Stabilisation of Methylene Radicals by Cob(II)alamin in Coenzyme B12 Dependent Mutases

ARTICLE *in* CHEMISTRY · JANUARY 2006

Impact Factor: 5.73 · DOI: 10.1002/chem.200501074 · Source: PubMed

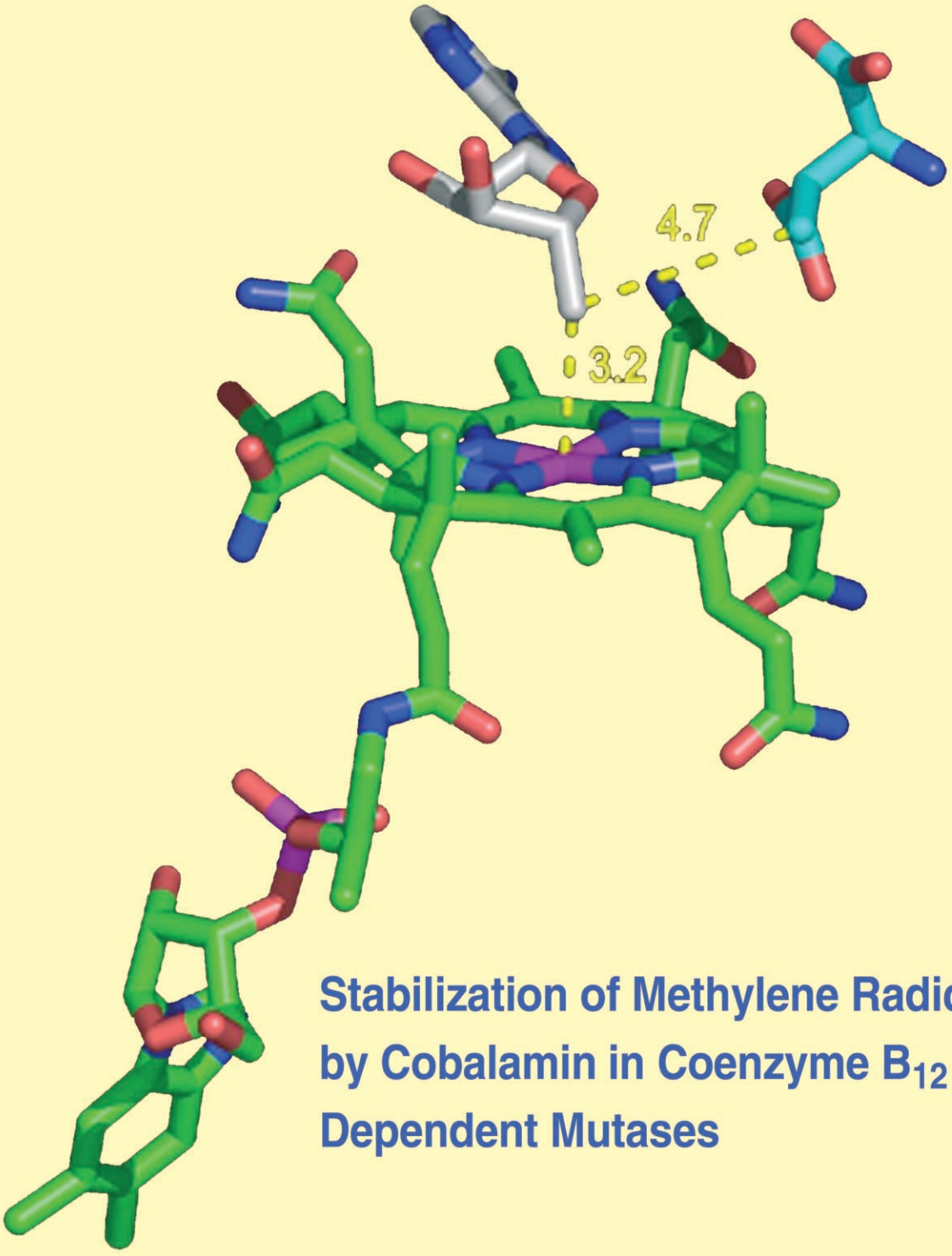
3 AUTHORS, INCLUDING:

