Facilitation of Enzyme-Catalyzed Reactions by Partial Proton Transfer: Application to Coenzyme-B_{12}-Dependent Methylmalonyl-CoA Mutase

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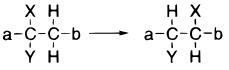
The cofactor adenosylcobalamin (coenzyme B_{12}) is associated with a set of remarkable enzyme-catalyzed transformations in which a group X formally changes places with a hydrogen atom on an adjacent carbon atom:[1]



(1)

Current[2] and previous[3,4] investigations have indicated that rearrangements of this type can be facilitated by substrate protonation. In the present study, we use high level ab initio molecular orbital theory to examine mechanisms that operate with and without protonation, as well as intermediate behavior. Our calculations reveal a continuous spectrum between the two extremes and demonstrate the potential importance of *partial proton transfer*. Although the results presented pertain to a specific B_{12}-dependent rearrangement, we believe that our conclusions may have widespread significance for enzymatic reactions requiring acidic catalysis.

Numerous proposals for the mechanisms of the B_{12}-dependent rearrangements have been put forward and include pathways via intermediate protein-bound free radicals,[5] carbocations,[4,6] carbanions,[7] and organocorrinoids.[8] EPR evidence has recently provided strong support for the mechanistic hypotheses involving free radicals,[9] and it is such intermediates that our calculations have been probing.[2,10] As a model for the radical mechanism in the context of the methylmalonyl-CoA-mutase-catalyzed rearrangement (reaction 1, X = COSCoA, a = b = H, Y = CO_2H), we have investigated the degenerate rearrangement of the 3-propanal radical (Figure 1).[11] This simplification replaces the 1,2-migration of a thioester group that occurs in the biological system with the computationally less demanding 1,2-shift of a formyl group in the model rearrangement.[12] Calculations[13] on this model

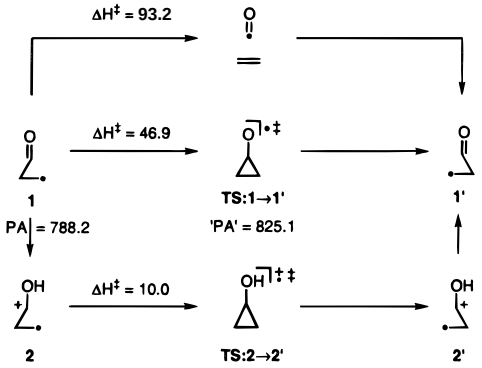
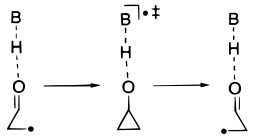


Figure 1. Mechanistic possibilities for the degenerate rearrangement of the 3-propanal radical (relative energies given in kJ mol^{-1}).

system show that an intermolecular fragmentation/recombination mechanism[14] (with a barrier of 93.2 kJ mol^{-1}) requires significantly more energy than alternative intramolecular pathways. Of the intramolecular mechanisms, protonation/deprotonation (barrier 10.0 kJ mol^{-1}) is energetically favored over addition/elimination (barrier 46.9 kJ mol^{-1}) (see Figure 1).

The concept of substrate protonation in the B_{12}-dependent rearrangements, while energetically attractive, carries with it the difficulty of achieving substantial protonation of a weak base with the weakly acidic groups available to enzymes.[15] In the context of the methylmalonyl-CoA-mutase-catalyzed rearrangement, the results of Figure 1 suggest that protonation of the thioester group will facilitate the rearrangement. However, the pK_a of the conjugate acid of a thioester carbonyl oxygen is estimated to be around −6,[16] so that even the strongest conceivable acid in a protein cannot be expected to generate a substantial concentration of protonated substrate.

