_{12} enzyme catalyzes dehydration of 1,2-dihydroxyethane. Its active site is buried within a hydrophobic cavity. Each of the three possibilities are examined by *ab initio* molecular orbital calculations. Total molecular energies have been calculated with full geometry optimization at the MP2(FC)/6-31G* level and, in addition, single point energies using the MP2(FC)/6-311++G** basis set. Vibrational frequencies were also calculated. Transfer of an HO group within a $HOCH-CH_2OH^{]\bullet}$ radical (postulated to be formed by the initial reaction of the diol with the deoxyadenosyl radical via a bridge structure) was ruled out because the activation energy is much too high in relation to the observed rate constant for the enzyme reaction. A carbocation mechanism also presents a problem, quite apart from the necessity of postulating some acceptor for the electron from the $HOCH-CH_2OH^{]\bullet}$ radical. In principle acetaldehyde could be formed via the 2,2-dihydroxyeth-1-yl cation which was found to undergo a spontaneous 1,2-hydride ion shift, giving protonated acetic acid. But, although the 1,2-dihydroxyethyl cation (otherwise protonated glycolaldehyde) is well established as a stable species, the intermediary bridge structure could not be found. The radical cation $HOCH-CH_2OH_2^{]\bullet+}$, formed by proton transfer from an active-site group, was found to be inherently unstable, transforming without activation into a stable hydrogen-bonded hydrate of the *anti*-vinyl alcohol radical cation, $H_2O\cdots HOCH-CH_2^{]\bullet+}$. Deprotonation and H-atom transfer (from $AdCH_3$) were then found to give stable hydrogen-bonded hydrates of the formylmethyl radical, protonated acetaldehyde, and acetaldehyde itself. The ultimate formation of acetaldehyde and water can be attributed either to the dissociation of acetaldehyde hydrate or the prior dissociation of the formylmethyl radical hydrate followed by the H-atom transfer step. Because H_2O is already formed as a discrete entity in the initial protonation step and no transfer of a bonded HO, H_2O, or H_2O^+ group from one carbon atom to the other actually occurs, this series of reactions may be termed a "predissociation" mechanism. The overall proton transfer and the formation of water are complicated interconnected processes. A consideration of whether or not the cobalt participates in one of the later reaction steps is underway.

Introduction

The enzyme−coenzyme B_{12}-catalyzed dehydration of 1,2-dihydroxyethane (ethane-1,2-diol), the "diol dehydrase" reaction, is one of several coenzyme B_{12} reactions that can be viewed as a rearrangement in which a hydrogen atom on one carbon atom changes places with an HO group on an adjacent carbon atom,



Studies over the last 30 years, however, have shown that the dehydration is initiated by the formation of the substrate radical $HO\dot{C}H-CH_2OH$, **1**, as a result of homolytic fission of the Co(III)−CH_2Ad bond in deoxyadenosylcobalamin (Ad = adenosyl) and subsequent reaction of the deoxyadenosyl radical so formed ($AdCH_2^{]\bullet}$) with the substrate $HOCH_2CH_2OH$.[1]



This substrate radical, **1**, then rearranges to acetaldehyde.[1f] The

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⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1997.

(1) (a) Babior, B. M. *Acc. Chem. Res.* **1975**, *8*, 376−384. (b) Abeles, R. H.; Dolphin, D. *Acc. Chem. Res.* **1976**, *9*, 114−120. (c) Golding, B. T. In *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1, *Chemistry*, Chapter 15, pp 543−582. (d) Rétey, J. In *Stereochemistry*; Tamm, C. H., Ed.; Elsevier, Biomedical Press: Amsterdam, 1982; Chapter 6. (e) Finke, R. G.; Schiraldi, D. A.; Mayer, J. M. *Coord. Chem. Rev.* **1984**, *54*, 1−22. (f) Halpern, J. *Science* **1985**, *277*, 869−875. (g) Golding, B. T. *Chem. Br.* **1990**, *26*, 950−954. (h) Glusker, J. P. *Vitamins Hormones* **1995**, *50*, 1−76.

S0002-7863(96)03424-5 CCC: $14.00

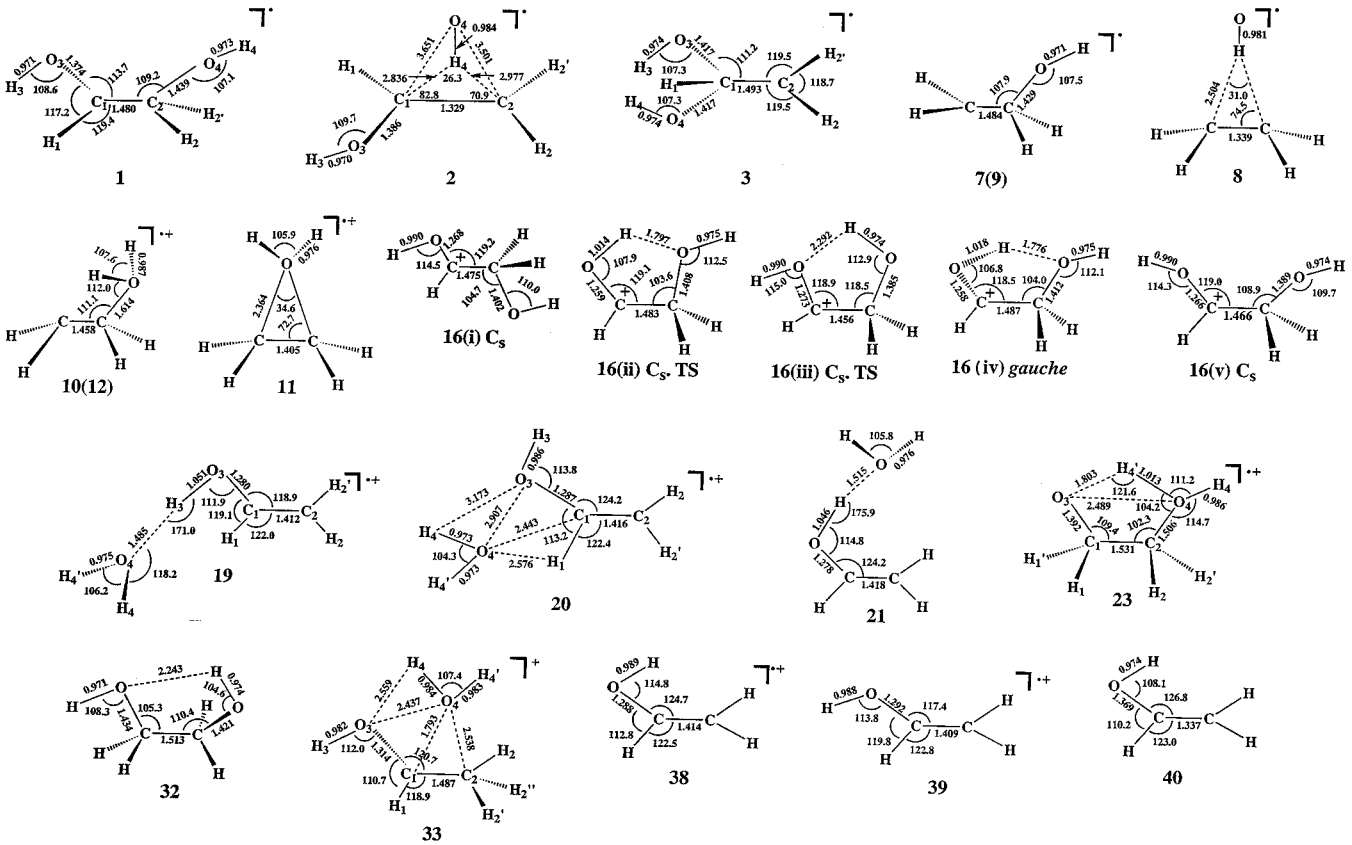
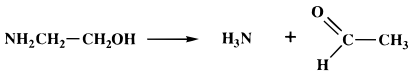


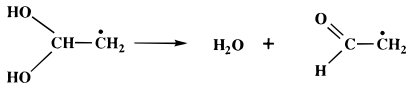
Figure 1. Geometries optimized using the MP2(FC)/6-31G* basis set: bond lengths in angstroms, bond angles in degrees.

formation of free radicals in the analogous adenosylcobalamin-mediated ethanolamine ammonia-lyase reaction

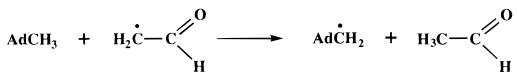


has been confirmed by studies on the effect of a magnetic field on the enzymatic reaction,[2] but the detailed mechanism by which these rearrangements occur has remained obscure.

