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Direct Participation of Potassium Ion in the Catalysis of Coenzyme B_{12}-Dependent Diol Dehydratase[1]

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The direct ion-dipolar interactions between potassium ion (K^+) and the two hydroxyl groups of the substrate are the most striking feature of the crystal structure of coenzyme B_{12}-dependent diol dehydratase. We carried out density-functional-theory computations to determine whether K^+ can assist the 1,2-shift of the hydroxyl group in the substrate-derived radical. Between a stepwise abstraction/recombination reaction proceeding *via* a direct hydroxide abstraction by K^+ and a concerted hydroxyl group migration assisted by K^+, only a transition state for the latter concerted mechanism was found from our computations. The barrier height for the transition state from the complexed radical decreases by only 2.3 kcal/mol upon coordination of the migrating hydroxyl group to K^+, which corresponds to a 42-fold rate acceleration at 37°C. The net binding energy upon replacement of the K^+-bound water for substrate was calculated to be 10.7 kcal/mol. It can be considered that such a large binding energy is at least partly used for the substrate-induced conformational changes in the enzyme that trigger the homolytic cleavage of the Co-C bond of the coenzyme and the subsequent catalysis by a radical mechanism. We propose here a new mechanism for diol dehydratase in which K^+ plays a direct role in the catalysis.

Key words: adenosylcobalamin, coenzyme B_{12}, density-functional-theory computation, diol dehydratase, potassium ion.

Certain enzymes utilize the high reactivity of free radicals to catalyze reactions that are chemically difficult to accomplish by ionic mechanisms (*1, 2*). Such radicals originate from either cofactors or protein radicals. AdoCbl (coenzyme B_{12}), a naturally occurring organometallic compound, is a cofactor for enzymatic radical reactions. AdoCbl-dependent reactions include carbon skeleton rearrangements, amino group migrations, and heteroatom elimination reactions. Diol dehydratase catalyzes the dehydration of 1,2-diols to the corresponding aldehydes (Eq. 1) (*3, 4*).

$$\begin{array}{l} \text{R-CHCH}_2\text{OH} \\ \quad\quad | \\ \quad\text{OH} \end{array} \longrightarrow \text{R-CH}_2\text{CHO} + \text{H}_2\text{O} \qquad (\text{R} = \text{CH}_3,\ \text{H},\ \text{HOCH}_2) \qquad (1)$$

Although a minimal mechanism has been established for AdoCbl-dependent rearrangements (Fig. 1) (*5-8*), the pathway for group X (X=OH on C(2) in the case of diol dehydratase) migration is not well understood. Some B_{12} enzymes, including diol dehydratase, share the absolute requirement for monovalent cations, such as K^+ or others with similar ionic radii (*9*), for catalytic activity, although their exact roles remain obscure. In the crystal structure of diol dehydratase as recently solved by Yasuoka and one of the present authors and their associates, the most striking feature is that both of the hydroxyl groups of the substrate coordinate directly to K^+, which is located in the inner part of the active-site cavity (Fig. 2) (*11*). This novel and surprising structure strongly suggests that K^+ participates directly in the enzymatic reaction.

AdoCbl-dependent enzymatic rearrangements involve the migration of a hydrogen atom from one carbon atom of the substrate to an adjacent carbon atom in exchange for group X, which moves in the opposite direction (*5-8*). As illustrated in the minimal mechanism (Fig. 1), the adenosyl radical introduced into the active site by homolytic cleavage of the Co-C bond of the coenzyme (Fig. 1A) triggers the reaction by activating substrates through abstraction of a hydrogen atom (Fig. 1B). The pathway of group X migration remains obscure. For the diol dehydratase reaction, several proposals concerning the hydroxyl group migration from C(2) to C(1) of the substrate radical have been made based on non-enzymatic model reactions and theoretical calculations (*12-14*). Recent crystallographic analysis of the structure of diol dehydratase in complex with cyano-

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Abbreviations: AdoCbl, adenosylcobalamin; DFT, density-functional-theory.

cobalamin and the substrate 1,2-propanediol revealed that the distances between Co and C(1) and C(2) of the substrate are 8.37 and 9.03 Å, respectively (*11*). This clearly excludes the possibility that Co(II) of cob(II)alamin is involved directly in the conversion of a substrate-derived radical to a product-derived radical. That is, Co(II) is a spectator in the 1,2-shift of the hydroxyl group in the diol dehydratase reaction.