Wolfgang Buckel
Max Planck Institute for Terrestrial Microbi…
247 PUBLICATIONS **6,903** CITATIONS

SEE PROFILE

Bernard Thomas Golding
Newcastle University
403 PUBLICATIONS **7,274** CITATIONS

SEE PROFILE



Stabilization of Methylene Radicals by Cobalamin in Coenzyme B_{12} Dependent Mutases

CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200501074

Stabilisation of Methylene Radicals by Cob(II)alamin in Coenzyme B_{12} Dependent Mutases

Wolfgang Buckel,*[a] Christoph Kratky,[b] and Bernard T. Golding*[c]

Dedicated to Professor János Rétey on the occasion of his 70th birthday

Abstract: Coenzyme B_{12} initiates radical chemistry in two types of enzymatic reactions, the irreversible eliminases (e.g., diol dehydratases) and the reversible mutases (e.g., methylmalonyl-CoA mutase). Whereas eliminases that use radical generators other than coenzyme B_{12} are known, no alternative coenzyme B_{12} independent mutases have been detected for substrates in which a methyl group is reversibly converted to a methylene radical. We predict that such mutases do not exist. However, coenzyme B_{12} independent pathways have been detected that circumvent the need for glutamate, β-lysine or methylmalonyl-CoA mutases by proceeding via different intermediates. In humans the methylcitrate cycle, which is ostensibly an alternative to the coenzyme B_{12} dependent methylmalonyl-CoA pathway for propionate oxidation, is not used because it would interfere with the Krebs cycle and thereby compromise the high-energy requirement of the nervous system. In the diol dehydratases the 5′-deoxyadenosyl radical generated by homolysis of the carbon–cobalt bond of coenzyme B_{12} moves about 10 Å away from the cobalt atom in cob(II)alamin. The substrate and product radicals are generated at a similar distance from cob(II)alamin, which acts solely as spectator of the catalysis. In glutamate and methylmalonyl-CoA mutases the 5′-deoxyadenosyl radical remains within 3–4 Å of the cobalt atom, with the substrate and product radicals approximately 3 Å further away. It is suggested that cob(II)alamin acts as a conductor by stabilising both the 5′-deoxyadenosyl radical and the product-related methylene radicals.

Keywords: cobalamins · enzymes · oxidation · radicals · reaction mechanisms

Introduction

Coenzyme B_{12}, also called adenosylcobalamin (Figure 1a), is the most complex cofactor for enzymatic reactions.[1,2] The special feature of this organometallic coenzyme is its weak cobalt–carbon σ bond with a dissociation energy of ~130 kJ mol^{-1}.[3] When the coenzyme is bound to an enzyme partner, the Co–C bond can be homolytically cleaved giving cob(II)alamin and the 5′-deoxyadenosyl radical. This highly reactive primary organic radical participates in the enzymatic catalysis of carbon skeleton rearrangements and 1,2-shifts of amino groups, as well as the elimination of water or ammonia from substrates such as glycerol or ethanolamine, respectively. Recently however, an alternative to the long-known coenzyme B_{12} dependent enzyme glycerol dehydratase, which catalyses the conversion of glycerol to 3-hydroxypropanal, was found in the anaerobic bacterium *Clostridium butyricum* (Schemes 1 and 2). This dehydratase contains a glycyl radical at the active site, which is formed by the action of the 5′-deoxyadenosyl radical on a specific glycine residue of the protein. The 5′-deoxyadenosyl radical is generated not from coenzyme B_{12}, but by one-electron reduction of the much simpler molecule *S*-adenosylmethionine (SAM; Figure 1b),[4,5] which Horace A. Barker called a