In its simplest form the diol dehydrase rearrangement reaction can be represented as a 1,2-HO shift;[3] see Scheme 1A. Loss of water from the *gem*-diol radical (2,2-dihydroxyeth-1-yl radical) **3** so formed then gives the formylmethyl radical,



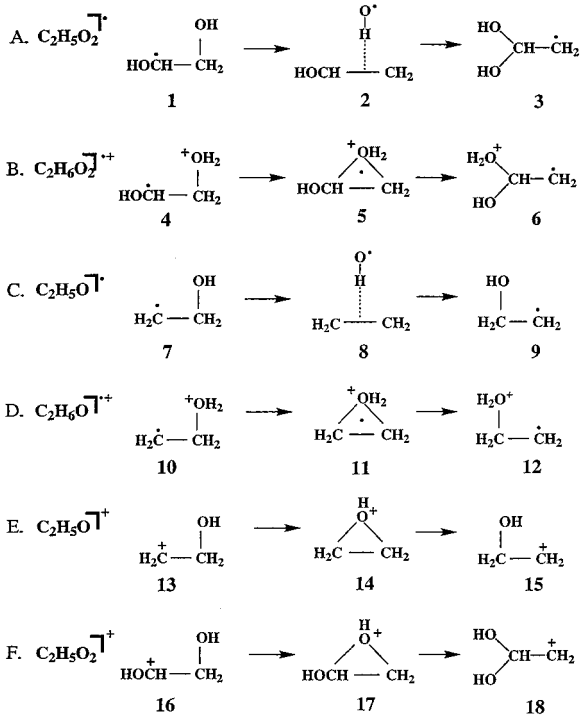
which, reacting with $AdCH_3$, gives acetaldehyde and regenerates the adenosyl radical.



However, the nonclassical bridge structure **2** (Figure 1), either as a stable intermediate or as a transition state, would entail one-electron occupancy of a relatively high energy antibonding orbital.[4] Therefore, the activation energy for the conversion of the substrate radical **1** into the product radical **3** could well be too high in relation to the quite rapid rate of the enzyme-catalyzed reaction,[5] $k_p = 150\ s^{-1}$, for the HO transfer to proceed in this manner.

To circumvent this difficulty, Golding and Radom[3,6] suggested that protonation might facilitate the 1,2-shift by increasing

Scheme 1



(2) (a) Harkins, T. T.; Grissom, C. B. *Science* **1994**, *263*, 958−960. (b) Harkins, T. T.; Grissom, C. B. *J. Am. Chem. Soc.* **1995**, *117*, 566−567.

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the carbocation character of the radical center as shown in Scheme 1B. To test this hypothesis, the energies of the "open" and "bridge" structures were calculated at the RHF/4-31G//RHF/STO-3G level for two model reactions—the degenerate transfer of the HO group in the 2-hydroxyethyl radical (see Scheme 1C) and the transfer of the H_2O^+ group in the protonated radical (see Scheme 1D). In the radical transfer reaction no bridge structure, **8** in Scheme 1C, was found *lower* in energy than the separated species, ethylene and the HO radical, and it was concluded that if transfer were to occur it would be by a dissociation−recombination mechanism. On the other hand, the protonated bridged structure **11** in Scheme 1D was found to be only 8.3 kcal/mol higher in energy than the protonated open structure **10** (**12**). Assuming **11** to be the transition state, the 1,2-H_2O^+ shift would be a very fast reaction in accord with the hypothesis. Later calculations[7] at the MP3/6-31G**//RHF/4-31G and the SDC1/6-31G**/RHF/4-31G levels gave even smaller energy differences, 3.1 and 1.7 kcal/mol, respectively; frequency calculations established that the open structure is a local minimum and the bridge structure a transition state on the potential energy surface (PES).

However, at the time these calculations[6] were carried out, a corresponding study of the transfer of the H_2O^+ group in the protonated ethane-1,2-diol radical, Scheme 1B, was feasible only with partial geometry optimization, assuming, *inter alia*, the optimized geometry of the $H_2O^+CH_2\dot{C}H_2$ skeleton from the protonated 2-hydroxyethyl radical calculations. The bridged structure **5** was found to be stabilized relative to the open structures **4** and **6** by 3.1 and 3.4 kcal/mol, respectively. Within the accuracy of the theoretical method used, it was difficult to predict whether the bridged or open structures would have the lower energy, and it was concluded that the 1,2-shift should occur quite readily. However, the possibility that the bridged structure has a lower energy than the open structures casts some doubt on the validity of the transfer mechanism. Moreover, if one or the other open structures is not stable, or if both are unstable, quite apart from the nature of the bridged structure, a different mechanism would have to be formulated.

To resolve this uncertainty, we have carried out calculations of total molecular energies with full geometry optimization at a much higher level, including polarization functions on the heavy atoms and taking electron correlation into account (MP2-(FC)/6-31G*). Vibrational frequencies were calculated to determine whether the computed structures are local minima or transition states on the PES. A brief account has been published.[8] No open structures corresponding to **4** or **6**, nor any bridged structure corresponding to **5**, could be identified on the PES. Instead, starting with several reasonable initial geometries for **4**, based on the geometry calculated for **1**, we found that they invariably optimized to a hydrogen-bonded hydrate of the *anti*-vinyl alcohol radical cation as a stable species, implying a transformation without activation. The existence of this complex between ionized vinyl alcohol and a water molecule, formed by the decomposition of ionized butane-1,4-diol in the gas phase, is well established.[9] Its structure and those of several isomeric hydrates, also various dissociation reactions in which the C−C bond is broken, have been investigated by high-level *ab initio* calculations.[10] In the present context of the dehydrase reaction, in which the C−C bond remains intact, the eventual formation of acetaldehyde can readily be accounted for by proton removal and H-atom addition. In this paper we give further details and examine the possibility of an alternative mechanism involving a hydrate of the *syn*-vinyl alcohol radical cation.

The results of similar calculations on the HO group transfer in the 2-hydroxyethyl and 1,2-dihydroxyethyl radicals, Scheme 1C, A, and H_2O^+ group transfer in protonated 2-hydroxyethyl radical, Scheme 1D, are also presented so that comparisons can be made at the same level of theory. Finally, we have considered transfer of the HO− group in the cationic species as shown in Scheme 1E, F. Since these rearrangements are specifically nucleophilic in character, the issue of one-electron occupancy of a (potentially) high-energy antibonding orbital does not arise,[4] and so they merit a close examination. The bridge structures **14** and **17** are the oxonium ion structures for protonated oxirane and protonated hydroxyoxirane, respectively, and the open structures **13** (**15**), **16**, and **18** are the corresponding carbocation structures that result from fission of one or the other C−O epoxide bond. However, previous calculations have shown there is a profound difference in the reactivity of these two bridge structures: **14** is a stable species,[11−13] whereas **17** could not be identified either as a stable species or as a transition state.[14] We have repeated these calculations on the protonated hydroxyoxirane structures with geometry optimization at the higher computational level, MP2(FC)/6-31G*, to explore further this difference in reactivity and determine whether there are stable species on the PES that could lead to the production of acetaldehyde.

