Ab Initio Study of Protonated Radical Species That May Be Involved in the Enzyme−Coenzyme B_{12} Catalyzed Dehydration of 1,2-Dihydroxyethane

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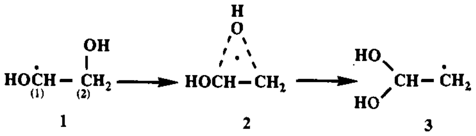
The effect of protonating the 1,2-dihydroxyethyl radical in the mechanism proposed for the enzyme−B_{12} catalyzed dehydration of 1,2-dihydroxyethane, has been studied by *ab initio*

$$\mathrm{HOCH_2CH_2OH \rightarrow CH_3CHO + H_2O}$$

molecular orbital calculations at the PMP2(FC)/6-311++G**//MP2(FC)/6-31G* level. No open or bridged protonated radical species corresponding to those involved in the 1,2 H_2O^+ shift in the protonated hydroxyethyl radical could be identified on the potential energy surface. Starting with a reasonable structure for the protonated 1,2-dihydroxyethyl radical, based on the structure calculated for the radical, we found that it transforms without activation to a hydrogen-bonded hydrate of the vinyl alcohol radical cation. Dissociation of this hydrate either into H_2O and $HOCHCH_2^{\bullet+}$ or into $H_2O^{\bullet+}$ and $HOCHCH_2$ is unlikely as a possible step in the dehydration pathway because the bonding energies are too high. Further reaction thus depends on deprotonation and the addition of a hydrogen atom. We found that these steps give hydrogen-bonded hydrates of the formylmethyl radical, protonated acetaldehyde, and acetaldehyde. The ultimate formation of acetaldehyde and water can be attributed to the dissociation of the acetaldehyde hydrate, or the prior dissociation of the formylmethyl radical hydrate followed by hydrogen atom addition, since both hydrates have very small dissociation energies of about 4 kcal/mol. Because H_2O is already formed as a discrete entity in the first step and no transfer of a *bonded* HO, H_2O, or H_2O^+ group *from one carbon to the other* actually occurs, a series of reactions of this kind can be termed a "predissociation" mechanism.

In the dehydration of 1,2-dihydroxyethane (ethane-1,2-diol), catalyzed by the B_{12}-utilizing enzyme diol dehydrase, the function of the coenzyme B_{12} is the generation of a bound radical form of the substrate. The radical substrate then undergoes a rearrangement leading to formation of acetaldehyde, the product of the reaction.[1] The detailed mechanism of this rearrangement has remained obscure. It can, for example, be represented as a 1,2 HO shift. But the bridged structure, **2**, whether as a stable intermediate or as a transition state, involves a one-electron occupancy of a relatively high-energy antibonding orbital,[2] and the activation energy for such a reaction could well be too high in relation to the quite rapid rate of the overall dehydration,[3] $k_p = 150\ \mathrm{s^{-1}}$, for HO transfer to proceed in this simple manner.



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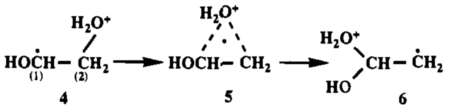
To avoid this difficulty, Golding and Radom[4] proposed protonation of the radical followed by a 1,2 shift of H_2O^+, which, since this shift is nucleophilic in character, would no longer require one-electron occupancy of an antibonding orbital.[2]



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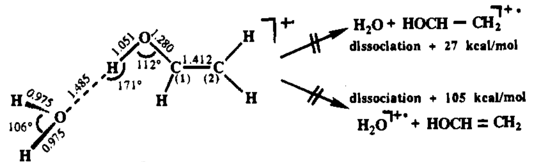
To investigate the feasibility of this mechanism (**4** → **5** → **6**) we have carried out calculations of total electronic energies with full geometry optimization at the MP2(FC)/6-31G* computational level.[5] Vibrational frequencies were also calculated to establish whether the computed structures relate to local minima or transition states on the potential energy surfaces,[6] and to evaluate total thermal energies at 298 K so that reaction enthalpies could be calculated that would correspond to experimental gas-phase data.[7]