[a] Prof. Dr. W. Buckel
Fachbereich Biologie, Philipps-Universität
35032 Marburg (Germany)
Fax: (+49) 6421-282-8979
E-mail: buckel@staff.uni-marburg.de

[b] Prof. Dr. C. Kratky
Institut für Chemie, Karl-Franzens-Universität
Graz (Austria)

[c] Prof. Dr. B. T. Golding
School of Natural Sciences—Chemistry
Bedson Building, Newcastle University
Newcastle upon Tyne (UK)
Fax: (+44) 191-222-6929
E-mail: b.t.golding@ncl.ac.uk

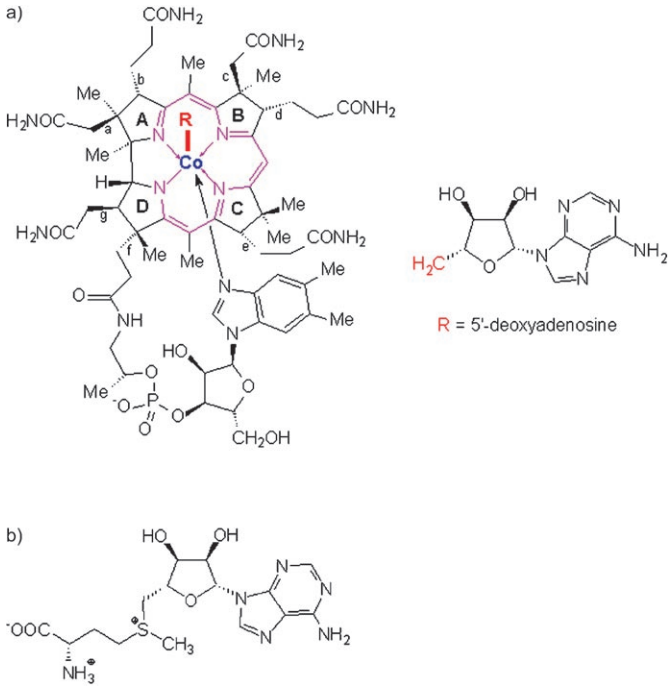
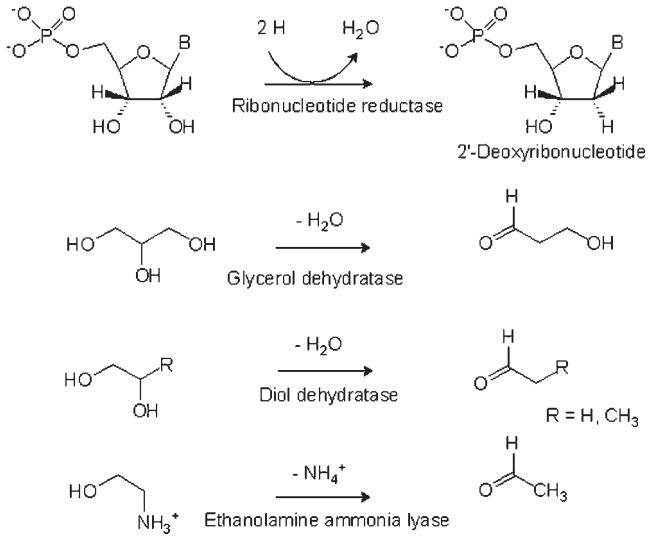
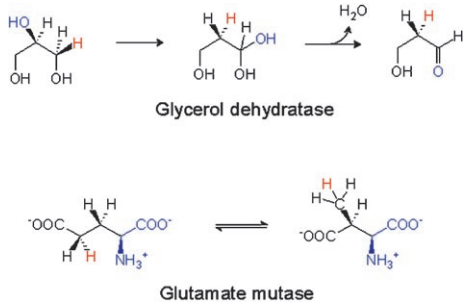


Figure 1. a) Coenzyme B_{12} or adenosylcobalamin in the base-on form. b) *S*-Adenosylmethionine (SAM).