Computational Methods

The calculations were performed at the Advanced Scientific Computing Laboratory, NCI-FCRF, using the GAUSSIAN 92 series of programs[15] on a Cray YMP computer. All structures were fully optimized using the split valence MP2/6-31G* (frozen core, valence orbitals active) basis set.[16] Total electronic energies were evaluated at three levels, **A**, PMP2(FC)/6-31G*, **B**, PMP2(FC)/6-311++G**, and **C**, QCISD(T)(FC)/6-311++G**, for the three heavy atom and smaller molecules and at levels **A** and **B** for the majority of the four heavy atom molecules (PMP2 indicating the projected MP2 energies for the radical and radical cation species). Vibrational frequencies were calculated at the MP2(FC)/6-31G*//MP2(FC)/6-31G* level to determine whether the computed structures correspond to local minima (stable intermediates) or saddle points (transition states) on the PESs[17] and to evaluate total thermal energies and entropies at 298 K. No scaling

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factors were used with the frequencies. Total molecular energies, E_{298}, the sum of the total electronic and thermal energies for the various molecules, and the entropies, S_{298}, are listed in Table 1S in the Supporting Information. The values for reaction and activation energies calculated from these data, with the incorporation of a ΔnRT term where needed, are listed in Tables 1−3, and would correspond to experimental gas-phase data.[18] Unless otherwise stated the values for the energies quoted in the text are those at the highest level **C** for the three heavy atom and smaller molecules and at level **B** for the four heavy atom molecules. Total atomic charges were calculated using Mulliken population analysis.[19]

Results

The molecular structures determined with full geometry optimization using the MP2(FC)/6-31G* basis set are depicted in Figure 1 and Figures 1S and 2S in the Supporting Information; see the listing in Table 1S. Selected bond lengths are given in angstroms and bond angles in degrees with some pertinent internuclear distances shown by dotted lines. The numbering scheme[20] adopted to identify the various atoms in Figure 1 is illustrated in **1**, **2**, **3**, and **19**. **Z**-matrix orientations and *X*, *Y*, and *Z* coordinates are available in Tables 2S−20S in the Supporting Information.

In tracking reaction pathways that could in principle lead to the production of acetaldehyde and water, it was necessary to study, *inter alia*, the *syn*- and *anti*-conformers of vinyl alcohol and the corresponding radical cations, also the radical cation of acetaldehyde, all of which have been the subject of many *ab initio* calculations.[21] Reaction enthalpies, ΔH_{298}, for rotation and isomerization reactions of these and other species, calculated using the E_{298} values in Table 1S, are listed in Table 21S of the Supporting Information. There is good agreement between these values and those obtained at similar calculation levels—and experimental values derived from $\Delta_f H$ data.[21b] Notably, the *syn*-conformer of vinyl alcohol is slightly more stable than the *anti*-conformer, whereas the *anti*-conformer of the radical cation is slightly more stable than the *syn*-conformer, and while the enol → keto isomerization is favorable to the extent of 12 kcal/mol for the neutral species, it is unfavorable to about the same extent for the radical cations.

A. Transfer of the HO Group in the 2-Hydroxyethyl Radical (Scheme 1C). At the RHF/STO-3G//RHF/STO-3G level Golding and Radom[6] could find no structure for the hypothetical bridged radical lower in energy than that of the separated species, ethylene and the hydroxyl radical, and concluded that if degenerate rearrangement of the open structure, **7** → **9**, does occur it would proceed by a dissociation−recombination mechanism. With the more extended basis set including electron correlation in the geometry optimization, MP2(FC)/6-31G*, we find the bridge structure **8** to be a local minimum on the $C_2H_5O^{\bullet}$ PES, 22.6 kcal/mol above the stable open structure(s) **7** (**9**); see Table 1A. Therefore, there must be transition states on either side, linking the bridge structure with the open structure. Furthermore, since the combined energies of the separated species is only 1.4 kcal/mol higher than that of the bridge structure, it must lie in a very shallow well, and HO group transfer via these transition states could scarcely be distinguished from a discrete dissociation−recombination mechanism. Either way the transfer would be quite a slow process.[22,23]

The orientation of the HO group in the bridge radical structure **8** with the H-atom nearer to and the O-atom away from the C−C bond is characteristic of the well-known OH−π interaction.[24] Comparison of the distribution of total atomic charge in this region with that in the separated species, see Figure 2A, shows that in the bridge structure the H-atom has become more positive while the O-atom and C-atoms have gained electronic charge. This type of charge redistribution is a well-established characteristic of hydrogen-bonding between electronegative atoms.[25] The dissociation energy of the "bond" in the bridge structure, 1.4 kcal/mol, is, as might be expected, significantly smaller.

Table 1. ΔH_{298}, Reaction Enthalpies (kcal/mol) Calculated Using the E_{298} Values in Table 1S

reaction	energy calculation level[a] A	B	C
(A) 2-Hydroxyethyl Radical			
open$^{\bullet}$ **7** → bridge$^{\bullet}$ **8**	+26.1	+27.2	+22.6
open$^{\bullet}$ **7** → HO$^{\bullet}$ **44** + $H_2C{=}CH_2$ **42**	+28.5	+28.7	+24.0
bridge$^{\bullet}$ **8** → HO$^{\bullet}$ **44** + $H_2C{=}CH_2$ **42**	+2.4	+1.5	+1.4
(B) Dihydroxyethyl Radicals			
1,2-open$^{\bullet}$ **1** → 1,1-open$^{\bullet}$ **3**	−5.7	−4.2	
1,2-open$^{\bullet}$ **1** → bridge$^{\bullet}$ **2**	+26.0	+28.6	
1,1-open$^{\bullet}$ **3** → bridge$^{\bullet}$ **2**	+31.7	+32.8	
1,2-open$^{\bullet}$ **1** → HO$^{\bullet}$ **44** + *syn*-vinyl alcohol **40**	+26.4	+27.7	
1,1-open$^{\bullet}$ **3** → HO$^{\bullet}$ **44** + *syn*-vinyl alcohol **40**	+32.1	+31.9	

[a] E_{298}, the sum of single-point energy calculations at **A**, the PMP2(FC)/6-31G* level, **B**, the PMP2(FC)/6-311++G** level, and **C**, the QCISD(T)(FC)/6-311++G** level, using MP2(FC)/6-31G* optimized geometries, plus the total thermal energy at 298 K evaluated from vibrational frequencies calculated at the MP2(FC)/6-31G* level.

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(19) Mulliken, R. S. *J. Chem. Phys.* **1955**, *23*, 1833−1840.

(20) C_1 and C_2 are, respectively, the left-hand and right-hand carbon atoms: O_3 is bonded to C_1 and O_4 to C_2, except in structures **3**, **28**, **29**, **30**, and **31**, where both O-atoms are bonded to C_1. Hydrogen atoms are given the same number as the heavy atom to which they are bonded. In the radical bridge structures **2** and **8**, H_4−O_4 forms the bridging group with H_4 nearer to the C_1−C_2 bond, whereas in the radical cation bridge structure **11**, O_4 is nearer to the C_1−C_2 bond. In the hydrate structures O_4 has become the oxygen atom of the water molecule.

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(22) The dissociation energy of the open structure at the RHF/STO-3G//RHF/STO-3G level calculated from Golding and Radom's value for its total electronic energy[6] and values for the HO radical and ethylene from Lathan, Curtiss, Hehre, Lisle, and Pople[23] is 31.6 kcal/mol, compared to our ΔH_{298} values of 28.5 and 28.7 kcal/mol at levels **A** and **B**. Their value[6] for the total electronic energy, however, gives an anomalously low dissociation energy of 6 kcal/mol.

