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TOWARDS THE UNIFICATION OF COENZYME B_{12}-DEPENDENT DIOL DEHYDRATASE STEREOCHEMICAL AND MODEL STUDIES: THE BOUND RADICAL MECHANISM

RICHARD G. FINKE *, DAVID A. SCHIRALDI and BARBARA J. MAYER
Department of Chemistry, University of Oregon, Eugene, Oregon 97403 (U.S.A.)
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A. INTRODUCTION

The reactions catalyzed by the adenosylcobalamin (coenzyme B_{12})-dependent enzymes [1] such as diol dehydratase, ethanolamine ammonia lyase, or methylmalonyl-CoA mutase are noted for their diverse stereochemical requirements [2,3]. For the reaction catalyzed by diol dehydratase (eqn. 1), early stereochemical and ^{18}O labeling studies [3] occupied a special place in subsequent mechanistic B_{12} chemistry. They provided evidence that was interpreted as requiring the formation of the *gem*-diol (eqn. 1) via cobalt-assisted OH migration followed by its stereospecific dehydration.

$$\underset{\text{R CH(OH)–CH}_2\text{(OH)}}{\text{OH OH}} \xrightarrow{\text{B}_{12}\text{-dependent diol dehydratase}} \text{RCH}_2\text{CHO} + \text{H}_2\text{O} \qquad (1)$$

$$\text{R CH(OH)–CH}_2\text{(OH)} \rightarrow [\text{RCH}_2\text{CH(OH)}_2] \rightarrow \text{RCH}_2\text{CHO} + \text{H}_2\text{O}$$

gem-diol intermediate

* Author to whom correspondence should be addressed.

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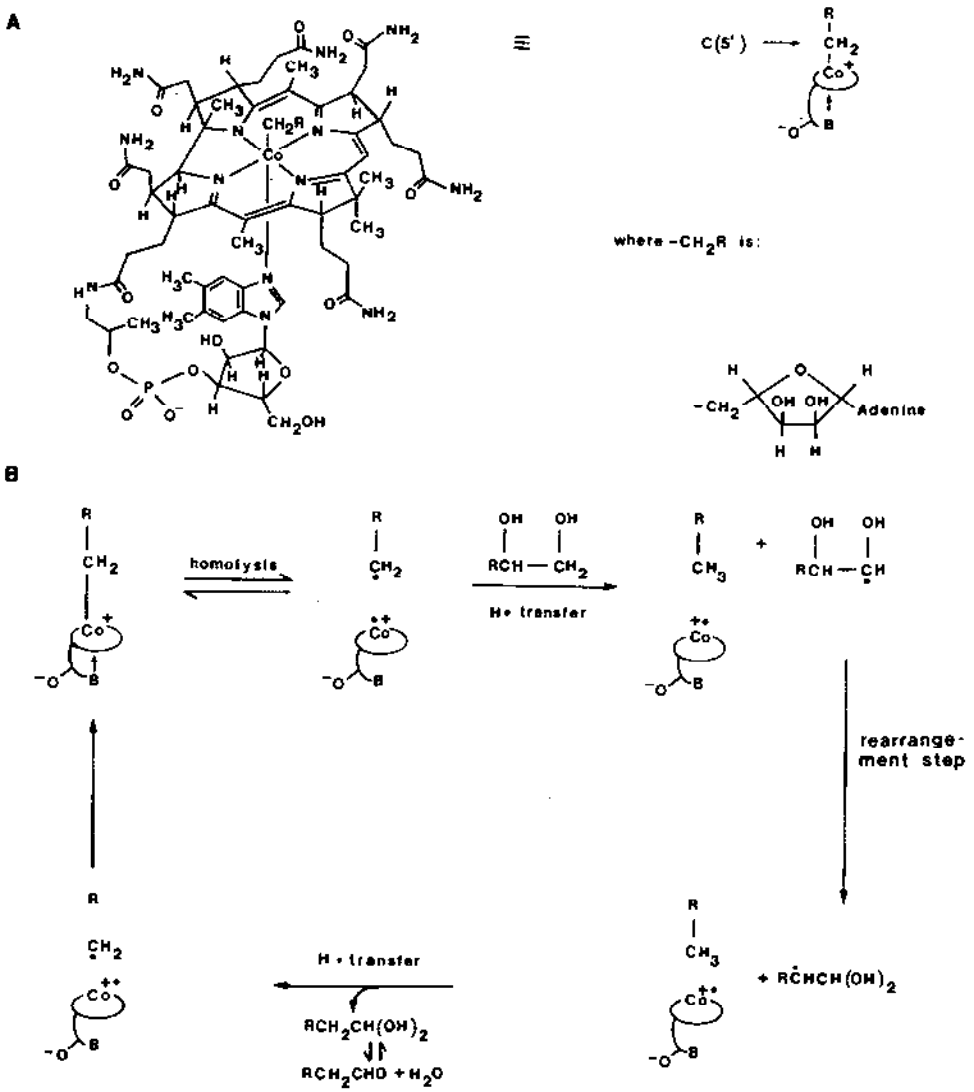
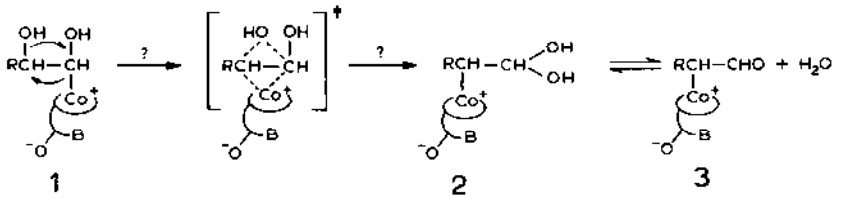


Scheme 1. (A) The structure of adenosylcobalamin (coenzyme B_{12}). (B) The most frequently cited mechanism of action for coenzyme B_{12}-dependent diol dehydratase.

Additional studies of diol dehydratase then followed that provided the well known, but occasionally neglected [4], evidence for homolysis [5] of the Co–C(5′) bond and participation of the C(5′) center as an obligatory H · transfer site [6] (steps 1 and 2, Scheme 1).

With the Co–C bond homolysis, H · transfer step and the stereochemical requirements all apparently in place, recent theoretical [7], pulse radiolysis [8], Co(III)–π complex [9] and other model studies [10,11] have generally

focused on the putative intermediate **1**, with the hope of ultimately demonstrating the cobalt-assisted OH migration rearrangement step (eqn. 2)



1 2 3 (2)

It is important to note that there is no convincing evidence for **1**, only that it was apparently necessary to explain the early diol dehydratase ^{18}O labeling and stereochemical studies. In fact, all attempts to detect the putative intermediate **1** using the enzyme have failed and the available ESR evidence argues against the formation of **1**, the data being consistent with a Co · and substrate R · interacting at distances > 10 Å [12a,b].

Recent model and pulse radiolysis studies have provided the first preparations of **1**. Our model study [11] showed unequivocally that **3** is not on the reaction pathway thereby ruling out the often-cited but unverified π-complex mechanism (eqn. 2) in the model system. Furthermore, the model study agreed with pulse radiolysis studies [8a] that used B_{12}, showing that the formation of base-on **1** yields only a side reaction to $HOCH_2CHO + Co(I)$ but no rearrangement product CH_3CHO, i.e. participation by base-on cobalamin is not only unnecessary, it is a hindrance! Under conditions free of axial 1,5,6-trimethylbenzimidazole, the model study demonstrated a 95% CH_3CHO yield and provided mechanistic evidence consistent with homolysis of the weak Co–C bond [13] in **1** followed by a simple, free radical fragmentation reaction without cobalt participation; $HO\dot{C}HCH_2OH \rightarrow H_2O + OHCCH_2\cdot$, then $OHCCH_2\cdot + S{-}H$ ($S{-}H = HOCH_2{-}H$ solvent) $\rightarrow CH_3CHO + S\cdot$. The lack of cobalt participation even when the Co–C bond in **1** was preformed seemingly inviting such participation is noteworthy and is consistent with eight studies [14] of $HO\dot{C}HCH_2OH$ and related α-hydroxy radicals. These studies show that α-hydroxy radicals are up to 10^5 times more acidic than the corresponding alcohols and rapidly undergo a base or acid catalyzed fragmentation to $\cdot CH_2CHO + H_2O$; $HO\dot{C}HCH_2OH + HO^- \rightleftharpoons H_2O + {}^-O\dot{C}HCHOH \rightarrow OHCCH_2\cdot + OH^-$; $HO\dot{C}HCH_2OH + H^+ \rightleftharpoons HO\dot{C}HCH_2\overset{+}{O}H_2 \rightarrow OHCCH_2\cdot + H^+ + H_2O$.