Scheme 1. The family of eliminases. B = nucleobase. The coenzyme B_{12} dependent ribonucleotide reductase takes ribonucleoside triphosphates as substrates.

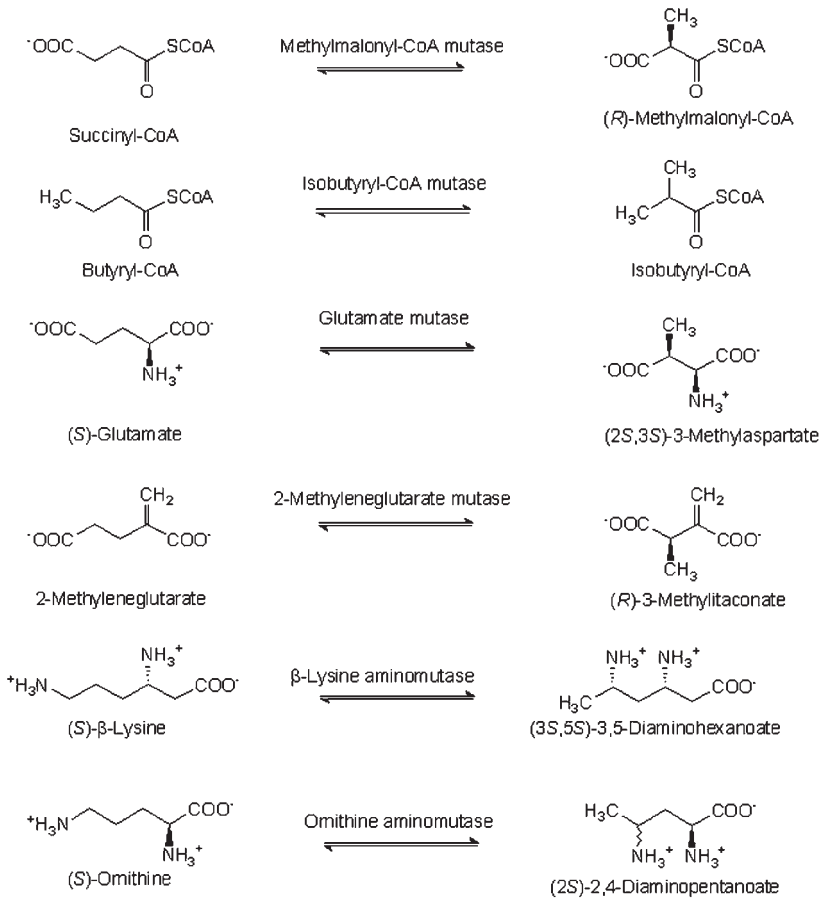
"poor man's adenosylcobalamin", cited in reference [6]. The discovery of the SAM-dependent glycerol dehydratase raises the question as to why nature uses the complex coenzyme B_{12} at all. This article provides an answer to this question. We propose that coenzyme B_{12} is only replaceable in the family of enzymes called eliminases (Scheme 1), whereas it is a chemical necessity in the mutase family because a methyl group has to be reversibly converted to a methylene radical (Scheme 3).



Scheme 2. Reactions catalyzed by coenzyme B_{12} dependent glycerol dehydratase and glutamate mutase. The migrating hydrogen atom is shown in red and the migrating group in blue. The SAM-dependent glycerol dehydratase catalyses an exchange of the red hydrogen atom with the solvent.