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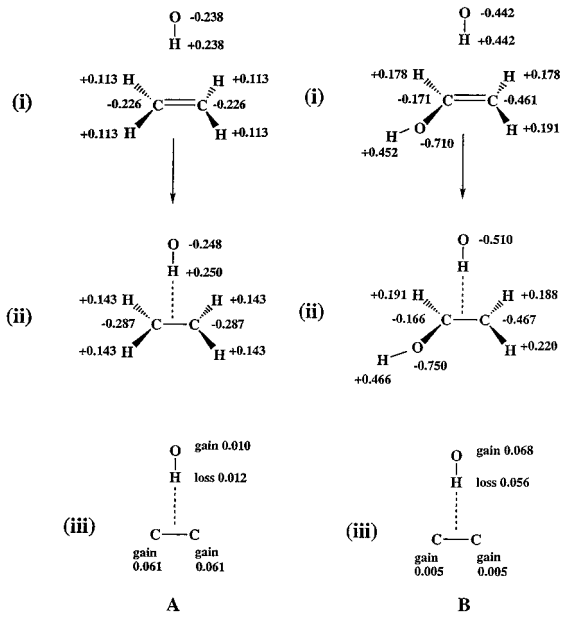


Figure 2. Total atomic charges on the various atoms, calculated from the SCF densities using the MP2(FC)/6-311++G** basis set: (**A**) (i) in the HO radical, **44**, and the ethylene molecule, **42**, (ii) in the hydroxyethyl radical bridge structure **8**, and (iii) the gain and loss of electronic charge in the region of the HO group and the C−C bond which accompany the formation of the bridge structure; (**B**) (i) in the HO radical, **44**, and *anti*-vinyl alcohol, **41**, (ii) in the dihydroxyethyl radical bridge structure **2**, and (iii) the gain and loss of electronic charge in the region of the HO group and the C−C bond which accompany the formation of the bridge structure.

B. Transfer of the HO Group in the Dihydroxyethyl Radical (Scheme 1A). As a consequence of the hydroxyl group substitution, the two open structures are no longer identical, and we find the 1,1-isomer **3** to be 4.2 kcal/mol lower in energy than the 1,2-isomer **1**. Furthermore, the bridge structure **2** is a transition state (not a local minimum on the PES, like **8**) 28.6 kcal/mol above the 1,2-open structure; see Table 1B. HO group transfer via this transition state is thus a formal possibility. However, the combined energies of the separated species, the HO radical and *anti*-vinyl alcohol, are a little lower in energy than **2**—by 0.9 kcal/mol—and we conclude that the $C_2H_6O_2^{\bullet+}$ PES is nearly flat in this region, and no distinction can be made between HO group transfer as such and the dissociation−recombination mechanism. With an activation energy of 28.6 kcal/mol the transfer would have a rate constant of about 10^{-7} s^{-1} at 37 °C, far smaller than the observed rate constant for the enzyme reaction[5] of 150 s^{-1}.

Whereas the H−O bond in the bridge structure **8** is perpendicular to the plane containing the hydrogen atoms bonded to the carbon, with the H-atom and the O-atom each equidistant from the C-atoms, there is considerable distortion in the bridge structure **2**. Not only is the H_4-O_4 group tilted with respect to the two C-atoms (see Figure 1, formula **2**) but it lies way over toward the hydroxy-substituted side of the molecule, i.e., $H_4\cdots H_2 = 2.443$ Å while $H_4\cdots H_{2'} = 4.045$ Å, and $O_4\cdots H_2 = 2.694$ Å while $O_4\cdots H_{2'} = 4.500$ Å. Comparison of internuclear distances in the two bridge structures shows the H−O group to be further away from the C−C bond in structure **2**, in accord with its transition state rather than a local minimum character. Even so the charge redistribution in this region of the molecule as the bridge structure is formed from the separated species is similar to that found for structure **8**; compare parts A and B of Figure 2.

C. Transfer of the H_2O^+ Group in the 2-Hydroxyethyl Radical Cation (Scheme 1D). In agreement with the work of others[6,7] we find the bridge structure **11** to be only a few kilocalories per mole higher in energy than the open structure **10** (**12**), by 4.3 kcal/mol at level **C** (Table 2). Frequency calculations confirm that **10** (**12**) is a local minimum and **11** a transition state on the $C_2H_6O^{\bullet+}$ PES. Hence, protonation of the 2-hydroxyethyl radical would induce a very rapid degenerate H_2O^+ group transfer, in accord with the hypothesis of Golding and Radom.[3] On the other hand, the dissociation of the open structure **10** into separated species, $H_2O + H_2C{-}CH_2^{\bullet+}$ or $H_2O^{\bullet+} + H_2C{=}CH_2$, is a far less favorable process; see Table 2A. The $C_2H_6O^{\bullet+}$ radical cation observed in a mass spectrometric study of the fragmentation of the propane-1,3-diol radical cation is thus more likely to have the open structure **10** (**12**) and not the bridge structure **11**.[26]

Table 2. ΔH_{298}, Reaction Enthalpies (kcal/mol) Calculated Using the E_{298} Values in Table 1S[a]

	energy calculation level[b]		
reaction	**A**	**B**	**C**
(A) 2-Hydroxyethyl Radical Cation			
open$^{\bullet+}$ **10** → bridge$^{\bullet+}$ **11** (TS)[c]	+8.1	+5.5	+4.3
open$^{\bullet+}$ **10** → H_2O **45** + $H_2C{-}CH_2^{\bullet+}$ **43**	+29.4	+23.9	+23.1
open$^{\bullet+}$ **10** → $H_2O^{\bullet+}$ **46** + $H_2C{=}CH_2$ **42**	+74.7	+72.3	+68.4
(B) Dissociation of Hydrogen-Bonded Hydrates			
19 → H_2O + **39**	+30.0	+26.5	
19 + $H_2O^{\bullet+}$ → **40**	+106.6	+104.8	
27 → H_2O + **37**	+28.6	+24.9	
27 → H_3O^+ + **35**	+43.1	+43.6	
25 → $HO^{\bullet}$ + **40**	+38.6	+38.5	
25 → H_2O + **34**	+6.0	+3.7	
26 → H_2O + **35**	+5.6	+3.5	
(C) Dissociation of Other Hydrate Structures			
20 → H_2O + **38**	+14.7	+13.0	
33 → H_2O + **37**	+14.4	+9.2	
(D) Dehydration of Dihydroxyethanes			
32 → H_2O + **35**	−4.3	−5.5	
30 → H_2O + **35**	+4.4	+2.6	

[a] More details are given in deposited Table 23S. See Schemes 1 and 2 and deposited formulas for molecular diagrams. [b] E_{298}, the sum of single-point energy calculations at **A**, the PMP2(FC)/6-31G* level, **B**, the PMP2(FC)/6-311++G** level, and **C**, the QCISD(T)(FC)/6-311++G** level, using MP2(FC)/6-31G* optimized geometries, plus the total thermal energy at 298 K evaluated from vibrational frequencies calculated at the MP2(FC)/6-31G* level. [c] Transition state.

D. Feasibility of H_2O^+ Group Transfer in the Dihydroxyethyl Radical Cation (Scheme 1B). Having confirmed that H_2O^+ group transfer could occur very readily in the 2-hydroxyethyl radical cation **10**, we set up an initial structure for the dihydroxyethyl radical cation **4** taking $C_1-O = 1.400$ Å and $C_2-O = 1.614$ Å as in **10** to explore the feasibility of H_2O^+ group transfer, **4** → **5** → **6**. Contrary to expectation, geometry optimization did not lead to the identification of local minima corresponding to structures **4**, **5**, and **6** or to a transition state corresponding to structure **5**. Instead, we found that the initial structure for **4** transformed without activation to a hydrogen-bonded hydrate of the *anti*-vinyl alcohol radical cation, **19**, 27.2 kcal/mol lower in energy. Frequency analysis confirms that **19** is a local minimum on the $C_2H_6O_2^{\bullet+}$ PES.[10a,d] The transformation involves the H_2O group on the right-hand carbon atom, C_2, moving far to the left of C_1 without the formation of the protonated *gem*-diol radical structure **6** as an intermediate. In the optimized structure, $C_2\cdots O_4 = 4.540$ Å and $C_1\cdots O_4 = 3.144$ Å.

To check the validity of this result, in case structure **6** was inadvertently bypassed in the optimization procedure, we set

(26) Terlouw, J. K.; Heerma, W.; Dijkstra, G. *Org. Mass Spectrom.* **1981**, *16*, 326−327.