The question remains, however, of the relevance of the model studies and such α-hydroxy radical chemistry to diol dehydratase, where stereochemical studies require some type of *gem*-diol intermediate, for example. The literature indicates that there is confusion, at least among some segments, regarding the interpretation of the stereochemical studies, the *gem*-diol inter-

mediate, the relevance of α-hydroxy radical chemistry, and the requirement or non-requirement of the cobalt-assisted rearrangement step (eqn. 2). For example, all of the focus [4,7–9] on the putative intermediate **1**, generally without mention of the protein, is, by itself, convincing evidence that it is generally thought that the B_{12} cofactor or intimate, "organic" mechanism is responsible for the stereochemistry of the rearrangements. This is in spite of available literature such as Arigoni's concluding statement [10a] that "many of the stereochemical details may well be determined by local circumstances at the active site rather than represent a compulsory mechanistic requirement of the underlying basic type of reaction" or Golding's statement [10b] that, "the terminology, 'free radical mechanism', in the context of AdoCbl-dependent rearrangements is simplistic, for it implies the dissociation of radical intermediates from the enzyme, which is not envisaged by its proponents. It is presumed that the radicals are bound by the protein...". The neglect of the proteins role in B_{12} chemistry occurs in spite of the fact that tight substrate–enzyme binding is a crucial component of major concepts in enzymatic catalysis such as transition state binding induced fit, or induced strain.

It is not surprising that non-experts find mechanistic B_{12} chemistry confusing given the voluminous, often conflicting and/or controversial nature of the B_{12} literature [15c], the incorrect statements in some texts [15d,e] and the fact that some of the most important stereochemical experiments are available only in a specialized volume of the proceedings of a 1979 meeting of B_{12} scientists [1a,3]. Thus on one hand, at least a few mechanistic B_{12} scientists are beginning to perceive a clarification and simplification of the mechanism of action of some of the B_{12}-dependent rearrangements, with the Co–C(5′) bond of coenzyme B_{12} simply serving as nature's source of protein-bound C(5′) radicals. The resultant stereochemistry of the H· abstraction step, the formation of the *gem*-diol, and the stereochemistry of the dehydration step are all determined by the protein in this simplified picture, although this view is by no means generally accepted at the present time. On the other hand, citations in the literature indicate that confusion continues to exist about: (a) whether only an HO-migration or maybe also an HO- (or H_2O^+-) fragmentation/recombination mechanism is consistent with the stereochemical data; (b) whether or not the "crucial intermediate in B_{12} chemistry is a Co(III)–π complex" [15d,h]; (c) whether or not a π-complex mechanism can explain the stereochemical data [15e]; and (d) whether the observed stereochemical data in the related B_{12}-dependent ethanolamine ammonia-lyase requires "complete stereospecificity" [15f] or just stereoselectivity. Claims continue to appear that (e) the bound diol intermediate **1** "must exist" [15d] or is a "solidly based" [4] point, and (f) that the vicinal OH migration is "difficult to reconcile with chemical experience" [4] or that

model studies are imperfect as they have not demonstrated the "hydroxyl migration as seen in the enzyme-catalyzed reaction" [15g].

The purpose of the present review is to try and remove the confusion by examining both the early and the more recent stereochemical studies in light of recent model studies [11] and the relevant free radical literature with the goal of obtaining a simplified and also a unified mechanism of action for diol dehydratase. The results, presented herein, show that in fact the mechanism alluded to or suggested by many B_{12} workers including Arigoni [16], Abeles and co-workers [17], Babior [2a], Retéy and co-workers [2b] and Golding [1g] is fully consistent with all of the available stereochemical model, and chemical (radical) mechanistic data relevant to diol dehydratase. The recent works by Arigoni [16], Golding [1g], Babior [2a], and Rétey and co-workers [2b] are especially recommended, as are the comments of Abeles [17b], who discovered diol dehydratase, and who noted from the beginning that "highly oriented" [17a] tightly bound radicals were probably involved in diol dehydratase. We emphasize that with the proposed name, the "bound radical mechanism", no claim of originality for this proposed mechanism is being made, although we believe that the simple change from a *free* radical to a *bound* radical nomenclature is significant and will help avoid future confusion. In what follows, (a) a more detailed, speculative version of the bound radical mechanism is proposed, (b) a reason for the apparently inefficient HO-migration followed by its elimination as H_2O ($+CH_3CHO$) is presented, (c) the stereoselective dehydration activity of diol dehydratase is accounted for by the postulation of acidic and basic sites, and (d) the apparent stereoselectivity and not stereospecificity in the early stereochemical studies using propanediol is pointed out, a result that requires a mechanism other than a stereospecific, cobalt-assisted migration mechanism, or a concerted stereospecific migration of a protonated hydroxyl group.

B. CONSTRUCTION OF A WORKING MODEL OF THE ACTIVE SITE OF DIOL DEHYDRATASE

(i) Cofactor binding

The structure of the adenosylcobalamin cofactor (see Scheme 1) has been well known since its X-ray diffraction structural analysis in 1961 [18]. As the result of a number of studies of chemically modified cofactor analogues [19], it is now known that the cofactor is bound to the apoprotein at the amide side chains, the adenine or sugar of the "upper" 5′-deoxyadenosyl moiety, and at the appended or "lower" sugar and/or the 5,6-dimethylbenzimidazole. Clearly, the coenzyme is tightly held [20] ($K_{\text{Association}}$ = ca. 10^5) as schematically shown in Fig. 1, so that following homolysis of the Co–C(5′) bond, the resultant Co(II) and C(5′) radicals must be bound and are not free

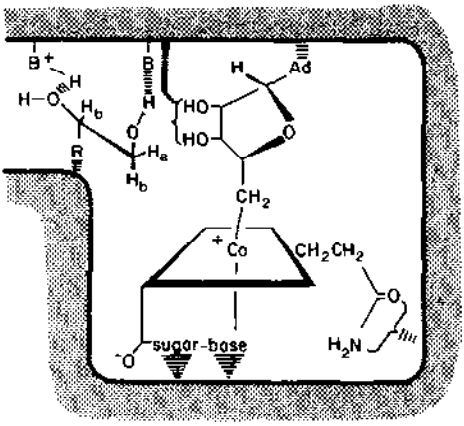


Fig. 1. A schematic, working model of the active site of diol dehydratase. The hatched lines indicate probable protein–cofactor or protein–substrate binding sites based on modified cofactor or stereochemical studies, respectively.

radicals. Evidence for the substrate binding as shown in Fig. 1 will be developed in the next section.

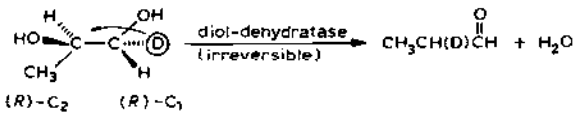
(ii) Stereochemical studies and substrate binding

Some impressively thorough and elegant stereochemical studies on diol dehydratase [3,16] have revealed detailed information on how the diol substrate and resultant radicals must be bound at the active site. It is not our purpose nor necessary to present herein all the stereochemical studies, as they have been summarized recently [3,16]. Rather, the key findings will be presented with emphasis upon points or interpretations where ambiguities still exist.

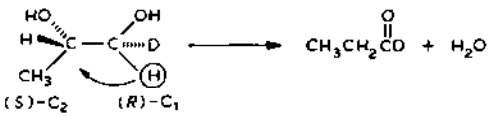
Early studies in 1966 by the laboratories of Abeles [17] (eqns. 3 and 4), and Arigoni [3] (eqns. 5 and 6), demonstrated that both (*R*)- and (*S*)-propanediol are accepted by the enzyme, that the stereochemistry at C_2 controls which H or D is transferred from C_1 (eqns. 3 and 4), and that there is net inversion of configuration at the carbon (C_2) to which H or D migrates (eqns. 5 and 6). The % e.e. (enantiomeric excess) of the reactions in eqns. 3–6 were not, or could not *, be established, however. Both groups interpreted these studies as evidence for a three-point attachment of the substrate to the protein and Fig. 2, due to Arigoni [3,16], shows how such a three-point binding could present different hydrogens, H_R and H_S, to the C(5′) position

* Loss of deuterium from the α position of α-d_1-propionaldehyde occurred during distillation preventing the determination of the % e.e. in the reaction. Kinetic isotope effect arguments presented on p. 3032 of ref. 17 appear to set the observed stereoselectivity at ≥ 88%, but do not demand stereospecificity (100%).