There are four enzyme families, which require derivatives of vitamin B_{12} as cofactors: methyltransferases, dehalogenases, eliminases and mutases.[1] Here we only consider the latter two families, which are dependent on the adenosylcobalamin form of vitamin B_{12}. The eliminase family comprises ribonucleotide reductase and ethanolamine ammonia lyase, as well as diol (e.g. propane-1,2-diol) and glycerol dehydratases (Scheme 1). The mutase family can be divided into the carbon skeleton mutase and aminomutase sub-families. The carbon skeleton mutase sub-family consists of glutamate mutase, methylmalonyl-CoA mutase, 2-methyleneglutarate mutase and isobutyryl-CoA mutase, whereas β-lysine-5,6-aminomutase and D-ornithine-4,5-aminomutase are the only known members of the aminomutase sub-family (Scheme 3).[7] In addition to coenzyme B_{12} both aminomutases contain pyridoxal-5′-phosphate, which assists through imine formation the migration of the amino group to the adjacent carbon.[8] Note that the name mutase is used for enzymes catalysing various isomerisations, for example, phosphoglucomutase,[9] but, unless otherwise indicated, in this paper we regard mutases only as coenzyme B_{12} dependent.

Both the eliminases and mutases appear to act by similar overall mechanisms. These can be described as the exchange between two adjacent carbon atoms of a group X (OH, NH_3^+ or a carbon-containing residue) and a hydrogen atom (Scheme 2). Initial homolysis of the cobalt–carbon bond of enzyme-bound coenzyme B_{12} generates cob(II)alamin and the 5′-deoxyadenosyl radical, which abstracts a specific hydrogen atom from a substrate molecule to form 5′-deoxyadenosine and a substrate-derived radical. Rearrangement of the substrate-derived radical yields the product-related radical, which reclaims a hydrogen atom from the 5′-methyl group of deoxyadenosine to regenerate the 5′-deoxyadenosyl radical and afford the product. In this way the migrating hydrogen atom always remains bound to carbon atom and therefore does not exchange with the solvent. In this generally accepted mechanism the initially generated cob(II)alamin has no function during the rearrangement and therefore is considered as a mere spectator until it recombines with the 5′-deoxyadenosyl radical.[10,11]



Scheme 3. The family of coenzyme B_{12} dependent mutases. Note that in each reaction a methylene group is reversibly converted into a methyl group via a methylene radical, which according to our proposal is stabilised by cob(II)alamin. The absolute configuration of (3*S*,5*S*)-3,5-diaminohexanoate has been tentatively assigned.[88]

The name eliminase[7] stems from the fact that the product of the rearrangement catalysed by enzymes of this subfamily, that is, a 1,1-diol or carbinolamine (geminal aminoalcohol), eliminates water or ammonia, respectively, to form an aldehyde (Scheme 2). Although ribonucleotide reductase does not fit completely into this generalisation, it shares with the other eliminases the homolysis of the cobalt–carbon bond of the coenzyme, the hydrogen abstraction from substrate and the elimination of the OH group from the carbon atom adjacent to the initially formed radical.

The classification into eliminases and mutases correlates with the mode of attachment of the coenzyme at the *apo*-enzyme. In the eliminase family the axial base 5,6-dimethylbenzimidazole remains coordinated to the cobalt atom,[12–14] a mode of attachment that has been called "base-on" (Figure 1a). In the mutase family the histidine residue in the conserved DXHXXG motif of the protein[15] replaces the 5,6-dimethylbenzimidazole base; this base exchange has been named "base-off" or more precisely "base-off, his-on".[16–20] It is unclear whether the "base-off, his-on" mode affects the participation of cob(II)alamin. Since the methyltransferases also use the conserved histidine residue as axial base,[21] this mode appears to be a hint for a common evolutionary origin rather than a functional necessity. Furthermore, site-directed mutagenesis of the histidine residue indicates a role in the binding of the coenzyme to methylmalonyl-CoA mutase rather than in catalysis.[22]