Among the reaction pathways that have been proposed for the hydroxyl group migration from C(2) to C(1), two seem to be consistent with the X-ray structure of the enzyme. In one pathway, it was postulated that the hydroxyl group migrates from C(2) to C(1) *via* a cyclic transition state. In this mechanism, K^+ may serve as a Lewis acid to lower the energy of the transition state. It has been proposed by Golding and coworkers (*12, 13*) that the barrier in the hydroxyl group migration from C(2) to C(1) is reduced by protonation. The second pathway involves a radical anion intermediate. The pK_a values of hydroxyl groups attached to a carbon radical are reported to be ~5 pH units lower than those of the corresponding alcohols (*15*). Formation of such a radical anion intermediate would be favored by K^+ through electrostatic stabilization. This may ensure the deprotonation of the hydroxyl group on C(1) by COO^- of Gluα170. The removal of the hydroxyl group from C(2) is facilitated by the resulting oxyanion on C(1) through σ-π overlapping, as well as by its coordination to K^+. The carbon atom of C=O, which is polarized by interaction with the general acid COOH of Gluα170, undergoes suprafacial nucleophilic attack by the eliminated hydroxide ion. This alternative mechanism is essentially similar to the hypothetical one proposed by Buckel and coworkers (*14*). It is difficult to determine from the three-dimensional structure of the active site which pathway is more likely, although the latter pathway seems to be incompatible with the constant activity of diol dehydratase in the pH range from 6.0 to 10.0 (*3*).

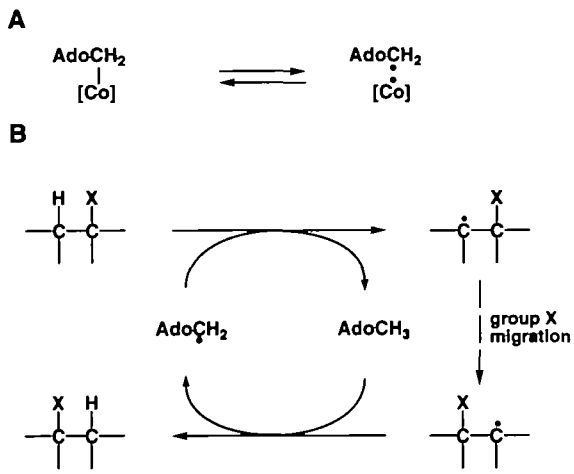

Fig. 1. **The minimal mechanism for coenzyme B_{12}-dependent rearrangements.** A: Homolytic cleavage of the Co-C bond of enzyme-bound AdoCbl. B: Adenosyl radical-catalyzed enzymatic rearrangements. [Co], cobalamin; $AdoCH_2$, adenosyl group; X represents a generic migrating group.

In the present paper, we report the results of DFT computations to determine whether K^+ can assist the 1,2-shift of the hydroxyl group in the substrate-derived radical. Based on the computational results as well as the three-dimensional structure of the enzyme, we propose a new mechanism for diol dehydratase in which K^+ plays a novel, essential role in the enzymatic catalysis.

MATERIALS AND METHODS

DFT Computations—To obtain a better understanding of the role of K^+ in the catalytic function of this B_{12} enzyme, we carried out DFT computations on the hydroxyl group migration from C(2) to C(1) in the 1,2-dihydroxypropyl radical. The role of proton and ammonium ion in the hydroxyl group migration has been well-documented from *ab initio* computations by Golding and coworkers (*12, 13*). They suggested that protonation of the migrating hydroxyl group may lead to a reduction in the barrier height and hence facilitate the rearrangement. Our DFT computations were carried out using Becke's three parameter hybrid functional (*16, 17*) combined with the Lee, Yang, and Parr (LYP) correlation functional (*18*), denoted as B3LYP, implemented with the Gaussian 94 *ab initio* program package (*19*). The triple-zeta 6-311G* basis set of Pople and coworkers (*20*) was used for carbon, oxygen, and