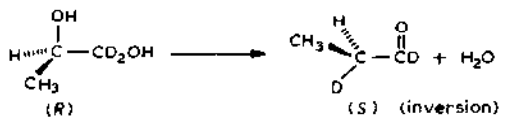
of the cofactor for activation. The studies of Moore and Richards [21] on butanediols which have methyl groups in the positions occupied by the



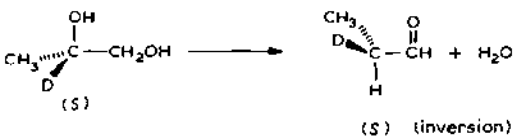
(3)



(4)



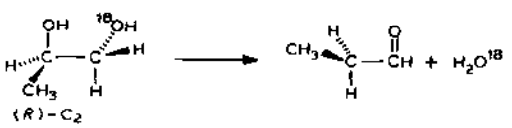
(5)



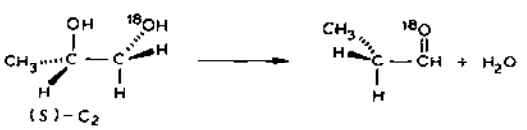
(6)

hydrogens abstracted in the propanediols provide further support for a binding of substrates as shown in Fig. 2.

Labeling experiments employing ^{18}O appeared in 1966 and proved central to subsequent mechanistic thought about the rearrangements [3]. These ^{18}O studies (eqns. 7 and 8) clearly show that the configuration at C_2 influences whether the ^{16}O or ^{18}O is lost.



(7)



(8)

	% ^{18}O Content		% Retention [a]
	Reactant	Product	
(*R*)-1-^{18}O-propan-1,2-diol	12	1	8 ± 4
(*S*)-1-^{18}O-propan-1,2-diol	9	8	88 ± 7
(*R*,*S*)-2-^{18}O-propan-1,2-diol	12.8	5.5	43 ± 4

[a] Error bars based on the published statement of ±0.5% precision in the mass spectrometric analysis.

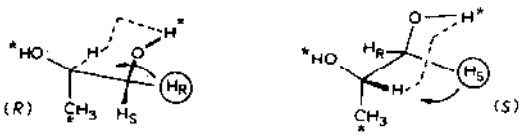
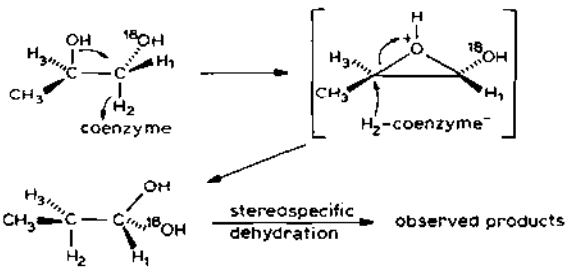


Fig. 2. Binding of propanediol at the active site of diol dehydratase. Protein–substrate binding sites are shown with a star.

These studies were interpreted at the time, 6 years before the evidence for radicals in diol dehydratase was obtained [5], as cofactor-assisted OH migration to the *gem*-diol, followed by its stereospecific dehydration (eqn. 9).



(9)

From 1966 until now, cobalt-assisted OH migration to the *gem*-diol, i.e. intermediate **1** and eqn. (2), have dominated the mechanistic picture of the rearrangement step. However, with a knowledge of the involvement of radicals and 16 years of hindsight, it can now be seen that the ^{18}O-labeling studies (eqns. 7 and 8), really only demand stereoselectivity and not stereospecificity in these reactions. Stated differently, the observation of stereoselectivity in reactions (7) and (8) requires a different mechanism than the cobalt coenzyme-assisted, stereospecific one shown in eqn. 9. Moreover,

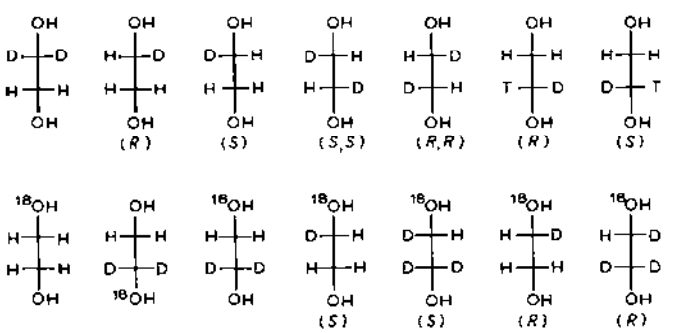
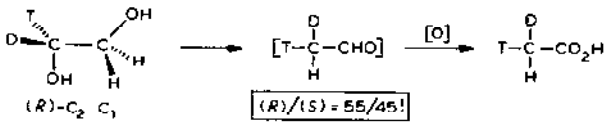
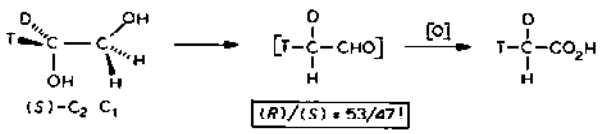


Fig. 3. Labeled ethylene glycol substrates used by Arigoni [16] to elucidate the mechanism of action of diol dehydratase.

Arigoni and co-workers have exhaustively utilized the stereochemical technique and the 14 labeled ethylene glycol substrates (Fig. 3), in a remarkable series of experiments to greatly clarify our understanding of diol dehydratase [16]. One of the most informative experiments was the use of (*R*)- or (*S*)- D and T labeled ethylene glycol. The results (eqns. 10 and 11) show that the stereoselectivity exhibited at C_2 for propanediol disappears when the sterically more demanding methyl group ("methyl binding handle") of propanediol is replaced by the H present in ethylene glycol.

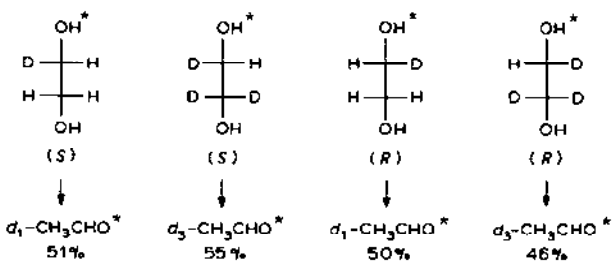


(10)



(11)

When the ^{18}O-labeling experiments were carried out to test the stereochemistry at the other carbon, C_1, the results showed random (statistical) retention of the ^{18}O label (eqn. 12) in spite of the $k_H/k_D = 10 \pm 1$ isotope effect imposing stereoselectivity on the preceding H · abstraction step. A control

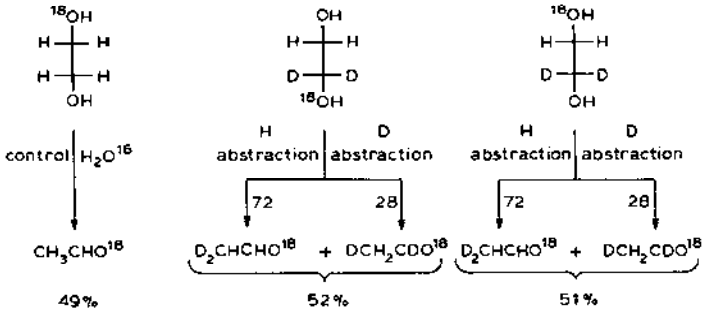


(12)

reaction showed that the solvent H_2O was not exchanging with the acetaldehyde released into solution and the use of dideuterated, ^{18}O-labeled glycols provided evidence demanding* the formation of a *gem*-diol prior to its dehydration to acetaldehyde and H_2O (eqn. 13).