Owing to the problems associated with mechanisms involving full protonation, we have considered whether *partial proton transfer* would be sufficient to activate the formyl group (and therefore the thioester group) to migration. To investigate such behavior, we have examined the interaction of the 3-propanal radical with three acids (BH = HF, NH_4^+, and H_3O^+ in reaction 2) whose conjugate bases (B = F^-, NH_3, and H_2O) present a



(2)

range of proton affinities (PAs) (F^- = 1556.0, NH_3 = 848.6, and H_2O = 680.1 kJ mol^{-1}).[17] The distance between the acidic proton

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(11) (a) Full details will be reported elsewhere. (b) Another model study of this reaction has been reported in Keese, R.; Dabre, T.; v. Arx, U.; Müller, S.; Wolleb-Gygi, A.; Hirschi, D.; Siljeovic, V.; Pfammatter, M.; Amolins, A.; Otten, T. In *Vitamin B_{12} and B_{12}-Proteins*; Krautler, B., Arigoni, D., Golding, B. T., Eds.; Wiley-VCH: Weinheim, 1998; pp 289−301.
(12) A similar simplification has been found to be a useful approximation in calculations on the 2-methyleneglutarate-mutase-catalyzed reaction.[2]
(13) Unless otherwise noted, all energy comparisons in this paper refer to CBS−RAD calculations at 0 K obtained with B3-LYP/6-31G(d,p) geometries and scaled zero-point energies. For details and leading references, see ref 10.
(14) Beatrix, B.; Zelder, O.; Kroll, F. K.; Orlygsson, G.; Golding, B. T.; Buckel, W. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2398−2401.
(15) See, for example: Thibblin, A.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 4963−4973.
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(17) These species, although not physiologically significant themselves, were chosen to demonstrate how the migration behavior depends on the strength of the interacting acid. On this basis, the amino acids His−H^+ [18] and Lys−H^+ could be expected to show behavior similar to NH_4^+, whereas Asp and Glu should be closer to H_3O^+, and Cys and Tyr closer to HF.

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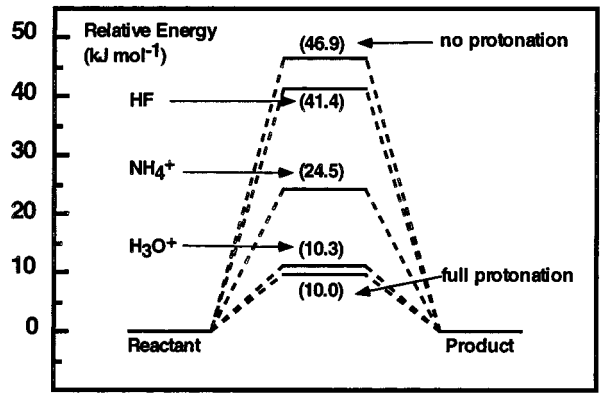

Figure 2. Schematic energy profile for the degenerate rearrangement of the 3-propanal radical showing barriers (kJ mol^{-1}) associated with varying degrees of protonation provided by acids BH (= HF, NH_4^+, or H_3O^+).

and the carbonyl oxygen of the 3-propanal radical is indicative of the degree of proton transfer to oxygen. We find that this distance decreases across the acid series from infinity (no protonation), to 1.727 Å (HF), 1.503 Å (NH_4^+), 1.046 Å (H_3O^+), and 0.976 Å (full protonation).

The most striking consequence of the transition from nonprotonation to complete protonation of the carbonyl oxygen of the 3-propanal radical is the monotonic lowering of the barrier to migration of the formyl group (see Figure 2). As might have been expected from the barriers in the extreme cases (Figure 1), a greater degree of proton transfer is associated with a lower barrier to rearrangement. The acidity of H_3O^+ is sufficient to result in a barrier (10.3 kJ mol^{-1}) virtually identical to that calculated for full protonation, whereas the barrier with HF as the acid (41.4 kJ mol^{-1}) shows that even a small amount of proton transfer can result in a significant decrease in the barrier for migration. With the ammonium ion, the moderately high proton affinity of ammonia maintains the relatively strong binding of the proton while allowing sufficient proton transfer to facilitate the rearrangement, to the extent that the barrier is reduced to 24.5 kJ mol^{-1}. In the context of enzymatic catalysis, this situation might be regarded as ideal since significant barrier lowering can be achieved without deprotonation of the enzyme.