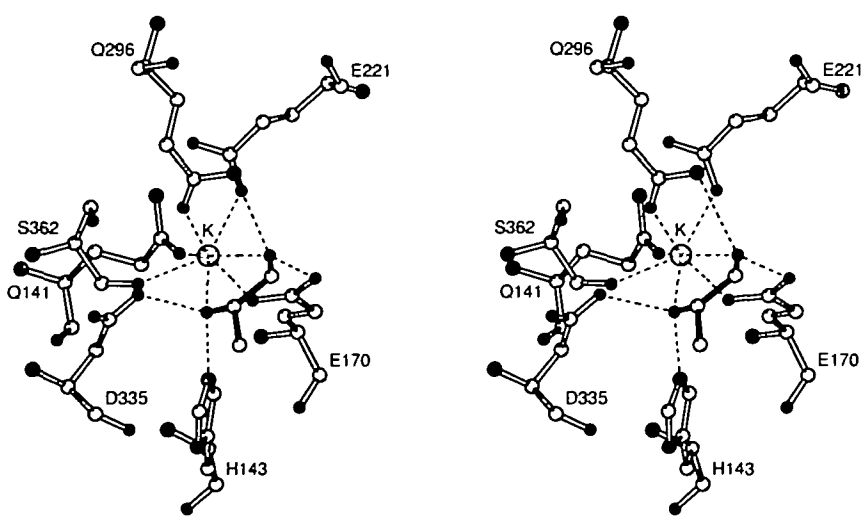

Fig. 2. **A stereoview of the active-site residues interacting with the substrate and K^+.** Drawn with MOLSCRIPT (*10*) using the coordinates obtained by Yasuoka and coworkers (*11*). ○, carbon atom; ●, oxygen atom; ●, nitrogen atom. Bonds in the enzyme and substrate are shown as open and closed sticks, respectively.

hydrogen, and the primitive set of Wachters (*21*) was used for potassium. We performed systematic vibrational analyses and took zero-point vibrational energies into account in making the energy diagram for the reaction pathway.

RESULTS AND DISCUSSION

The Role of Potassium Ion in Enzymatic Catalysis—We considered two kinds of hydroxyl group migrations; the first is a stepwise abstraction/recombination reaction that can proceed *via* a direct OH abstraction by K^+, and the second is a concerted OH migration assisted by K^+. Despite our best efforts, we could not find a transition state for the direct OH abstraction by K^+. Only a transition state for the latter concerted mechanism was found from our DFT computations. The migration of the hydroxyl group requires 20.6 kcal/mol in the absence of K^+ at the B3LYP level of theory, and this electronic process *via* a three-centered transition state is thus energetically unfavorable. Figure 3 presents the potential energy diagram. In the initial stages of the reaction, the substrate replaces the K^+-bound H_2O to form a stable complex with K^+ with a net binding energy of 10.7 kcal/mol. The most notable geometric change in the complexation is the rotation of the dihedral angle of O-C(1)-C(2)-O in the substrate due to the direct ion-dipolar interactions between K^+ and the two hydroxyl groups. The barrier height for the transition state was computed to be 18.3 kcal/mol if measured from the stabilized K^+ complex. A decrease in the barrier height by 2.3 kcal/mol corresponds to a 42-fold rate acceleration at 37°C by coordination of the migrating hydroxyl group to K^+. It seems to us that the hydroxyl group migration still requires high activation energies, but this might not be unreasonable, because the hydroxyl group migration is not a rate-determining step (*22*). Thus, we conclude that the hydroxyl group migration will undergo only a partial rate acceleration by coordination to K^+. If K^+ is assumed not to bind H_2O, catalytic turnovers would be difficult, because dissociation of the product radical complex to a dissociation limit requires high energies. In addition, it should be noted that the relatively large binding energy released upon complexation of the substrate with K^+ would be utilized, at least in part, for the substrate-induced conformational change in the enzyme, as discussed below.