* Arigoni states (ref. 16, p. 404) that, "the data are conclusive in showing that migration of the hydroxyl group to the electron-deficient center created by hydrogen abstraction is a compulsory feature of the reaction mechanism". We understand this to mean, and the results to require, that a concerted OH group migration is not demanded but rather, that $\cdot CH_2CH(OH)(^{18}OH)$ formation (see ref. 16, p. 407, Fig. 20) and/or $CH_3CH(OH)(^{18}OH)$ formation is required prior to dehydration and release of $CH_3CHO(CH_3C^{18}O)$ from the enzyme.

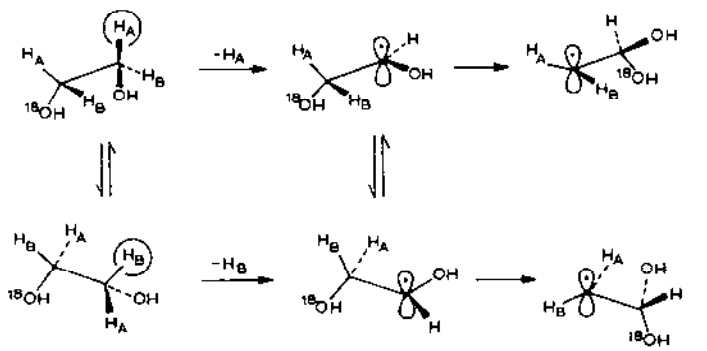
These labeling results (eqns. 10–13), require racemization pathways at both



(13)

C_1 and C_2 of ethylene glycol in any proposed diol dehydratase mechanism.

For racemization at C_1 there are two possibilities: (1) the formation of a chiral ^{18}O-labeled $CH_3CH(OH)(^{18}OH)$ intermediate that spontaneously and non-stereospecifically dehydrates in solution; or (2) a mechanism that results in the formation of a racemic *gem*-diol. Noting that the first possibility is "very improbable" due to "the well certified dehydratase activity of the enzyme" (the stereoselectivity shown in the ^{18}O-labeling studies with propanediols), Arigoni [22] chose the second alternative and presents the following (eqn. 14)



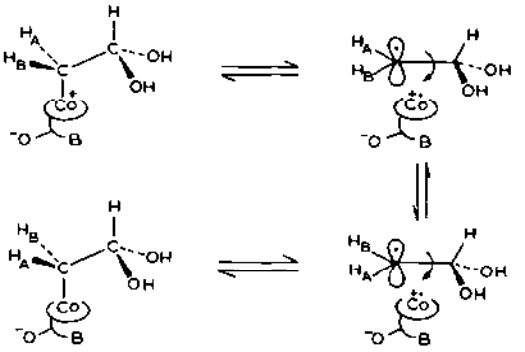
(14)

In the next section on the bound radical mechanism, one part will simply expand eqn. 14 to make it consistent with the known, ca. 10^5 greater, acidity and rapid fragmentation chemistry of α-hydroxy radicals, $HOCH_2\dot{C}HOH$ + base → HO^- + · CH_2CHO + H base$^+$.

To account for the observed racemization at C_2, Arigoni writes the cobalt assisted chemistry shown in eqn. (15) [22].

Since we now have model precedent demonstrating that cobalt participation is not only unnecessary but can introduce a rearrangement-inhibiting side reaction [11b,c], eqn. 15 can be updated by simply leaving out cobalt and retaining its right-hand side showing only rotation about the C–C bond

to affect racemization at C_2.



(15)

Using the other 14 labeled ethylene glycol substrates, Arigoni [16] came to five additional conclusions, summarized below.

(1) At the active site, the substrate partitions rapidly between two binding arrangements which are energetically equivalent, or nearly so, and correspond to the binding modes of (*R*)- and (*S*)-propanediol, respectively.

(2) After fixation to the enzyme, no equilibration is possible between the two homotopic halves of the substrate which undergo regiospecific attack by the cofactor.

(3) Different enantiotopic H atoms are offered to the attack of the cofactor in the two binding arrangements.

(4) In the absence of isotopic label, 4 out of 5 molecules in the enzyme–substrate complex undergo H · abstraction prior to dissociation, and they react with $k_R = k_S$.

(5) Since the influence of deuterium substitution on the v_{max} of the reaction has a value $k_H/k_D = 2.6$, the hydrogen-abstracting step with its $k_H/k_D = 10 \pm 1$ cannot be the rate-determining event of the overall reaction sequence.

From these stereochemical studies of diol dehydratase showing that the substrate is initimately bound at the active site by the protein, a working model of this active site emerges (Fig. 1). Substrate binding appears to be primarily a two-point attachment via the two hydroxyl groups, a view reinforced by noting that inhibitors also require two hydroxyl groups [23]. In the case of propanediol, additional restriction to rotation due to the sterically more demanding methyl group ("methyl group binding") in key radical intermediates will also be postulated as part of the "bound radical mechanism".

C. THE BOUND RADICAL MECHANISM

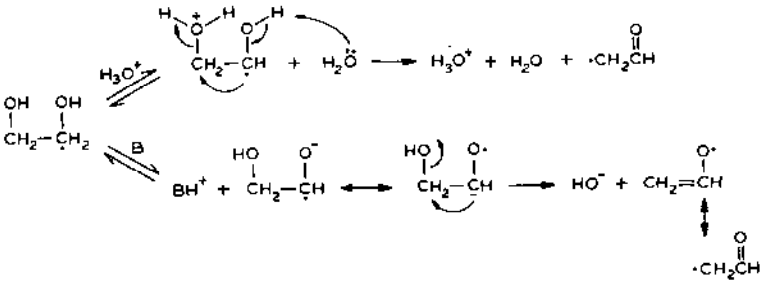
Prior to presenting this mechanism, the key pieces of experimental evidence that any proposed diol dehydratase mechanism must accomodate are summarized.

(1) Studies of chemically modified forms of the B_{12} cofactor showing that the cofactor is tightly bound to the protein and, therefore, that the radicals produced by Co–C bond homolysis are also bound.

(2) Stereochemical studies of labeled ethylene and propylene glycols requiring substrate binding to the protein through the two OH groups and, therefore, that the radicals produced by H · abstraction from the substrate are bound. Complete racemization at both carbons of ethylene glycol yet only partial racemization and thus stereoselectivity at both C_1 and C_2 for propylene glycol emphasize the greater rigidity and less rotation in the bound radical intermediates formed from propylene glycol.

(3) Independent chemical model and pulse radiolysis studies which both show that the often-cited, cobalt-bound intermediate $Co–CH(OH)CH_2OH$ (**1**, eqn. 2) and subsequent cobalt participation are not only unnecessary, they lead to a side reaction producing Co(I) and $OHCCH_2OH$ in the case of base-on cobalt participation. Additional arguments against cobalt participation in the rearrangement steps are the lack of any evidence, in spite of many searches, for the formation of $Co–CH(OH)CH_2OH$ (**1**, eqn. 2) and the stability towards homolysis and thus kinetic non-competence (assuming negligible enzyme effects) of all known [9,11b,11c] $Co–CH_2CH(OH)_2$ and $Co–CH_2CHO$ intermediates (**2** and **3**, eqn. 2).

(4) Numerous studies of α-hydroxy, $HOCH_2\dot{C}HOH$, radicals showing that they are ca. 10^5 more acidic than their corresponding alcohols and that they undergo rapid acid and base catalyzed β-OH cleavage (H_2O loss) reactions.