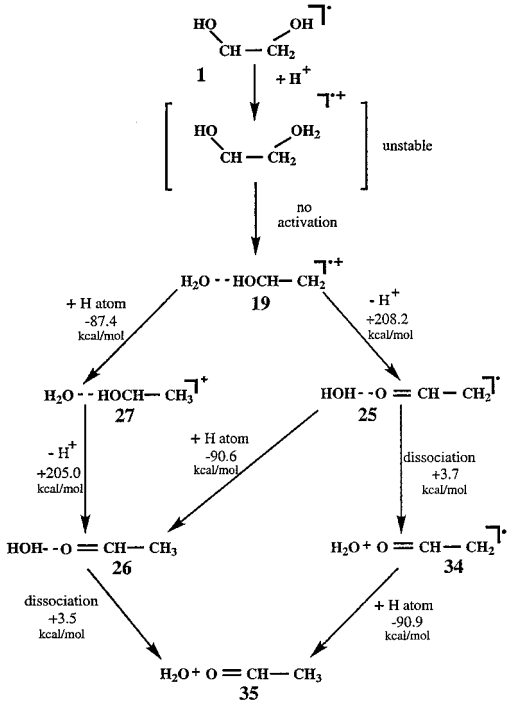
up an initial structure for **6** taking the C−O bonds to be 1.417 Å as in the 1,1-dihydroxyethyl radical, **3**. However, upon optimizing this structure C_1-O_3 was found to decrease to 1.287 Å, whereas $C_2\cdots O_4$ increased to 2.443 Å, with (again) no indication that **6** participates as an intermediate. The resulting structure **20**, found to be another local minimum on the $C_2H_6O_2^{\bullet+}$ PES, is evidently a hydrate of the *syn*-vinyl alcohol radical cation, an isomer of **19**. But the orientation of the water molecule excludes hydrogen-bond formation, and the interaction can be characterized as ion−dipole. At level **B**, **20** is found to be 15.8 kcal/mol higher in energy than **19**, of which 2.3 kcal/mol can be attributed to the difference in stability between the *syn*- and *anti*-rotamers of the vinyl alcohol radical cation; see Table 21S. The hydrogen-bonded hydrate of the *syn*-vinyl alcohol radical cation, **21**, which results from a 180° rotation of the H_2OHO- grouping about the C−O bond or inversion of the HO group with respect to the C−O bond in the *anti*-structure,[10a,d] is considerably more stable—only 1.8 kcal/mol higher in energy than **19** at level **A**; see Table 21S.[27−29]

Thus, although H_2O^+ transfer **4** → **5** → **6** can be ruled out as an integral part of the mechanism for the dehydration of 1,2-dihydroxyethane, the collapse of **4** without activation to give a hydrogen-bonded hydrate of the *anti*-vinyl alcohol radical cation and the possibility that the ion−dipole hydrate of the *syn*-vinyl alcohol radical cation could be formed from the stable isomer of **4** suggest that a different type of mechanism is operative. All that would remain would be appropriate deprotonation and H-atom addition steps, with dissociation of the water molecule at some stage. This mechanism will be elaborated in the following sections.

E. Deprotonation and Hydrogen-Atom Addition Reactions Following the Formation of the Hydrogen-Bonded Hydrate of the *anti*-Vinyl Alcohol Radical Cation. Deprotonation of **19** was found to give a hydrogen-bonded hydrate of the formylmethyl radical, **25**, and the subsequent addition of a hydrogen atom was found to give a hydrogen-bonded hydrate of acetaldehyde, **26**. In the reverse order, addition of a hydrogen atom to **19** was found to give a hydrogen-bonded hydrate of protonated acetaldehyde, **27**, which upon deprotonation gives **26**. Frequency analyses show that all three hydrates, **25**, **26**, and **27**, are local minima on the $C_2H_5O_2^{\bullet}$, $C_2H_6O_2$, and $C_2H_7O_2^{+}$ potential energy surfaces, respectively.

F. A "Predissociation" Mechanism for the Dehydration of 1,2-Dihydroxyethane. Following the formation of the hydrogen-bonded hydrate of the *anti*-vinyl alcohol radical cation, an acceptable mechanism for the dehydration in the context of the diol dehydrase reaction must be compatible with the observed rate constant for product formation.[5] Taking the entropy of activation in the putative rate-determining step to be zero as a lower limit, and 10 eu as an upper limit, the values for the enthalpies of activation commensurate with a rate constant of 150 s^{-1} at 37 °C are 13.5 and 16.2 kcal/mol, respectively. Any dissociation process that has an energy exceeding about 15 kcal/mol can therefore be disregarded as a potential step in the diol dehydrase pathway.

Scheme 2



Most of the hydrogen-bonded hydrates come into this category, namely, the hydrogen-bonded hydrate of the *anti*-vinyl alcohol radical cation, **19** ↛ H_2O + HO−CH−$CH_2^{\bullet+}$ (dissociation +27 kcal/mol) and **19** ↛ $H_2O^{\bullet+}$ + HOCH=CH_2 (dissociation +105 kcal/mol), the hydrogen-bonded hydrate of protonated acetaldehyde, **27** ↛ H_2O + HOCH−CH_3^{+} (dissociation +27 kcal/mol) and **27** ↛ H_3O^{+} + O=CH−CH_3 (dissociation +44 kcal/mol), and the hydrogen-bonded hydrate of the formylmethyl radical, **25** ↛ $HO^{\bullet}$ + HOCH=CH_2 (dissociation +39 kcal/mol). *Only two of the hydrates have sufficiently low dissociation energies for them to qualify as intermediates:* the hydrogen-bonded hydrate of the formylmethyl radical, **25**, giving water and the formylmethyl radical (dissociation +3.7 kcal/mol), and the hydrogen-bonded acetaldehydrate hydrate itself, **26**, giving water and acetaldehyde (dissociation +3.5 kcal/mol); see Table 2B.

Following protonation of the 1,2-dihydroxyethyl radical and its collapse to give the hydrogen-bonded hydrate of *anti*-vinyl alcohol radical cation, a sequence of reactions leading to the production of water and acetaldehyde via these two dissociation steps and the necessary deprotonation and H-atom addition is set out in Scheme 2. The very large (adverse) enthalpy changes for the deprotonation and the very large (favorable) enthalpy changes for the H-atom addition reactions, noted beside the arrows in Scheme 2, relate to gas-phase processes. It should perhaps be emphasized that in the enzyme−coenzyme B_{12}-catalyzed dehydration these steps are to be understood as involving *H^+ transfer* to a proton acceptor at the active site on the enzyme and *H-atom transfer* from the 5′-deoxyadenosyl group of the coenzyme—transfer steps that would be characterized by far smaller enthalpy changes.

(27) We have also characterized three other structures on the $C_2H_6O_2^{\bullet+}$ PES that have the same O−C−C−O grouping as the protonated 1,2-dihydroxyethyl radical, **4**. In the first, H_3-O_3 is rotated about C_1-O_3 to give an intramolecular hydrogen-bonded structure which might be expected to be more stable than **4**, much as hydrogen-bonded structures are the more stable forms of 1,2-dihydroxyethane.[28] Yet, although a geometry-optimized structure was obtained, **22** (not drawn), 48 kcal/mol higher in energy than **19** at level **A**, frequency analysis showed it to have two negative eigenvalues in the force constant matrix, so its role on the $C_2H_6O_2^{\bullet+}$ PES is neither as a local minimum nor as a transition state. In the second, a 1,4-H-atom shift in ionized 1,2-dihydroxyethane[29] gives the radical cation **23**, 48 kcal/mol higher in energy than **19**. In the third, **24**, the isomer is formed by protonation of the HOĊH grouping, instead of the CH_2OH grouping which is an essential feature of the mechanism proposed by Golding and Radom.[3,6] Rather surprisingly, in view of the transient nature of **4** collapsing to give **19**, frequency analysis shows this isomer to be a local minimum on the $C_2H_6O_2^{\bullet+}$ PES, 35 kcal/mol higher in energy than **19**.

(28) (a) van Alsenoy, C.; van den Enden, L. *J. Mol. Struct.: THEOCHEM* **1984**, *108*, 121−128. (b) Murcko, M. A.; DiPaola, R. A. *J. Am. Chem. Soc.* **1992**, *114*, 10010−10018. (c) Oie, T.; Topol, I. A.; Burt, S. K. *J. Phys. Chem.* **1994**, *98*, 1121−1128.