The Proposed Overall Mechanism for Diol Dehydratase—Figure 4 illustrates a new mechanism for diol dehydratase that we propose based on the computational results as well as the three-dimensional structure of the enzyme. Although the crystal structure of the substrate-free enzyme (**1**) has not yet been determined, it is likely that the binding of 1,2-propanediol to the active site converts a hexa-coordinated complex of K^+ into the hepta-coordinated complex (**2**) through coordination of its two hydroxyl groups to K^+ displacing the sixth ligand H_2O (*11*). It has been suggested that this causes a conformational change in the enzyme that triggers homolytic cleavage of the Co-C

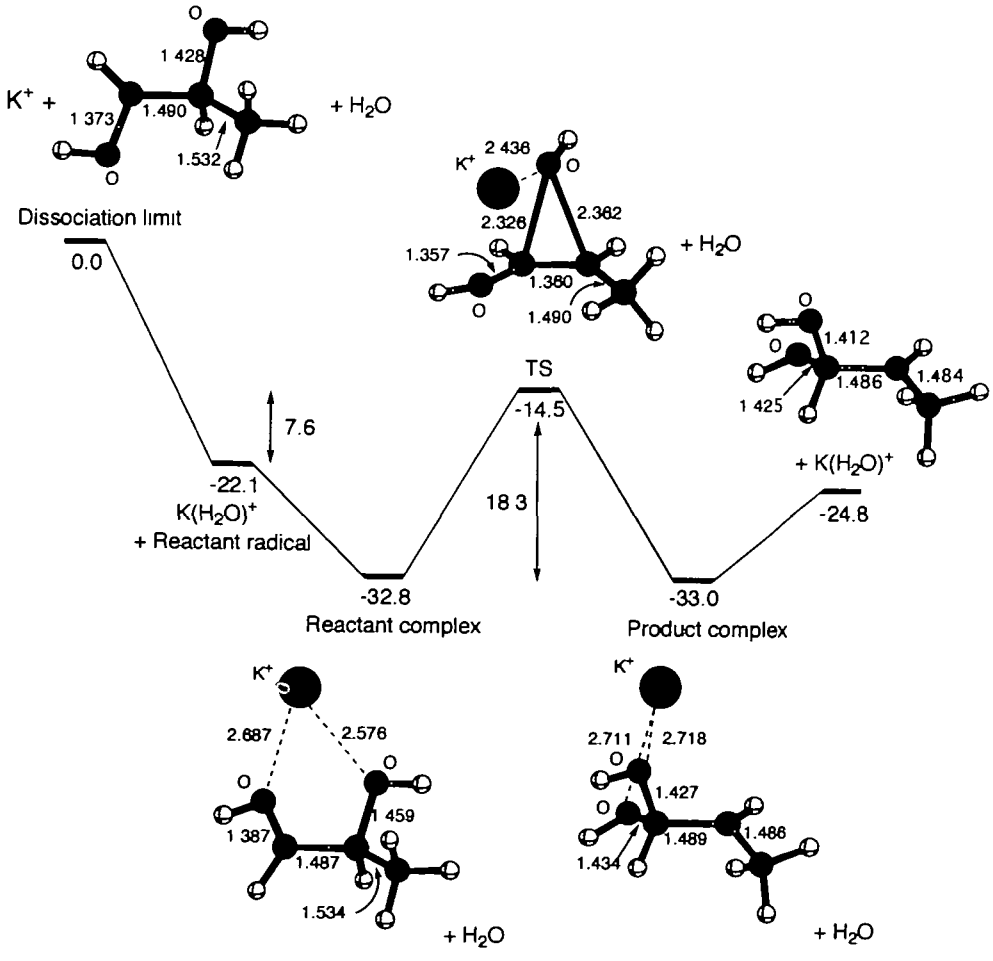

Fig. 3. **Energy diagram for the migration of the hydroxyl group from C(2) to C(1) in the 1,2-dihydroxypropyl radical in the presence of K^+.** Bond lengths in Å and energies in kcal/mol.