It is relatively easy to postulate the bound radical mechanism in its general form (Fig. 4) consistent with all of the above modified coenzyme, stereochemical, chemical model, and known radical chemistry data. Refinement of minor, if not major, details of this mechanism may prove necessary however, i.e. it is somewhat speculative. It consists of dual modes of substrate binding (only one of which is illustrated in Fig. 4 for clarity), with different, enantiotopic hydrogens H_A and H_B being offered to the coenzyme's C(5′) position. Molecular models show that if the two OH binding sites are essentially fixed, the interconversion of the two binding modes is basically a

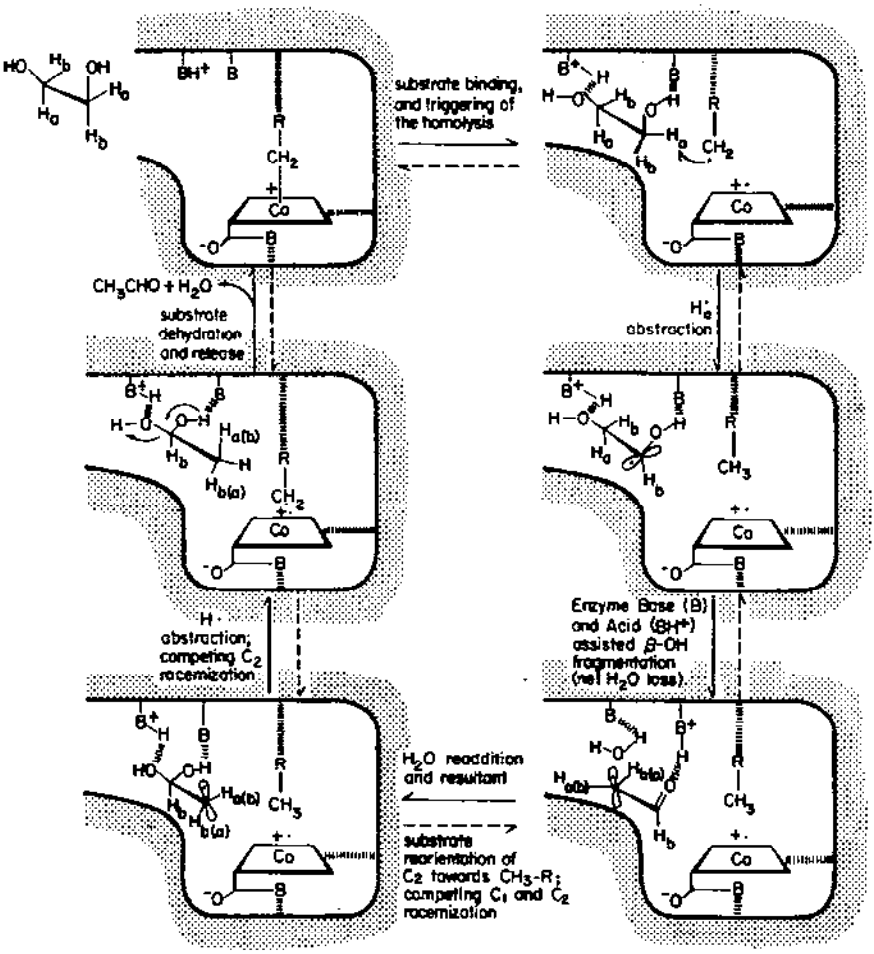


Fig. 4. A schematic diagram of the bound radical mechanism, illustrated only for the binding mode that results in H_A abstraction (the other known binding mode that results in H_B abstraction is shown in Fig. 2 and eqn. 14 in the text). Relatively rigid acidic (BH^+) and basic (B) diol OH-binding sites are postulated and questions of base-on vs. base-off cobalt or the importance of the reverse of individual steps have not been considered in detail. No attempt has been made to illustrate fully the periplanar conformations known to be optimum for radical β-fission, readdition reactions. (See Beckwith, A.L.J., Tetrahedron, 37 (1981) 3073, and p. 3088.) Discussion of the mechanism is provided in the text.

rocking motion concomitant with a partial rotation about the C–C bond (as shown in the left most equilibrium of eqn. 14). Following H · abstraction, there should be a very rapid β-OH fragmentation (net H_2O loss), especially if both acidic (BH^+) and basic (B) OH binding and activation are present, ($\overset{\frown}{BH^+}$ HO–CH_2CHO–H------B), to provide a better leaving group and

abstraction of the ca. 10^5 more acidic proton, respectively. The acidic and basic diol binding sites have been assumed solely on the basis of known $HOCH_2\dot{C}HOH$ radical chemistry but, as we will see shortly, they also allow us to explain the stereoselective dehydratase activity of the enzyme operating on *gem*-diol intermediates.

It is presumably at the next stage, where a weakly bound, $\cdot CH_2CHO\cdots HB^+$—, radical is shown, that racemization about the carbonyl carbon, C_1, can occur by simply providing either face of the planar carbonyl for the next step, H_2O readdition. The exact extent of C_1 racemization depends upon the rates of (intramolecular) H_2O readdition vs. the racemization pathway, with the latter proving less competitive in the case of propylene glycol with its sterically more demanding methyl group in the place of the hydrogen present in ethylene glycol. To the extent that resonance stabilization [24], $R\dot{C}H-CH=O \leftrightarrow RCH=CH-O\cdot$, is present it will hinder rotation about the C–C bond. The contribution of the $RCH=CH-O\cdot$ resonance structure is thought [22] to be small, however, although this point has been somewhat controversial.

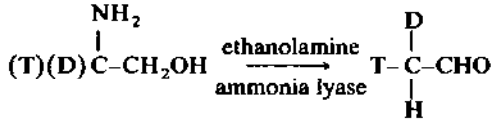
Racemization at the other carbon, C_2, may also occur at this stage but is seemingly more likely at the next stage, where a $\cdot CH_2CH(OH)_2$ intermediate is formed by H_2O readdition and probably has a longer lifetime due to a difficult $H\cdot$ abstraction from an unactivated CH_3–R group as its next step. In the case of propanediol, the high ($\geqslant 88\%$) stereoselectivity at C_2 [17] requires that only a small amount of rotation and racemization occur and that the stereochemistry at C_2 be predominantly inversion (as shown in Fig. 4, if no interconversion of H_A and H_B via rotation occurs (where H_A or $H_B=CH_3$)).

The last step, *gem*-diol dehydration, completes the cycle. With one acidic and one basic OH binding site, stereoselective dehydration becomes a chemically reasonable possibility. One would predict that the OH bound to the acidic site is preferentially lost, a prediction consistent with the known ^{18}O-labeling data on propanediol (eqns. 7, 8 and Fig. 4). It is rather amazing to realize that the whole diol dehydratase cycle is completed 12 times each second!

It may seem puzzling, and initially puzzled us, that the enzyme would prefer a seemingly inefficient mechanism with H_2O elimination, followed by H_2O readdition, followed by H_2O re-elimination once more just one step later. It is possible that the H_2O readdition step is non-essential, a side reaction driven by a possibly hydrophobic pocket in the protein. Alternatively, we would like to suggest that it is probably an essential step, the *gem*-diol $\cdot CH_2CH(OH)_2$ being bound by the enzyme through the same two, essentially rigid, OH binding groups. These groups would, according to molecular models, reorient the carbon radical towards the cofactor for $H\cdot$

transfer as shown in the H_2O readdition step (Fig. 4) thereby preventing indiscriminate radical abstraction reactions with the protein. (An examination of this point using molecular models and two rigid binding sites is recommended.) The readdition of H_2O, $\cdot CH_2CHO + H_2O \rightleftharpoons \cdot CH_2CH(OH)_2$, would also eliminate any resonance stabilization [24], $\cdot CH_2CHO \leftrightarrow CH_2{=}CHO\cdot$, thereby providing additional driving force for the next, rather difficult, step of $H\cdot$ removal from an unactivated methyl group, $RCH_3 + \cdot CH_2CH(OH)_2 \rightarrow RCH_2\cdot + CH_3CH(OH)_2$.

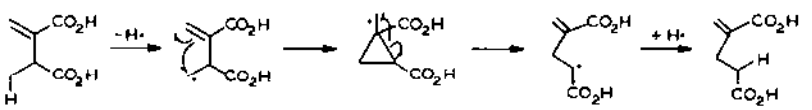
An important question is the possible generality of the bound radical mechanism for the other B_{12}-dependent rearrangements. For ethanolamine ammonia lyase [25] (eqn. 16) radicals are observed in the studies of the enzymes [26], the ESR data best fits a $R\cdot$ and $Co\cdot$ at a 10–12 Å separation [12b], analogous radical fragmentations are well known [27] ($H_2N{-}CH_2\dot{C}HOH \rightarrow NH_3 + \cdot CH_2CHO$) and a change from racemization (eqn. 16) to at least stereoselectivity was recently observed using (2*S*)- or (2*R*)-2-aminopropan-1-ols [2b,15f], so that the bound radical mechanism is consistent with the above data* and with other studies [28]. In fact, much of the bound radical mechanism was recently proposed for ethanolamine ammonia lyase by others [2b].



(16)

(Both (*R*) and (*S*)) (racemic)

In the case of α-methylene glutarate mutase, cobalt participation at the rearrangement step is unnecessary as allylcarbonyl ⇌ cyclopropyl carbonyl radical rearrangements are well established [29] (eqn. 17).