Contrary to expectation, geometry optimization did not lead to the identification of local minima corresponding to structures **4**, **5**, or **6** or to a transition state corresponding to structure **5**. Starting with a reasonable structure for the protonated 1,2-dihydroxyethane radical, **4**, based on the structure calculated for radical **1**, we found that it transforms without activation to a hydrogen-bonded hydrate of the vinyl alcohol radical cation, **7**, 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, **6**, as an intermediate. (The bond lengths in **7**−**10** are in angstroms, the bond angles in degrees). A frequency analysis confirms that **7** is a local minimum on the potential energy surface. The values of ΔH^{298} for the dissociation of **7** into H_2O and $HOCHCH_2^{\bullet+}$ and into $H_2O^{\bullet+}$ and $HOCH{=}CH_2$, calculated at the PMP2(FC)/6-311++G**//MP2(FC)/6-31G*



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(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. Mechanisms of Action of the B_{12} Coenzyme: Theory and Models. In *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1. Chapter 15, pp 543−582. (d) Rétey, J.; Vitamin B_{12}: Stereochemical Aspects of its Biological Functions and of its Biosynthesis. 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**, *227*, 869−875. (g) Golding, B. T. The B_{12} Mystery. *Chem. Br.* **1990**, *26*, 950−954.

(2) Marsh, J. *Advanced Organic Chemistry. Reactions, Mechanisms and Structure*, 4th ed.; John Wiley and Sons: New York, 1992; Chapter 18, pp 1051−1052, 1064−1067.

(3) Bachovchin, W. W.; Eager, R. G.; Moore, K. W.; Richards, J. H. *Biochemistry* **1977**, *16*, 1082−1092.

(4) (a) Golding, B. T.; Radom, L. *J. Chem. Soc., Chem. Commun.* **1973**, 939−941. (b) Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1976**, *98*, 6331−6338.

(5) (a) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213−222. (b) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257−2261. (c) Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618−622. (d) Schlegel, H. B. *J. Chem. Phys.* **1986**, *84*, 4530−4534. (e) Schlegel, H. B. *J. Phys. Chem.* **1988**, *92*, 3075−3078. (f) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Anders, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *Gaussian 92*, Revision A; Gaussian, Inc.: Pittsburgh, PA, 1992.

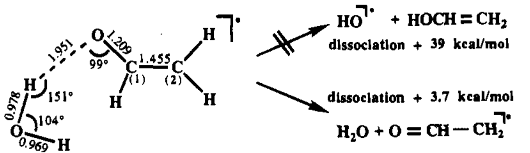
(6) Schlegel, H. B. Some Practical Suggestions for Optimizing Geometries and Locating Transition States. In *New Theoretical Concepts for Understanding Organic Reactions*; Bertran, J., Csizmadia, I. G., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1989; pp 33−53.

(7) Del Bene, J. E.; Quantum Mechanical Reaction Enthalpies. In *Molecular Structure and Energetics*; Liebman, J. F., Greenberg, A., Eds.; VCH Publishers: Deerfield Beach, FL, 1986; Vol. 1, Chapter 9.

0002-7863/95/1517-10131$09.00/0

level, are 27 and 105 kcal/mol, respectively. The latter is certainly too high and the former probably too high for these dissociations to be steps in the overall dehydration. The formation of CH_3CHO and H_2O as separate entities is more likely to occur later, following a reversal of the initial activation, i.e., deprotonation and the addition of a hydrogen atom.

Deprotonation of **7** was found to give a hydrogen-bonded hydrate of the formylmethyl radical, **8**, and the subsequent addition of a hydrogen atom was found to give a hydrogen-bonded hydrate of acetaldehyde, **9**. In the reverse order, addition of a hydrogen atom to **7** was found to give a hydrogen-bonded hydrate of protonated acetaldehyde **10**, which upon deprotonation gives **9**. Frequency analyses show that all three hydrates **8**, **9**, and **10**, like **7**, are local minima on the potential energy surface. The values of ΔH^{298} for the dissociation of **8** into $HO^\bullet$ and $HOCH{=}CH_2$, and **10** into H_2O and CH_3CHOH^+ or H_3O^+ and CH_3CHO, calculated at the PMP2(FC)/6-311++G**//MP2-(FC)/6-31G* level, are 39, 21, and 40 kcal/mol, respectively, so these dissociations are also unlikely as steps in the dehydration pathway. On the other hand, the dissociation of **8** into H_2O and $CH_2CHO^\bullet$, or **9** into H_2O and CH_3CHO, requires only 3.7 or 3.5 kcal/mol, respectively, at 298 K, so dissociation would be extremely rapid.