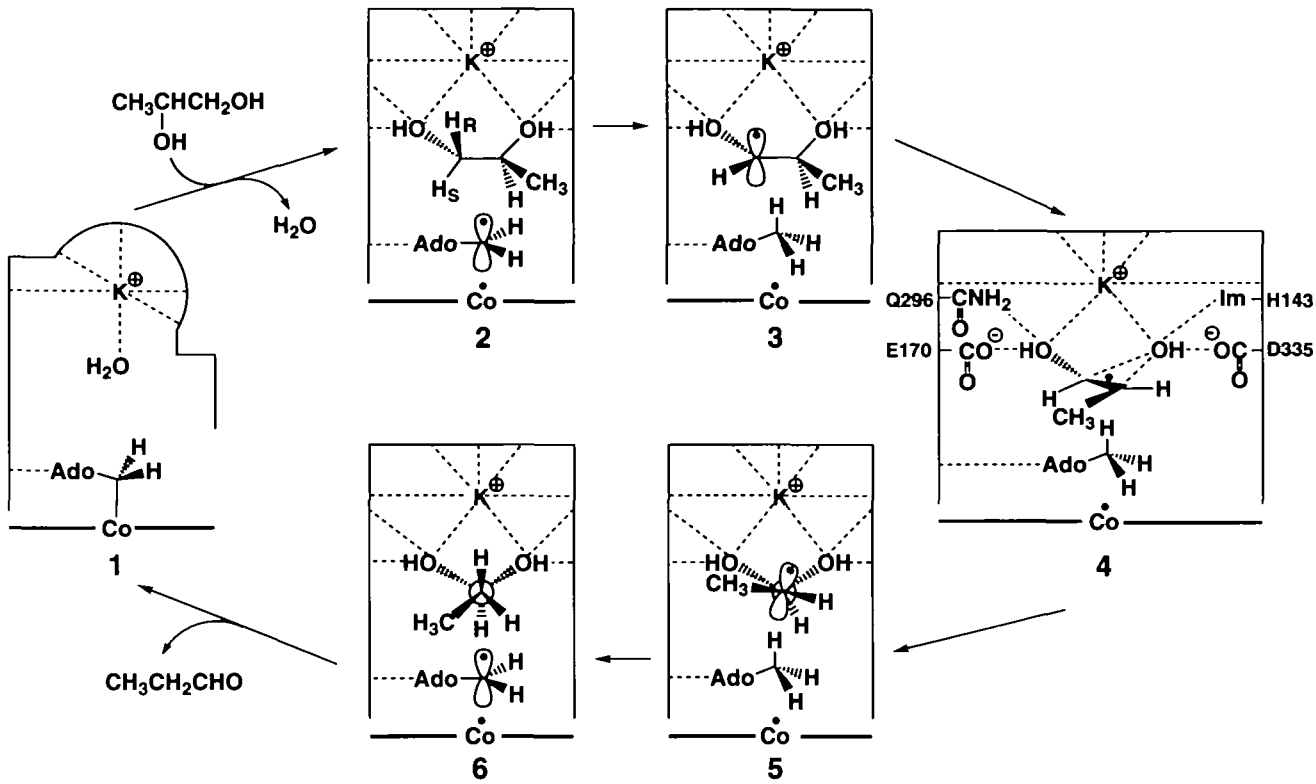

Fig. 4. **The proposed mechanism for diol dehydratase.** The reaction with (*S*)-1,2-propanediol is illustrated. -Co-, cobalamin; AdoCH$_2$, adenosyl group; Im, imidazole group of His143.

bond of AdoCbl, forming an adenosyl radical and cob(II)-alamin. In the presence of substrate, the Co-C bond cleavage undergoes rate acceleration ranging from 10^{11} to 10^{12}-fold. In the absence of substrate, the rate acceleration of the bond cleavage was calculated to be approximately 10^6 to 10^7-fold. Therefore, the substrate enhances the rate of Co-C bond cleavage by a factor of 10^4 to 10^6. This corresponds to $\Delta\Delta G^{\ddagger}$ of 6-9 kcal/mol. Such energy for rate enhancement can be considered to be supplied at least in part by a large binding energy upon complexation of the substrate with K^+ in the active site.