The eliminases, including ribonucleotide reductase, catalyse *irreversible* reactions, for example, the elimination of ammonia from ethanolamine [Eq. (1)]** or water from ethane-1,2-diol [Eq. (2)]. Based on thermodynamic considerations two steps can describe these eliminations. Firstly, ethanolamine and ethane-1,2-diol are reversibly converted to vinyl alcohol, a reaction analogous to the reversible dehydration of malate to fumarate catalysed by fumarase. Secondly, vinyl alcohol irreversibly tautomerises to acetaldehyde. As shown below, however, the actual mechanism of the eliminases is different. On the other hand, the mutases catalyse *reversible* rearrangements, in which neither the substrate nor the product undergoes a subsequent elimination mediated by the same enzyme, for example, the rearrangement of (*S*)-glutamate to (2*S*,3*S*)-3-methylaspartate [Eq. (3)[23]; Schemes 2 and 3].

$$HOCH_2CH_2NH_3^+ \rightarrow CH_3CH(OH)NH_3^+ \rightarrow CH_3CHO + NH_4^+ \quad \Delta G^{o'} = -32\ kJ\,mol^{-1} \tag{1}$$

$$HOCH_2CH_2OH \rightarrow CH_3CH(OH)OH \rightarrow CH_3CHO + H_2O \quad \Delta G^{o'} = -47\ kJ\,mol^{-1} \tag{2}$$

$$(S)\text{-glutamate} \rightleftharpoons (2S,3S)\text{-3-methylaspartate} \quad \Delta G^{o'} = +6.3\ kJ\,mol^{-1} \tag{3}$$

Proposal

Here we postulate that eliminases, exemplified by the diol dehydratases, but *not* mutases, can use radical initiators

[**] $\Delta G^{o\prime}$ of the reactions were calculated from ΔG_f^o values (ΔG° of formation from the elements).[17] Since the ΔG_f^o value of 2-ethanolamine was not available, it was estimated from that of L-serine as $-188\ kJ\,mol^{-1}$ assuming $\Delta G^{o\prime} = -23\ kJ\,mol^{-1}$ for the decarboxylation (calculated from oxaloacetate $+ H^+ = CO_2 +$ pyruvate).

other than coenzyme B_{12}. The reason is that the mutases require assistance from cob(II)alamin, the "conductor" rather than a "spectator",[10] by stabilising intermediate radicals and lowering transition states that interconvert these radicals.

Alternatives to Coenzyme B_{12} Dependent Eliminases

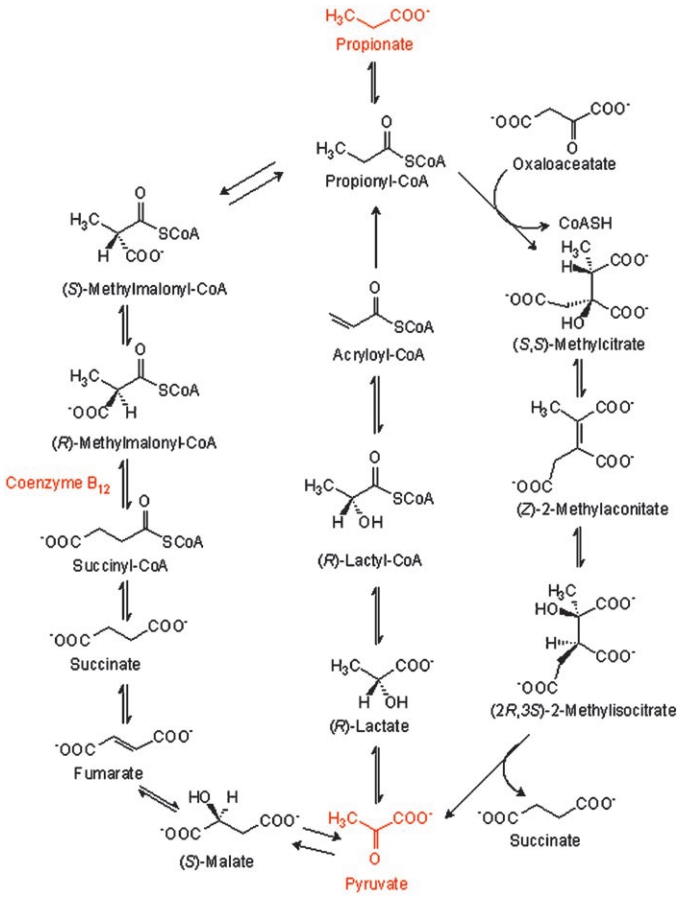
The best studied case of alternative radical enzymes among the eliminases are the ribonucleotide reductases, for which three different generators are known: dioxygen and SAM, both of which require additional cofactors, and coenzyme B_{12}.[24] The radicals derived from these species are formed in unrelated subunits or domains and are submitted to the active site located in a subunit, which is related among all three types of ribonucleotide reductase. At the active site a conserved cysteine residue is converted to a thiyl radical, which initiates catalysis by hydrogen atom abstraction from C-3 of the ribose moiety of a ribonucleotide.[25,26]