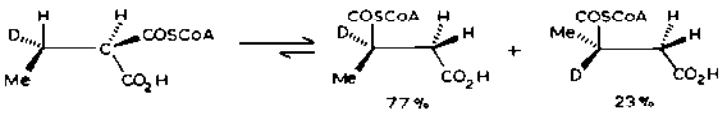
(17)

The available data [30] is again consistent with the bound radical mechanism, although the enzyme, α-methylene glutarate mutase, is less well studied. The case of methylmalonyl-CoA mutase is interesting, since while early

* The bound radical mechanism combined with the poorer leaving group ability of $-NH_2(NH_3)$ vs. $-OH(H_2O)$ suggests a longer lifetime and greater ease of detection for $H_2NCH_2\dot{C}HOH$ vs. $HOCH_2\dot{C}HOH$, predictions which appear consistent with ESR studies of ethanolamine ammonia lyase vs. those of diol dehydratase.

stereochemical work [31a] was interpreted as 100% retention at the carbon to which the hydrogen is transferred, more recent work using ethylmalonyl as a substrate shows that in this case there is, again, only stereoselectivity (eqn. 18) [31b]. Model studies [32] as well as chemical evidence [33] suggest that methylmalonyl-CoA mutase may involve cobalt participation as a reducing agent giving a substrate carbanion in the rearrangement step, although this point deserves clarification.

A final, interesting point is that our understanding of diol dehydratase has



(18)

advanced only as far as our basic chemical knowledge has progressed. The question of concerted 1,2-migration vs. fragmentation–readdition mechanisms appears to be a general one in current radical chemistry [7c,34]. Moreover, it is only very recently that we have learned that Co–C bonds are thermodynamically weak, often with bond dissociation energies (BDE) < 25–30 kcal mol^{-1} [13], and, therefore, that the bond in $Co–CH(OH)CH_2OH$ (1) should be quite weak.

D. SUMMARY

Herein, stereochemical data, modified cofactor studies, chemical model studies, and well known radical chemistry have been shown to be consistent with a simple, unified mechanism of action for diol dehydratase, the bound radical mechanism. The key features are that the cofactor and its weak Co–C bond [13,35] serve as nature's source* of $RCH_2\cdot$ radicals. The $RCH_2\cdot$ then removes an $H\cdot$ from the substrate and it is the protein–substrate binding that controls the subsequent chemistry, not the cofactor. The bound radical mechanism naturally focuses interest back to the initial, poorly understood, homolysis step.

A measurement of the Co–C bond dissociation energy of the cofactor in vitro** and its apparent bond dissociation energy in vivo are needed, as are answers to questions concerning how the homolysis is triggered. In addition, the corrin conformation, steric, electronic and axial benzimidazole effects all

*Recently it has been suggested by Eschenmoser [36] that nature may have chosen B_{12} because of the ease of the synthesis of corrins in nature, an interesting notion in light of the 11 year Woodward–Eschenmoser total synthesis of B_{12}, involving ca. 100 co-workers.

**This has recently been accomplished, the adenosylcobalmin Co–C BDE is $\simeq 29$ (rigorously $\leqslant 29$) kcal mol^{-1}; R.G. Finke and B.P. Hay, J. Am. Chem. Soc., submitted.

remain to be quantified [13,35]. The role of thiols [37] and the large isotope effects [38,39c], especially on the second H · transfer step (see Scheme 1), are presently poorly understood and are likely to lead to revision of the proposed mechanism once they become better understood. The challenge of designing and preparing a synthetic system capable of binding glycol substrates and mimicking diol dehydratase also remains to be met. The most significant challenge is in the hands of those working with protein and the protein crystallographers, since structural data is the crucial underpinning of mechanistic work, yet only limited amino acid composition, and sequence [39a,b] but no crystallographic studies [40] of B_{12}-dependent enzymes have appeared to date. In support of the above statement one can cite recent data where an improved isolation procedure yields 16 times more of a ca. 20–40% more active, less proteolyzed form of diol dehydratase as well as evidence for membrane association and a revised number of subunits for this enzyme [39a,b]. Especially intriguing are recent isotope effect studies [39c] which suggest that a second H · acceptor/donor site may exist in addition to the C(5′) position of the coenzyme established by Abeles' classic studies [6].

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REFERENCES

1 Recent reviews:
(a) B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979.
(b) B.M. Babior and J.S. Krouwer, CRC Crit. Rev. Biochem., 6 (1979) 35.
(c) B.T. Golding in H.A.O. Hill (Ed.), Inorganic Biochemistry, The Chemical Society, London, 1979, pp. 75–87.
(d) R.H. Abeles and D. Dolphin, Acc. Chem. Res., 9 (1976) 114.
(e) B.M. Babior, Acc. Chem. Res., 8 (1975) 376.
(f) D. Dolphin (Ed.), B_{12}, Vols I and II, Wiley–Interscience, New York, 1982.
(g) B.T. Golding, in D. Dolphin (Ed.), B_{12}, Vol. I, Wiley Insterscience, New York, 1982, Chap. 15.
2 (a) B.M. Babior, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 168, and references cited therein.
(b) P. Diziol, H. Haas, J. Rétey and S.W. Graves, Eur. J. Biochem., 106 (1980) 211.
3 J. Rétey, A. Umani-Ronchi, J. Seibl and D. Arigoni, Experientia, 22 (1966) 502. (To our knowledge, subsequent full papers on this work have not been published.) *Note added in proof*: Rétey has recently summarized the stereochemical studies of the B_{12}-dependent

rearrangement reactions; J. Rétey, in Ch. Tamm (Ed.), Stereochemistry, Elsevier Biomedical Press, Amsterdam, 1982, Chap. 6.

4 E.J. Corey, N.J. Cooper and M.L.H. Green, Proc. Natl. Acad. Sci. U.S.A., 74 (1977) 811.

5 (a) J.E. Valinsky, R.H. Abeles and J.A. Fee, J. Am. Chem. Soc., 96 (1974) 4709.
(b) S.A. Cockle, H.A.O. Hill, R.J.P. Williams, S.P. Davies and M.A. Foster, J. Am. Chem. Soc., 94 (1972) 275.
(c) J.E. Valinsky, R.H. Abeles and A.S. Mildvan, J. Biol. Chem., 249 (1974) 2751.
(d) T.H. Finlay, J. Valinsky, A.S. Mildvan and R.H. Abeles, J. Biol. Chem., 248 (1973) 1285.
(e) R.G. Eagar, Jr., W.W. Bachovchin and J.H. Richards, Biochemistry, 14 (1975) 5523.

6 (a) R.H. Abeles, in W.O. Milligan (Ed.), Proc. Robert A. Welch Found. Conf. Chem. Res., Vol. XV, Bioorganic Chemistry and Mechanisms, 1972, pp. 95–127.
(b) R.H. Abeles, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 375.

7 (a) L. Salem, O. Eisenstein, N.T. Anh, H.B. Burgi, A. Davequet, G. Segal and A. Veillard, Nouv. J. Chim., 1 (1977) 335.
(b) B.T. Golding and L. Radom, J. Am. Chem. Soc., 98 (1976) 6331.
(c) J.J. Russell, H.S. Rzepa and D.A. Widdowson, J. Chem. Soc., Chem. Commun., (1983) 625. This work addresses the dissociation/recombination vs. concerted migration mechanisms (at 0 K in the gas phase) for the various B_{12} substrates using MNDO/UHF calculations. For $H_2O^+ CH_2\dot{C}HOH$, a weak π-allyl complex of H_2O and the radical cation of vinyl alcohol is favored over dissociation although the other, potentially more relevant extreme (assuming basic and acidic diol bond sites), of $H_2O^+CH_2\dot{C}HO^-$ ($\cdot CH_2CHO + H_2O$) was not examined. Again it must be stressed that for diol dehydratase the stereochemical studies rule out a stereospecific H_2O^+-migration for ethylene glycol as a substrate and require that at least part of the reaction for propanediol also proceed through some non-stereospecific mechanism.