A specific substrate hydrogen atom (*proS* and *proR* hydrogen atoms of the *S*- and *R*-isomers, respectively) (*22*) is abstracted by the adenosyl radical, producing a substrate-derived radical and 5′-deoxyadenosine (**3**). As described above, our computational results indicate that the 1,2-shift of the hydroxyl group in the substrate radical takes place through a cyclic transition state. In this mechanism, it should be noted that the activation energy for the transition state from the complexed substrate radical decreases only partially, and still remains high. Smith *et al.* (*13*) indicated by *ab initio* calculations that the barrier height for the transition state in the hydroxyl group migration can be lowered by partial protonation with NH_4^+. The X-ray structure of the active site reveals that the hydroxyl group on C(2) is hydrogen-bonded to Hisα143 and Aspα335 (Fig. 2). However, a possibility that, in the diol dehydratase reaction, the interaction of the migrating hydroxyl group with the imidazolium ion of Hisα143 might be essential for stabilization of the transition state is less likely, because the rate of diol dehydratase reaction is constant in the pH range from 6.0 to 10.0 (*3*). The hydroxyl group seems to migrate from C(2) to C(1) with hydrogen-bonding to these residues and with the turning of the C(2)-C(1) bond through the transition state illustrated as **4** in Fig. 4. It seems reasonable to assume that the enzyme-bound substrate changes its orientation during the reaction, because the two hydroxyl groups are much more strongly bound to the active site through ion-dipolar interactions as well as hydrogen bonding than the C-C-C backbone of the substrate is bound through hydrophobic or van der Waals interactions.

The *gem*-diol radical (**5**) that is formed abstracts a hydrogen atom back from 5′-deoxyadenosine, producing *gem*-diol and the adenosyl radical (**6**). The *gem*-diol is then dehydrated, producing propionaldehyde and H_2O. Removal of the migrating hydroxyl group would also be facilitated by its coordination to K^+. The product aldehyde loses its affinity for K^+ because of the loss of the hydroxyl groups and is thus released from the active site. A decrease in the coordination number of K^+ would return the conformation back to the substrate-free form (**1**); this accompanies recombination of the adenosyl radical with the Co(II) of cob(II)alamin to regenerate the coenzyme. A bond energy liberated upon the reformation of the Co-C bond ensures the release of the product from the active site and the conformational change in the enzyme to the substrate-free form.

The mechanism proposed here can explain all of the biochemical results obtained so far as well as the stereochemistry of the reaction catalyzed by diol dehydratase (*5, 6*). Our computational results provide strong support for a

novel role of K^+ in the catalytic function of this B_{12} enzyme. We show here that the monovalent cation is not a spectator but an active conductor in the enzyme catalysis.