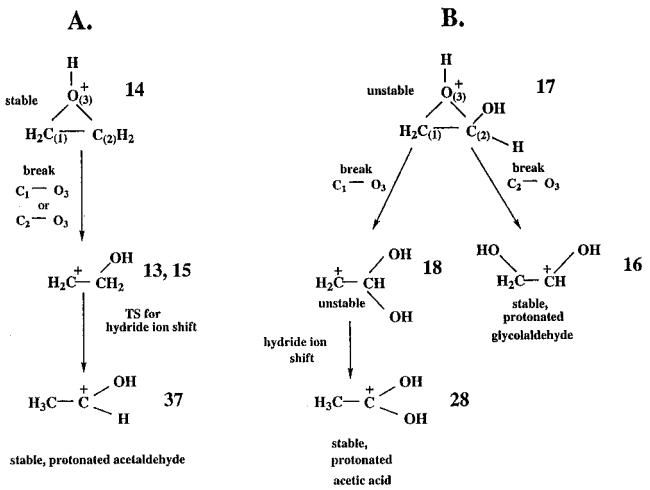
(29) Yates, B. F.; Bouma, W. J.; MacLeod, J. K.; Radom, L. *J. Chem. Soc., Chem. Commun.* **1987**, 204−205.

Table 3. Reaction Enthalpies, ΔH_{298} (kcal/mol), Calculated Using the E_{298} Values in Table 1S, Compared with Experimental Values Derived from $\Delta_f H$ Data[a]

reaction (location)	energy calculation level A	B	exptl
(i) dehydration (introduction): *tGg′* $HOCH_2CH_2OH \rightarrow CH_3CHO + H_2O$	−4.3	−5.5	−4.9
(ii) Dehydration (Scheme 4A): $CH_3C(OH)_2^{+} + e^- + H^{\bullet} \rightarrow CH_3CHO + H_2O$	−203.6	−220.3	−221.7
(iii) fission (Scheme 4B): $CH_3C(OH)_2^{+} + H^{\bullet} \rightarrow CH_3^{\bullet} + HC(OH)_2^{+}$	+11.2	+5.7	+7.3
(iv) isomerization (Scheme 3A):  $\rightarrow$ *cis/syn*-CH_3CHOH^{+}	−24.9[b]		−25.8

[a] (i) from ref 30, (ii) from ref 30 and 31, (iii) from ref 30−32, (iv) from ref 31. [b] Energies calculated at level **A**, but with the thermal energy corrections obtained from frequency analyses[14] using the RHF/6-31G* basis set.

Scheme 3

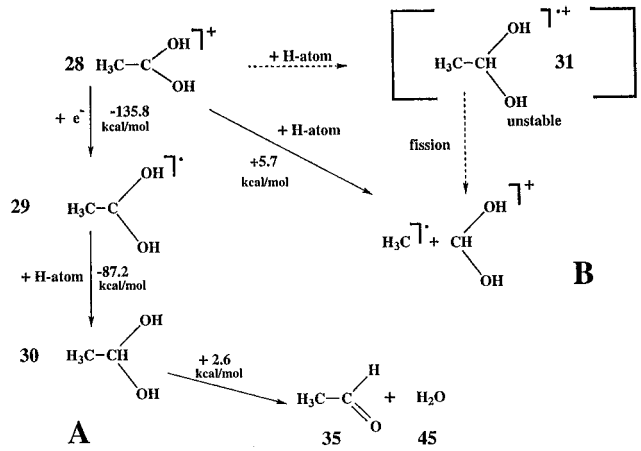


A sequence of reactions of this kind can be termed a "predissociation" mechanism because only hydrate structures are involved and *at no stage is there an actual transfer of a bonded* HO, H_2O, or H_2O^+ group *from one carbon atom to the other*. A very similar mechanism has been postulated for the decomposition of the 1,2-dihydroxyethyl radical generated by HO radical attack in acidic aqueous solution,[30] except that in this case dissociation of the H_2O was presumed to accompany the protonation.

G. Reactivity of the Open and Bridge Structures of the Hydroxyethyl and Dihydroxyethyl Cations (Scheme 1E, F). Several calculations have established that the reactivity of these species is very different from that set out in Scheme 1E, F.[11−14] Calculations at both the MP2(FC)/6-31G**//RHF/6-31G*[13] and MP2(FC)/6-31G*//MP2(FC)/6-31G*[14] levels have found that the bridge structure **14** for the hydroxyethyl cation (otherwise the oxonium ion form of protonated oxirane) is a stable species 28 kcal/mol lower in energy than the open structure, **13** (**15**) (otherwise the carbocation form of protonated oxirane that results from fission of the C_1-O_3 or C_2-O_3 bond in the epoxide ring). This carbocation is a transition state for the production of protonated acetaldehyde via a 1,2-hydride ion shift; see Scheme 3A.

Furthermore, hydroxyl group substitution in the protonated oxirane results in a remarkable alteration in the reactivity, see Scheme 3B. With full geometry optimization at the RHF/6-31G* level, the bridge structure **17** could not be identified either as a stable entity or as a transition state. Two stable carbocation structures were characterized—an extended open *trans*-conformer and a *cis*-conformer with a plane of symmetry and an intramolecular hydrogen bond with the O-atom of the CH_2OH group as the acceptor. A *cis*-conformer which also has a plane of symmetry, but with the O-atom of the CHOH group as acceptor, was identified as the transition state for rotation of HO in the CH_2OH group about the C−O bond.[11]

Scheme 4



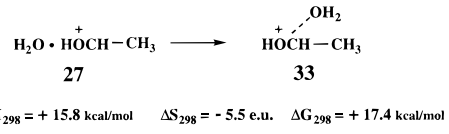
Repeating these calculations at the MP2(FC)/6-31G* level the extended open *trans*-structure **16**(i) and the latter *cis*-structure **16**(ii) were again identified as a stable species and a transition state, respectively; see Figure 1. However, at this higher level the former *cis*-structure **16**(iii) was also found to be a transition state, in this case for rotation of HO in the CHOH group about the C−O bond. The stable structure with respect to this rotation was found to be a *gauche*-conformer, **16**(iv). The energies of **16**(iii) and **16**(iv) are nearly identical, indicating that the PES is almost flat in this region and that a mixture of the two enantiomeric forms of the *gauche*-structure would be present. Further attempts to characterize **17**, starting with the HO group in a bridge position, led, upon optimization, to the formation of yet another stable *cis*-structure, which has the open configuration, **16**(v), very similar in energy to that of the open *trans*-structure and about 10 kcal/mol less stable than the *gauche*-structure **16**(iv); see Table 3A. There would appear to be no way for any of these stable 1,2-dihydroxyethyl carbocation structures to serve as a precursor for the formation of acetaldehyde.

The isomeric carbocation structure **18**, in which both HO groups are bonded to the same carbon atom, was found to be unstable, reverting without activation via a 1,2-hydride ion shift to give protonated acetic acid, **28**. Electron addition giving the 1,1-dihydroxyeth-1-yl radical, **29**, followed by H-atom addition giving 1,1-dihydroxyethane (the *gem*-diol), **30**, and the subsequent elimination of water could thus account for the dehydration process; see Scheme 4A. However, even though these reactions are quite straightforward the formation of **18** presents a difficulty. The bridge structure **17** from which it could in principle be formed by fission of the C_1-O_3 epoxide bond (Scheme 3B) could not be identified either as a stable species or as a transition state on the PES. Electron removal from the

(30) Walling, C.; Johnson, R. A. *J. Am. Chem. Soc.* **1975**, *97*, 2405−2407.

2,2-dihydroxyeth-1-yl radical, **3**, would also appear to be ruled out because this structure in turn would have to be derived from the initial substrate radical **1** via the HO group transfer, **1** → **2** → **3**, which, as shown in section B (above), is an exceedingly slow process. There is the same difficulty with the alternative pathway in which H-atom addition occurs first, giving the 1,1-dihydroxyethane radical cation, **31**, see Scheme 4B, followed by electron addition giving 1,1-dihydroxyethane, **30**, and then water elimination as before. Furthermore, starting with a reasonable structure for the radical cation based upon that for the *gem*-diol, it was found to be unstable, the C−C bond breaking spontaneously to give the methyl radical $\dot{C}H_3$ and protonated formic acid $HC^+(OH)_2$. The formation of acetaldehyde and water by further reaction of these separated species is improbable, and for this reason alone Scheme 4B can be neglected.