8 (a) W.A. Mulac and D. Meyerstein, J. Am. Chem. Soc., 104 (1982) 4124. (We thank Prof. Meyerstein for a preprint.)
(b) H. Elroi and D. Meyerstein, J. Am. Chem. Soc., 100 (1978) 5540.
(c) R. Blackburn, M. Kyaw and G.O. Phillips, J. Chem. Soc., Faraday Trans. 1, 71 (1975) 2277.

9 (a) R.B. Silverman and D. Dolphin, J. Am. Chem. Soc., 98 (1976) 4626; 96 (1974) 7094; 95 (1973) 1686; 97 (1975) 2924.
(b) R.B. Silverman and D. Dolphin, J. Organomet. Chem., 101 (1975) C14.
(c) R.B. Silverman, D. Dolphin and B.M. Babior, J. Am. Chem. Soc., 94 (1972) 4028.
(d) R.B. Silverman, D. Dolphin, T.J. Carty, E.K. Krodel and R.H. Abeles, J. Am. Chem. Soc., 96 (1974) 7096.
(e) T.M. Vickrey, R.N. Katz and G.N. Schrauzer, J. Am. Chem. Soc., 97 (1975) 7248.
(f) G.N. Schrauzer, W.J. Michaely and R.J. Holland, J. Am. Chem. Soc., 95 (1973) 2024.
(g) W.J. Michaely and G.N. Schrauzer, J. Am. Chem. Soc., 95 (1973) 5771.

10 The following model studies focus primarily upon the H· transfer steps and do not address the question of cobalt participation in the rearrangement step.
(a) B.T. Golding, C.S. Sell and P.J. Sellars, J. Chem. Soc., Perkin Trans. 2, (1980) 961.
(b) B.T. Golding, T.J. Kemp, C.S. Sell, P.J. Sellars and W.P. Watson, J. Chem. Soc., Perkin Trans. 2, (1978) 839.
(c) I.P. Rudakova, T.E. Ershova, A.B. Belikov and A.M. Yurkevich, J. Chem. Soc., Chem. Commun., (1978) 592.

11 (a) R.G. Finke and W. McKenna, J. Chem. Soc., Chem. Commun., (1980) 460.

(b) R.G. Finke, W.B. McKenna, D.A. Schiraldi and B.L. Smith, J. Am. Chem. Soc., 105 (1983) in press.
(c) R.G. Finke and D.A. Schiraldi, J. Am. Chem. Soc., 105 (1983) in press.
(d) For an electrochemical comparison of B_{12} models see: C.M. Elliott, E. Hershenhart, R.G. Finke and B.L. Smith, J. Am. Chem. Soc., 103 (1981) 5558.

12 (a) K.L. Schepler, W.R. Dunham, R.H. Sands, J.A. Fee and R.H. Abeles, Biochim. Biophys. Acta, 397 (1975) 510.
(b) J.F. Boas, P.R. Hicks, J.R. Pilbrow and T.D. Smith, J. Chem. Soc., Faraday Trans. 2, 74 (1978) 417.
(c) In ribonucleotide reductase, similar ESR signals are observed, some of which are not kinetically competent (refs. 12(d) and (e), and see the discussion in ref. 1(b), pp. 65 and 68), while others are kinetically competent (refs. 12(f) and (g)).
(d) J.A. Hamilton, Y. Tamao, R.L. Blakley and R.E. Coffman, Biochemistry, 11 (1972) 4696.
(e) G.R. Buettner and R.E. Coffman, Biochim. Biophys. Acta, 480 (1977) 495.
(f) R.E. Coffman, Y. Ishikawa, R.L. Blakley, M. Beinert and W.H. Orme-Johnson, Biochim. Biophys. Acta, 444 (1976) 307.
(g) R.L. Blakley, W.H. Orme-Johnson and J.M. Bozdech, Biochemistry, 18 (1979) 2335.

13 (a) J. Halpern, Acc. Chem. Res., 15 (1982) 238, and references cited therein.
(b) J. Halpern, in D. Dolphin (Ed.), B_{12}, Vol. I, Wiley Interscience, New York, 1982, Chap. 14.
(c) J. Halpern, F.T.T. Ng and G.L. Rempel, J. Am. Chem. Soc., 101 (1979) 7124.
(d) G.N. Schrauzer and J.H. Grate, J. Am. Chem. Soc., 103 (1981) 541.
(e) F.T.T. Ng, G.L. Rempel and J. Halpern, J. Am. Chem. Soc., 104 (1982) 621.
(f) T.-T. Tsou, M. Loots and J. Halpern, J. Am. Chem. Soc., 104 (1982) 623.
(g) H.B. Gjerde and J.H. Espenson, Organometallics, 1 (1982) 435.
(h) R.G. Finke, B.L. Smith, B.J. Mayer and A.A. Molinero, Inorg. Chem., 22 (1983) in press.

14 (a) A.K. Pikaev and L.I. Kartasheva, Int. J. Radiat. Phys. Chem., 7 (1975) 395.
(b) A.L. Buley, R.O.C. Norman and R.J. Pritchett, J. Chem. Soc. B, (1966) 849.
(c) R. Livingstone and H. Zeldes, J. Am. Chem. Soc., 88 (1966) 4333.
(d) B.C. Gilbert, J.P. Larkin and R.O.C. Norman, J. Chem. Soc., Perkin Trans. 2, (1972) 794.
(e) K.M. Bansal, M. Grätzel, A. Henglein and E. Janata, J. Phys. Chem., 77 (1973) 16.
(f) P.J. Venter, H.J. van der Linde and R.A. Basson, J. Chem. Soc., Chem. Commun., (1972) 187.
(g) C.E. Burchill and K.M. Perron, Can. J. Chem., 49 (1971) 2382.
(h) S. Steenken and D. Schulte-Frohlinde, Tetrahedron Lett., (1973) 653.

15 (a) D. Arigoni, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 409.
(b) C.A. McAuliffe, in H.A.O. Hill (Ed.), Inorganic Biochemistry, The Chemical Society, London, 1979, p. 87.
(c) A.I. Scott, Science, 218 (1982) 674. (In this book review of the new 1982 volume on B_{12} (ref. 1(f)), Scott notes that the mechanism of action of Coenzyme B_{12} is "a topic still largely shrouded in mystery".)
(d) H. Dugas and C. Penney, Bioorganic Chemistry. A Chemical Approach to Enzyme Action, Springer Verlag, New York, 1981, pp. 374, 376–378. (See also ref. 15(h).)
(e) E.-I. Ochiai, Bioinorganic Chemistry. An Introduction, Allyn and Bacon, Boston, 1977, Chap. 12, pp. 311–366. (See also the discussion in ref. 1(c), Chap. 4, on the top of p. 84, regarding Scheme 2.4 and ref. 198 therein.)

(f) D. Gani, O.C. Wallis and D.W. Young, J. Chem. Soc., Chem. Commun., (1982) 898.
(g) B.M. Babior and J.S. Krouwer, CRC Crit. Rev. Biochem., 6 (1979) 79.
(h) R. Hamilton and J.J. Rooney, J. Mol. Catal., 17 (1982) 29. (Herein the authors state that a π complex is a "key intermediate".)

16 D. Arigoni, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 389.

17 (a) B. Zagalah, P.A. Frey, G.L. Karabatsos and R.H. Abeles, J. Biol. Chem., 241 (1966) 3028.
(b) R.H. Abeles, in W.O. Milligan (Ed.), Proc. Robert A. Welch Foundation Conf. Chem. Res. Vol. XV, Bioorganic Chemistry and Mechanisms, 1972, pp. 95–127 (see p. 118).

18 (a) D.C. Hodgkin, J. Kamper, M. MacKay, J. Pickworth, K.N. Trueblood and J.G. White, Nature (London), 178 (1956) 64.
(b) P.G. Lenhart and D.C. Hodgkin, Nature (London), 182 (1961) 937.

19 (a) T. Toraya and R.H. Abeles, Arch. Biochem. Biophys., 203 (1980) 174. (See also ref. 1(f), Vol. II, Chap. 9, p. 233.)
(b) T. Toraya, E. Krodel, A.S. Mildvan and R.H. Abeles, Biochemistry, 18 (1979) 417.
(c) S. Fukui and T. Toraya, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 413; T. Toraya and S. Fukui, in D. Dolphin, C. McKenna, Y. Murakami and I. Tabushi (Eds.), Biomimetic Chemistry, Adv. Chem. Ser., Vol. 191, 1980, p. 139.
(d) J.S. Krouwer, B. Homlquist, R.S. Kipnes and B.M. Babior, Biochim. Biophys. Acta, 612 (1980) 153.
(e) T. Toraya, K. Ushiko, S. Fukui and H.P.C. Hogenkamp, J. Biol. Chem., 252 (1977) 963.
(f) M.I. Yakusheva, A.A. Poznanskaya, T.A. Pospelova, I.P. Rudakova, A.M. Yurkevich and V.A. Yakovlev, Biochim. Biophys. Acta, 484 (1977) 216.