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REFERENCES

1. Stubbe, J. and van der Donk, W.A. (1998) Protein radicals in enzyme catalysis. *Chem. Rev.* **98**, 705-762
2. Sigel, H. and Sigel, A., eds. (1994) *Metalloenzymes Involving Amino Acid-Residue and Related Radicals*, Vol. 30, Marcel Dekker, New York
3. Lee, H.A. Jr. and Abeles, R.H. (1963) Purification and properties of dioldehydrase, an enzyme requiring a cobamide coenzyme. *J. Biol. Chem.* **238**, 2367-2373
4. Toraya, T., Shirakashi, T., Kosuga, T., and Fukui, S. (1976) Coenzyme B_{12}-dependent diol dehydrase: Glycerol as both a good substrate and a potent inactivator. *Biochem. Biophys. Res. Commun.* **69**, 475-480
5. Toraya, T. (1994) Diol dehydrase and glycerol dehydrase, coenzyme B_{12}-dependent isozymes. *Metal Ions Biol. Syst.* **30**, 217-254
6. Abeles, R.H. (1979) Current status of the mechanism of action of B_{12}-coenzyme in *Vitamin* B_{12} (Zagalak, B. and Friedrich, W., eds.) pp. 373-388, Walter de Gruyter, Berlin
7. Babior, B.M. (1988) The mechanism of adenosylcobalamin-dependent rearrangements. *Biofactors* **1**, 21-26
8. Buckel, W. and Golding, B.T. (1996) Glutamate and 2-methyleneglutarate mutase: From microbial curiosities to paradigms for coenzyme B_{12}-dependent enzymes. *Chem. Soc. Rev.* **25**, 329-337
9. Toraya, T., Sugimoto, Y., Tamao, Y., Shimizu, S., and Fukui, S. (1971) Propanediol dehydratase system. Role of monovalent cations in binding of vitamin B_{12} coenzyme or its analogs to apoenzyme. *Biochemistry* **10**, 3475-3484
10. Kraulis, P.J. (1991) MOLSCRIPT: A program to produce both detailed and schematic plots of protein structures. *J. Appl. Cryst.* **24**, 946-950
11. Shibata, N., Masuda, J., Tobimatsu, T., Toraya, T., Suto, K., Morimoto, Y., and Yasuoka, N. (1999) The new mode of B_{12} binding and direct participation of potassium ion in enzyme catalysis: X-ray structure of diol dehydratase. *Structure* **7**, 997-1008
12. Golding, B.T. and Radom, L. (1976) On the mechanism of action of adenosylcobalamin. *J. Am. Chem. Soc.* **98**, 6331-6338
13. Smith, D.M., Golding, B.T., and Radom, L. (1999) Toward a consistent mechanism for diol-dehydratase-catalyzed reactions: An application of the partial-proton-transfer concept. *J. Am. Chem. Soc.* **121**, 5700-5704
14. Bothe, H., Bröker, G., Müller, U., Schall, I., Textor, S., Golding, B.T., and Buckel, W. (1998) Mechanisms of coenzyme B_{12}-dependent carbon-carbon and carbon-oxygen rearrangements in *Vitamin* B_{12} *and* B_{12}*-Proteins* (Kräutler, B., Arigoni, D., and Golding, B.T., eds.) pp. 237-251, Wiley-VCH, Weinheim
15. Hayon, E. and Simic, M. (1974) Acid-base properties of free radicals in solution. *Acc. Chem. Res.* **7**, 144-121
16. Becke, A.D. (1988) Density-functional exchange-energy approximation with correct asymptotic behavior. *Phys. Rev.* **A38**, 3098-3100
17. Becke, A.D. (1993) Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **98**, 5648-5652
18. Lee, C., Yang, W., and Parr, R.G. (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev.* **B37**, 785-789
19. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Gill, P.M.W., Johnson, B.G., Robb, M.A., Cheeseman, J.R., Keith, T.A., Petersson, G.A., Montgomery, J.A., Raghavachari, K., Al-Laham, M.A., Zakrzewski, V.G., Ortiz, J.V., Foresman, J.B., Cioslowski, J., Stefanov, B.B., Nanayakkara, A., Challacombe, M., Peng, C.Y., Ayala, P.Y., Chen, W., Wong, M.W., Andres, J.L., Replogle, E.S., Gomperts, R., Martin, R.L., Fox, D.J., Binkley, J.S., Defrees, D.J., Baker, J., Stewart, J.J.P., Head-Gordon, M., Gonzalez, C., and Pople, J.A. (1995) in *Gaussian 94*, Gaussian Inc., Pittsburgh, PA
20. Krishnan, R., Binkley, J.S., Seegar, R., and Pople, J.A. (1980) Self-consistent molecular orbital methods. XX. A basis set for correlated wave functions. *J. Chem. Phys.* **72**, 650-654
21. Wachters, A.J.H. (1970) Gaussian basis set for molecular wavefunctions containing third-row atoms. *J. Chem. Phys.* **52**, 1033-1036
22. Zagalak, B., Frey, P.A., Karabatsos, G.L., and Abeles, R.H. (1966) The stereochemistry of the conversion of D and L 1,2-propanediols to propionaldehyde. *J. Biol. Chem.* **241**, 3028-3035