H. Relative Stability of Acetaldehyde Hydrate and Protonated Acetaldehyde Hydrate with Respect to the Isomeric *gem*-Diol Structures. The formation of acetaldehyde hydrate and protonated acetaldehyde hydrate rather than the isomeric *gem*-diol structures prompted a study of their relative stabilities. No structure corresponding to protonated *gem*-diol $HO(H_2O^+)$-$CHCH_3$ could be identified on the $C_2H_7O_2^+$ PES. Starting with a reasonable structure based on that for the unprotonated molecule **30**, upon optimization it was found to transform without activation into a distorted hydrate of protonated acetaldehyde, **33**, with the H_2O situated above C_1. This distorted hydrate is a local minimum on the $C_2H_7O_2^+$ PES, significantly higher in energy than the hydrogen-bonded hydrate **27** in terms of both ΔH_{298} and ΔG_{298}.



27 **33**

ΔH_{298} = + 15.8 kcal/mol ΔS_{298} = - 5.5 e.u. ΔG_{298} = + 17.4 kcal/mol

We note in passing that in a mass spectrometric study of alkene loss from ionized dialkyl carbonates and ortho esters at least eight stable isomeric $C_2H_7O_2^+$ ions were identified. *Ab initio* calculations at the RHF/3-21G//RHF/3-21G level found a structure that most closely resembles **27** to be the most stable, followed by a structure that most closely resembles **33** as the next most stable, 11.2 kcal/mol higher in energy.[31] Interconversion of these isomers is evidently prevented by high barriers.

Acetaldehyde hydrate OH_2•$OCH-CH_3$, **26**, was found to be more stable than the *gem*-diol $(HO)_2CH-CH_3$, **30**, due largely to the unfavorable entropy change (ΔH_{298} = +0.8 kcal/mol, ΔS_{298} = -10.8 eu, ΔG_{298} = +4.0 kcal/mol). Although it has been customary to account for the formation of acetaldehyde by the dehydration of the *gem*-diol $(HO)_2CH-CH_3 \rightarrow H_2O + O{=}CH-CH_3$, the retention of H_2O in hydrate structures and the ultimate dissociation of one of these structures are also consistent with the observation that acetaldehyde is released in the final step.[32]

I. A Comparison of Calculated and Experimental Values for Various Reaction Enthalpies, ΔH_{298}. With the exception of reactions i−iv in Table 3 there are insufficient $\Delta_f H$ data for reactant and/or product species in the other reactions in Schemes 1−4 from which ΔH_{298} could be evaluated. Nevertheless, there is close agreement between the calculated values at level **B** and the experimental values for reactions i, ii, and iii, and between the calculated value at level **A** and the experimental value for reaction iv. The differences (calculated minus experimental) amount, respectively, to −0.6, +1.4, −1.6, and +0.9 kcal/mol. Bearing in mind the uncertainties in the values for $\Delta_f H$, the cumulative uncertainty in the ΔH_{298} values is unlikely to be less than 0.5 kcal/mol.[33−35]

Discussion

An electron spin resonance study has shown that the distance of closest approach of water molecules to the magnetic center in the enzyme−coenzyme B_{12}-catalyzed dehydration of 1,2-dihydroxyethane, **32**, is about 10.2 Å.[36] The environment of the active site is thus essentially hydrophobic in nature. The calculated ΔH_{298} values found in the present study correspond to gas-phase values,[18] but they are, nonetheless, useful indicators of possible reactions steps. A large positive value for ΔH_{298} and hence an activation energy incompatible with the experimental kinetic data for the enzyme reaction would suffice to rule out any such step. A recent crystal structure determination of the related cobalamin-dependent methylmalonyl−coenzyme A (CoA) mutase has shown that the active site of this enzyme is deeply buried and inaccessible to solvent, except through the CoA channel along the barrel axis.[37] A similar inaccessibility has been seen in several other free radical enzymes such as galactose oxidase, copper amino oxidase, and prostaglandin H_2 synthase. This inaccessibility is believed to protect reactive radical intermediates from undesirable side reactions.

For a carbocation species to participate in the diol dehydrase reaction, an electron acceptor is required at the active site. While the obvious choice is electron transfer to the Co(II) giving Co(I), there seems to be no experimental evidence for the formation of Co(I) in the adenosylcobalamin-utilizing diol dehydrase reactions, unlike the synthesis of methionine catalyzed by the methylcobalamin-dependent enzyme methionine synthase.[38,39] The function of methionine synthase is the transfer of a methyl group to homocysteine, which it accomplishes by *heterolytic* fission of the Co(III)−CH_3 bond of methylcobalamin, generating Co(I) and CH_3^+.[40,41] By contrast, the function of the diol dehydrase system is the formation of the substrate free radical $HO\dot{C}H-CH_2OH$. *Homolytic* fission of the Co(III)−CH_2Ad bond in adenosylcobalamin gives Co(II) and the $\dot{C}H_2$Ad radical, which then abstracts a hydrogen atom from the diol to give a radical.

The predissociation mechanism for the dehydration set out in Scheme 2 requires a proton donor at the active site, presumably from an amino acid side chain, such as a carboxyl group implicated in the action of the closely related ethanolamine ammonia-lyase.[42] For diol dehydrase the proton transfer in its entirety and the formation of water are complicated,

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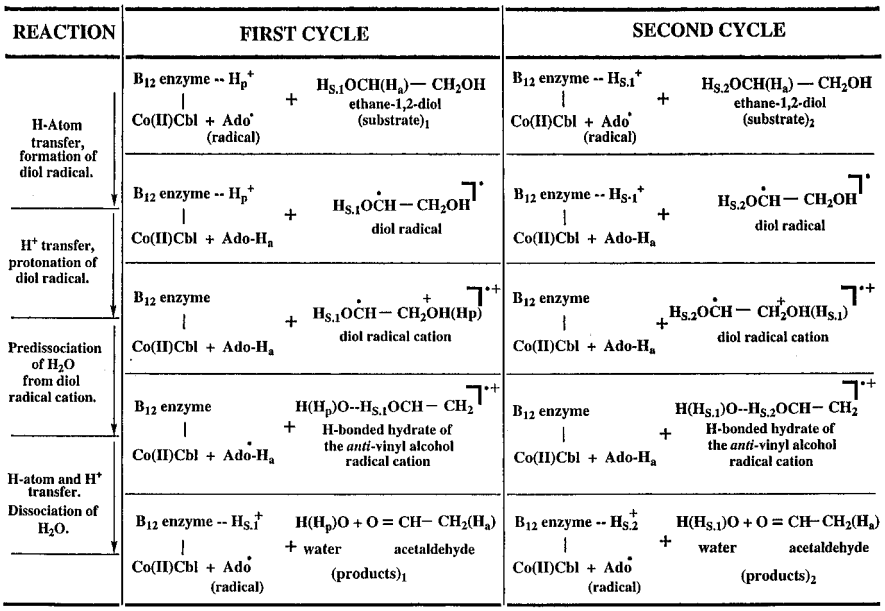
Scheme 5



interconnected processes as shown in Scheme 5. In the active site, transfer of the proton H_p^+ (from protein) to the diol radical initiates the first cycle, while reprotonation toward the end of the cycle occurs by transfer from the $H_{S.1}O$ group (substrate, in the first cycle) originally in the HOĊH grouping of the diol radical. The hydrogen atoms in the water molecule are, respectively, the H_p hydrogen from the protein and the OH hydrogen from the CH_2OH grouping in the diol radical. The second cycle is initiated by transfer of $H_{S.1}^+$ from the active site and the final reprotonation by transfer from the $H_{S.2}O$ group in the HOĊH grouping of the second diol radical. The hydrogen atoms in the second water molecule are, respectively, $H_{S.1}$ from the HOĊH grouping in the first diol radical and the OH hydrogen from the CH_2OH grouping in the second diol radical, and so on. Apart from the first cycle, the water molecules thus contain a hydrogen atom from the OH group in the CH_2OH grouping in one diol radical and a hydrogen atom from the OH group in the HOĊH grouping from the diol radical in the previous cycle.