The lowering of a reaction barrier by protonation is equivalent to saying that the transition structure interacts with the proton more favorably than does the reactant. For example, the energy of the transition structure (**TS:1→1′**) is lowered by 825.1 kJ mol^{-1} upon protonation while the 3-propanal radical (**1**) has a proton affinity of 788.2 kJ mol^{-1}. The difference between these two energies of 36.9 kJ mol^{-1} is exactly the reduction in barrier associated with protonation. The same concept applies to partial protonation. That is, the gas-phase hydrogen bond between the 3-propanal radical and NH_4^+ is quite strong (96.9 kJ mol^{-1}), despite the fact that the proton transfer between donor and acceptor is described by a *single*, *asymmetric* energy well. However, the 22.3 kJ mol^{-1} lowering of the rearrangement barrier (corresponding to a rate increase of ca. 5 orders of magnitude) by NH_4^+ comes not from the strength of this hydrogen bond but from the fact that the interaction between NH_4^+ and the transition structure (119.2 kJ mol^{-1}) is 22.3 kJ mol^{-1} stronger than its interaction with the reactant, due to the higher "proton affinity" of the former species.

In an enzymatic reaction facilitated by protonation, the proton-accepting site will generally carry some small amount of negative charge, making it a good candidate for binding to a proton donor in the protein via a hydrogen bond. If such a hydrogen bond exists and remains intact during the course of the reaction, then regardless of the strength of the H-bond donor, the barrier will be lowered simply because the transition structure interacts with the proton more strongly than does the substrate. Enzymes may therefore utilize substrate hydrogen bonding for both binding *and* catalysis.[18] The transition from a "weak" hydrogen bond to a "short-strong" hydrogen bond is continuous, and regardless of where a particular H-bonding interaction happens to lie on this scale, there will be a contribution to the lowering of the barrier made by partial proton transfer.[20] Our thesis is simple: *any reaction that is facilitated by protonation will also be facilitated (to a moderated extent) by the partial proton transfer that enzymatic hydrogen bonding can provide.*

In summary, we note that the present study has both specific and general implications. The degenerate rearrangement of the 3-propanal radical (as a model for the methylmalonyl-CoA-mutase-catalyzed reaction) is an example of a reaction whose barrier is lowered substantially by protonation (Figure 1). Application of the idea of partial proton transfer to this reaction demonstrates that complete protonation is not necessary to obtain a significant amount of proton-induced barrier lowering. We believe that these findings may provide some clue as to the ability of enzymes to catalyze certain categories of reactions. If a reaction is facilitated by protonation and the proton-accepting site is H-bonded to the enzyme, there will be a contribution to the lowering of the barrier made by partial proton transfer.[22,23] It is possible to view protonation as a "source" of energy for those reactions whose barriers are lowered by it. It would seem curious under such circumstances for nature not to tap this source, at least to some degree. We submit that such a tapping mechanism is provided by partial proton transfer.

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(18) In methylmalonyl-CoA mutase, the crystal structure[19] suggests that His^{A244} is in a position potentially to bind the carbonyl by means of a hydrogen bond and hence provide partial protonation for catalysis.

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(20) Recently, there has been much debate as to whether "low-barrier" hydrogen bonds (LBHBs) or "short strong" hydrogen bonds (SSHBs) can be important in enzymatic catalysis.[21] A SSHB is merely a specific case of partial proton transfer. Our results show that it is not necessary to have a centralized proton or matched donor and acceptor pK_as to gain significant rate enhancement through enzymatic H-bonding.

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(22) Various factors (e.g., solvent) may of course reduce the importance of this effect to an extent dependent on the surrounding environment. Interestingly, the crystal structure[19] of methylmalonyl-CoA mutase has shown that the active site is deeply buried and largely inaccessible to solvent, suggesting that partial proton transfer should be quite efficient in this protein.

(23) Further examples of reactions facilitated by protonation are the elimination processes catalyzed by diol dehydratase, for which our observations regarding partial proton transfer are similar to those made in the current study.[24]

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