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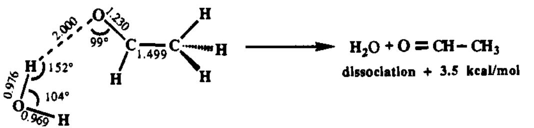

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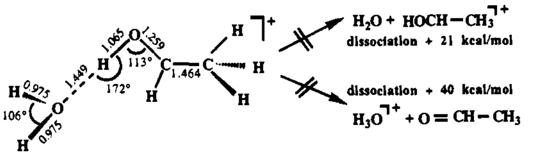

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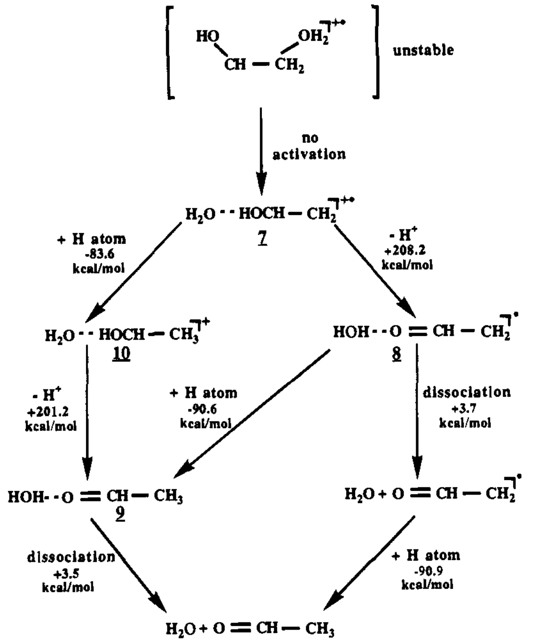
The sequence of reactions following protonation of the 1,2-dihydroxyethyl radical and leading to the production of acetaldehyde via these dissociation steps is set out in Scheme 1. A series of reactions of this kind can be termed a "predissociation" mechanism because only hydrate structures are involved and there is no actual transfer of a *bonded* HO, H_2O, or H_2O^+ group *from one carbon atom to the other*. A very similar mechanism, termed "a collapse of the radical to a radical cation", has been postulated for the decomposition of the 1,2-hydroxyethyl radical generated by HO radical attack in acidic solution,[8] except that in this case dissociation of the H_2O was presumed to accompany the protonation.

Scheme 1



It should perhaps be emphasized at this point that the very large (favorable) enthalpy changes for the reactions with $H^\bullet$ and the very large (adverse) enthalpy changes for the deprotonation noted beside the arrows in Scheme 1 relate to gas-phase processes. In the enzyme-catalyzed dehydration these steps are to be understood as involving $H^\bullet$ *transfer* from the 5′-deoxyadenosyl group of the coenzyme, and H^+ *transfer* to a proton acceptor at the active site on the enzyme, transfer steps that would be characterized by far smaller enthalpy changes.

This study has investigated the effect of protonation on the rearrangement of the free radical substrate in the dehydration of ethane-1,2-diol. The question now arises as to what mechanistic role other than stereoselective[1e] the diol dehydrase enzyme might play in addition to proton donation. Since *gem*-diols are generally accepted as obligatory intermediates in the dehydrase reaction,[1] contrary to Scheme 1, this suggests either that groups at the active site induce a changeover to OH, H_2O, or H_3O^+ transfer from C_2 to C_1 or that an entirely different mechanism is operative, such as a reaction of the substrate radical with Co^{II} giving Co^{I} and the corresponding carbocation in which the OH group is then transferred from C_2 to C_1. Calculations are currently being carried out to determine the energy barrier for this process.

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