20 R.H. Abeles, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 384.

21 K.W. Moore and J.H. Richards, Biochem. Biophys. Res. Commun., 87 (1979) 1052.

22 D. Arigoni, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, pp. 406–409, Figs. 20–22.

23 R.H. Abeles and H.A. Lee, Jr., Brookhaven Symp. Biol., 15 (1962) 310.

24 (a) A.H. Zimmerman, K.J. Reed and J.I. Brauman, J. Am. Chem. Soc., 99 (1977) 7203, and refs. 27 and 28 therein.
(b) E.S. Huyser, D. Feller, W.T. Borden and E.R. Davidson, J. Am. Chem. Soc., 104 (1982) 2956.

25 (a) J. Rétey, C.J. Suckling, D. Arigoni and B.M. Babior, J. Biol. Chem., 249 (1974) 6359.
(b) P. Diziol, H. Haas, J. Rétey, S. Graves and B.M. Babior, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 485.

26 (a) P.Y. Law, D.G. Brown, E.L. Lien, B.M. Babior and J.M. Wood, Biochemistry, 10 (1971) 3428.
(b) B.M. Babior, T.H. Moss and D.C. Gould, J. Biol. Chem., 247 (1972) 4389.
(c) B.M. Babior, T.H. Moss, W.H. Orme-Johnson and H. Beinert, J. Biol. Chem., 249 (1974) 4537.
(d) O.C. Wallis, R.C. Bray, S. Gutteridge and R. Hollaway, Eur. J. Biochem., 125 (1982) 299.

27 (a) T. Foster and P.R. West, Can. J. Chem., 51 (1973) 4009.
(b) T. Foster and P.R. West, Can. J. Chem., 52 (1974) 3589.

28 (a) J.S. Krouwer and B.M. Babior, J. Biol. Chem., 252 (1977) 5004.
(b) J.S. Krouwer, R.M. Schultz and B.M. Babior, J. Biol. Chem., 253 (1978) 1041.
(c) M.R. Hollaway, H.A. White, K.N. Joblin, A.W. Johnson, M.F. Lappert and O.C. Wallis, Eur. J. Biochem., 82 (1978) 143; see also M.R. Hollaway, H.A. White, K.N. Joblin, A.W. Johnson, M.F. Lappert and O.C. Wallis, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 471.
(d) B.M. Babior, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 461.
29 (a) B. Maillard, D. Forrest and K.U. Ingold, J. Am. Chem. Soc., 98 (1976) 7024.
(b) D. Griller and K.U. Ingold, Acc. Chem. Res., 13 (1980) 317, and references cited therein.
(c) R.Kh. Freidlina, in G.H. Williams (Ed.), Advances in Free Radical Chemistry, Vol. 1, Logos Press, London, 1965, p. 211.
30 (a) P. Dowd, M. Shapiro and K. Kang, J. Am. Chem. Soc., 97 (1975) 4754.
(b) P. Dowd, B.K. Trivedi, M. Shapiro and L.K. Marwaha, J. Am. Chem. Soc., 98 (1976) 7875.
(c) P. Dowd, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 555.
(d) A. Bury, M.R. Ashcroft and M.D. Johnson, J. Am. Chem. Soc., 100 (1978) 3217.
(e) M.P. Atkins, B.T. Golding and P.J. Sellars, J. Chem. Soc., Perkin Trans. 2, (1978) 954.
(f) M.P. Atkins, B.T. Golding, A. Bury, M.D. Johnson and P.J. Sellars, J. Am. Chem. Soc., 102 (1980) 3630.
(g) S. Chemaly and J.M. Pratt, J. Chem. Soc., Chem. Commun., (1976) 998.
(h) M.P. Atkins, B.T. Golding and P.J. Sellars, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 587.
31 (a) M. Sprecher, R.L. Switzer and D.B. Sprinson, J. Biol. Chem., 241 (1966) 864.
(b) J. Rétey, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 439.
32 (a) P. Dowd and M. Shapiro, J. Am. Chem. Soc., 98 (1976) 3724.
(b) P. Dowd, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 557.
(c) A.I. Scott, J. Kang, D. Dalton and S.K. Chung, J. Am. Chem. Soc., 100 (1978) 3603.
(d) A.I. Scott and K. Kang, J. Am. Chem. Soc., 99 (1977) 1997.
(e) G. Bidlingmaier, H. Flohr, U.M. Kempe, T. Krebs and J. Rétey, Angew. Chem. Int. Ed. Engl., 14 (1975) 822.
(f) H. Flohr, W. Pannhorst and J. Rétey, Angew. Chem. Int. Ed. Engl., 15 (1976) 561.
(g) H. Flohr, U.W. Kempe, W. Pannhorst and J. Rétey, Angew. Chem. Int. Ed. Engl., 15 (1976) 427.
(h) M. Fountoulakis and J. Rétey, Chem. Ber., 113 (1980) 650.
(i) J. Rétey, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 439.
(j) J.H. Grate, J.W. Grate and G.N. Schrauzer, J. Am. Chem. Soc., 104 (1982) 1588.
33 (a) R.M. Acheson, Acc. Chem. Res., 4 (1971) 177.
(b) S.N. Lewis, J.J. Miller and S. Winstein, J. Org. Chem., 37 (1972) 1478.
34 R.Kh. Freidlina and A.B. Terent'ev, in G.H. Williams (Ed.), Advances in Free Radical Chemistry, Vol. 6, Heyden, London, 1982, pp. 1–63.
35 For structural model studies related to the homolysis step see:
(a) L. Randaccio, N. Bresciani-Pahor, P.J. Toscano and L.G. Marzilli, J. Am. Chem. Soc., 103 (1981) 6347; 102 (1980) 7372.

(b) L.G. Marzilli, P.J. Toscano, L. Randaccio, N. Bresciani-Pahor and M. Calligaris, J. Am. Chem. Soc., 101 (1979) 6754.
(c) S.M. Chemaly and J.M. Pratt, J. Chem. Soc., Dalton Trans. (1980) 2259 (part 17); 2267 (part 18); 2274 (part 19).
36 A. Eschenmoser, Chem. Eng. News, Sept. 14, 1981, pp. 36–37.
37 (a) S. Kuno, T. Toraya and S. Fukui, Arch. Biochem. Biophys., 210 (1981) 474.
(b) L. Mauck and M. Babior, Biochim. Biophys. Acta, 480 (1977) 489.
(c) I.P. Rudakova, T.E. Ershova, A.B. Belikov and A.M. Yurkevich, J. Chem. Soc., Chem. Commun., (1978) 592.
(d) J.S. Krouwer and B.M. Babior, Mol. Cell. Biochem., 15 (1977) 89.
(e) F. Nome and J.H. Fendler, J. Chem. Soc., Dalton Trans. (1976) 1212.
(f) T. Frick, M.D. Francia and J.M. Wodd, Biochim. Biophys. Acta, 428 (1976) 808.
38 (a) R.H. Abeles, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, pp. 373–388, and references cited therein.
(b) B.M. Babior and J.S. Krouwer, CRC Crit. Rev. Biochem., 6 (1979) 35 (see discussion and refs. on p. 70).
39 (a) D.E. McGee and J.H. Richards, Biochemistry, 20 (1981) 4293–4298.
(b) D.E. McGee, S.S. Carroll, M.W. Bond and J.H. Richards, Biochem. Biophys. Res. Commun., 2 (1982) 547–551.
(c) J.H. Richards, personal communication. It is a pleasure to acknowledge informative discussions with Professor Richards and Professor Abeles.
40 Only methylmalonyl-CoA has been crystallized, although not in a form suitable for X-ray diffraction studies; J. Rétey, in B. Zagalah and W. Friedlich (Eds.), Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor, W. de Gruyter, Berlin, 1979, p. 439.