The steps in Scheme 2 can be regarded as the "primary" reactions in the overall enzyme mechanism, but in addition there are undoubtedly "secondary" reactions and/or interactions that contribute to and so define its individuality. Even in the preliminary formation of the substrate radical there is a possibility that another hydrogen acceptor besides $-CH_2Ad$ is involved in the hydrogen transfer, as in the case of ethanolamine ammonia-lyase.[43] Moreover, to explain the stereochemical aspects of the diol dehydrase reaction of 1,2-dihydroxypropane, a selective interaction between the substrate and some group or groups at the active site is necessary.[1d,e] Furthermore, the 5′-deoxyadenosyl group has its own special binding site.[44]

Summary and Conclusions

These calculations have led to the following conclusions on the apparent transfer of an HO group (or some equivalent species) from one carbon atom to an adjacent one in the production of acetaldehyde and water from 1,2-dihydroxyethane in the diol dehydrase-catalyzed reaction.

(i) Following the formation of the substrate radical HOĊH−CH_2OH, **1**, by reaction of the diol with the deoxyadenosyl radical, transfer of an HO group to give $(HO)_2CH-\dot{C}H_2$, **3**, can be ruled out because the bridge structure **2**, identified as the transition state in this reaction, is 28.6 kcal/mol higher in energy than **1**. Such a high-energy barrier would result in too slow a transfer compared to the observed rate constant for the enzyme reaction. Therefore, for the reaction to proceed, the substrate radical must be modified in some way. Two possibilities have been considered—protonation and carbocation formation.

(ii) Protonation of the substrate radical and transfer of H_2O^+ in the radical cation $HO\dot{C}H-CH_2(OH_2^+)$, **4**, to give $HO(H_2O^+)$-$CH-\dot{C}H_2$, **6**, as proposed by Golding and Radom,[3,6] can also be ruled out. Neither **4** nor **6** (Scheme 1) could be identified as a stable structure, nor the bridge structure **5** either as a stable structure or as a transition state. Instead the inherently unstable radical cation structure **4** can transform without activation into a hydrogen-bonded hydrate of the *anti*-vinyl alcohol radical cation, $H_2O\cdots HOCH-CH_2^{\bullet+}$, **19** (Scheme 2). The transformation involves the OH_2 grouping on the right-hand C-atom in **4**, in Scheme 2, moving far to the left of the left-hand C-atom without the formation of the protonated *gem*-diol radical **6** of Scheme 1, as an intermediate.

(iii) The formation of this hydrogen-bonded hydrate of the *anti*-vinyl alcohol radical cation accounts for the ultimate production of acetaldehyde and water. Deprotonation of **19** gives a hydrogen-bonded hydrate of the formylmethyl radical $HOH\cdots OCH-\dot{C}H_2$, **25**. Subsequent H-atom addition gives a hydrogen-bonded hydrate of acetaldehyde $HOH\cdots OCH-CH_3$ (diagrammed in Scheme 2).[26] In the reverse order, H-atom addition to **19** gives a hydrogen-bonded hydrate of protonated acetaldehyde $H_2O\cdots HO^+CH-CH_3$, **27**, and deprotonation then gives **26**. All that now remains is for the water molecule to separate from **26** at some stage to give **35**. *To be compatible with the observed rate constant for the enzyme reaction, any dissociation with an energy in excess of about 15 kcal/mol can be disregarded.* Either **25** or **26**, with dissociation energies of 3.7 and 3.5 kcal/mol, respectively, are thus singled out as acceptable intermediates in the dehydration pathway. This sequence of reactions, set out in Scheme 2, can be termed a "predissociation" mechanism because only hydrate structures are involved and *at no stage is there an actual transfer of a bonded* HO, H_2O, or H_2O^+ group *from one carbon atom to the other*. The overall proton transfer and the formation of water are complicated interconnected processes, as shown in Scheme

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5. Notably, apart from the first cycle, the water molecules contain a hydrogen atom from the OH group of the CH_2OH grouping in one diol radical and a hydrogen atom from the HO group in the $HO\dot{C}H$ grouping in the diol radical that was involved in the previous cycle.

(iv) On the other hand, carbocation formation by the removal of an electron from the radical species gives protonated glycolaldehyde HOC^+H-CH_2OH, **16**, corresponding to **1**, and the 2,2-dihydroxyeth-1-yl carbocation $(HO)_2CH-C^+H_2$, **18**, corresponding to **3** (see Scheme 1). The bridge structure **17** corresponding to **2** is an oxonium ion. Although several conformers of **16** have been identified as stable species, there would appear to be no way for any of these structures to serve as precursors for the production of acetaldehyde and water. The isomer **18**, on the other hand, is unstable, reverting without activation via a 1,2-hydride ion shift to give protonated acetic acid $(HO)_2C^+-CH_3$, **28**. Electron addition giving the 1,1-dihydroxyeth-1-yl radical $(HO)_2\dot{C}-CH_3$, **29**, followed by H-atom addition giving 1,1-dihydroxyethane $(HO)_2CH-CH_3$, **30**, the *gem*-diol, and subsequent elimination of water, could thus account for the dehydration process; see Scheme 4A. The alternative pathway in which H-atom addition occurs first, giving the 1,1-dihydroxyethane radical cation $(HO)_2CH-CH_3^{\bullet+}$, **31**, can be ruled out because fission of the C−C bond occurs spontaneously, giving protonated formic acid $(HO)_2C^+H$ and the methyl radical $\dot{C}H_3$, and the formation of acetaldehyde and water by further reaction of these separated species is improbable.

(v) However, although the formation of protonated acetic acid, **28**, from the (unstable) carbocation **18** and the subsequent reactions in Scheme 4A are straightforward, it is difficult to account for the formation of **18** in the first place. The removal of an electron from the 2,2-dihydroxyeth-1-yl radical, **3**, is the obvious choice, but since this structure in turn has to be derived from the initial substrate radical $HO\dot{C}H-CH_2OH$, **1**, there remains the difficulty that the HO group transfer, **1** → **2** → **3**, is too slow, see (i) above, quite apart from the issue of any involvement of Co(I) in forming the carbocations.

(vi) The sequence of primary reactions in Scheme 2 provides a more acceptable pathway for the dehydration process, recognizing that secondary reactions and/or selective interactions at the active site contribute to and so characterize the individuality of the enzyme−coenzyme system.

Thus, in the diol dehydrase-catalyzed reaction it appears that both the cofactor (adenosylcobalamin) and the enzyme itself play crucial roles in the catalytic mechanism. The cofactor produces a radical and the enzyme contributes a proton (in the initial cycle) to give a radical cation, the decomposition of which results in hydrates that can lead to the production of the acetaldehyde and water.

Acknowledgment. We thank the Advanced Scientific Computing Laboratory, NCI-FCRF, for providing time on the CRAY YMP supercomputer, and Carol Afshar for technical assistance. We also thank one of the reviewers for drawing our attention to several important papers in the literature. This work was supported by Grants CA10925 and CA06927 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

Supporting Information Available: Figure 1S, structures **24**, **28**−**30**, and **33**, Figure 2S, structures **34**−**47** inclusive, calculated using the MP2(FC)/6-31G* basis set with full geometry optimization, Table 1S, E_{298} and S_{298} values, (**Z**-matrix orientations) Tables 2S−15S for structures **1**, **3**, **7** (**9**), **8**, **10** (**12**), **11**, **16**(i)−**16**(v), **20**, **23**, **26**−**28**, **30**, **32**−**34**, and **38**−**43**, (*XYZ* coordinates) Tables 16S−20S for structures **2**, **19**, **21**, **24**, **25**, **29**, and **35**−**37**, Table 21S, reaction enthalpies, ΔH_{298}, for various rotation and isomerization reactions, and Tables 22S and 23S, additional information for Tables 1 and 2 (32 pages). See any current masthead page for ordering and Internet access instructions.

JA963424V