In addition to the glycerol dehydratase from *C. butyricum*[4,5] mentioned in the introduction, there is a glycol dehydratase from *Clostridium glycolicum* that appears not to require coenzyme B_{12},[27,28] although this result needs to be confirmed by purification of the membrane-bound enzyme. The dehydration of *meso*-butane-2,3-diol to butan-2-ol mediated by *Lactobacillus brevii* may also be coenzyme B_{12} independent, because exchange of the migrating hydrogen atom occurs with the solvent.[29] Such an exchange is also observed in the SAM-dependent glycyl radical enzyme *C. butyricum* glycerol dehydratase,[4,5] but not in the coenzyme B_{12} dependent diol/glycerol dehydratases.[30] The *C. butyricum* glycerol dehydratase uses a thiyl radical to abstract a hydrogen atom from substrate glycerol. The thiyl radical is derived by the action of the glycyl radical in the enzyme from a cysteine thiol, which can exchange with solvent. This glycyl radical is formed by hydrogen atom abstraction from a conserved glycine within the enzyme by the 5′-deoxyadenosyl radical generated by one-electron transfer to SAM.[4] A coenzyme-independent eliminase was recently found in the biosynthesis of TDP-D-desosamine (TDP = thymidine diphosphate). In this pathway the hydroxyl group at C-4 of TDP-6-deoxy-D-glucose was converted to an amino group by oxidation and amino transfer from glutamate. The amino group is then eliminated to yield TDP-4,6-dideoxy-3-oxo-D-glucose catalysed by DesII. This enzyme contains a [4Fe-4S] cluster and requires *S*-adenosylmethionine as cofactor.[31] Hence the elimination can be described as a SAM-dependent ethanolamine ammonia lyase-related reaction.

There is a coenzyme B_{12} independent carbon-skeleton mutase, littorine mutase, found in the alkaloid producing plant *Datura stramonium* (*Solanaceae*). In the rearrangement of littorine to hyoscyamine an ester group migrates from a carbon bearing a hydroxyl group to the adjacent benzylic carbon. Hence both intermediate radicals are stabilised and therefore coenzyme B_{12} is unnecessary. The reaction mediated by hairy root extracts is stimulated up to 30-fold by SAM, suggesting a participation of this coenzyme as radical generator.[32] This important discovery agrees well with the absence of cobalamins from plants and with our proposal on the function of coenzyme B_{12} in carbon-skeleton mutases.

Alternative Coenzyme B_{12} Independent Pathways

No carbon-skeleton mutase devoid of coenzyme B_{12} has yet been discovered that catalyses a reaction via an intermediate methylene radical. We predict that such enzymes do not exist. Several organisms lacking coenzyme B_{12} are forced to use *alternative pathways* for the assimilation of propionate or fermentation of glutamate. They do not use a coenzyme B_{12} independent mutase analogous to the coenzyme B_{12} independent eliminases. A well-known example is the reversible interconversion of pyruvate with propionate (Scheme 4), which in animals and in many anaerobic bacteria proceeds through the coenzyme B_{12} dependent rearrangement of (*R*)-methylmalonyl-CoA to succinyl-CoA.[33] However, in a few



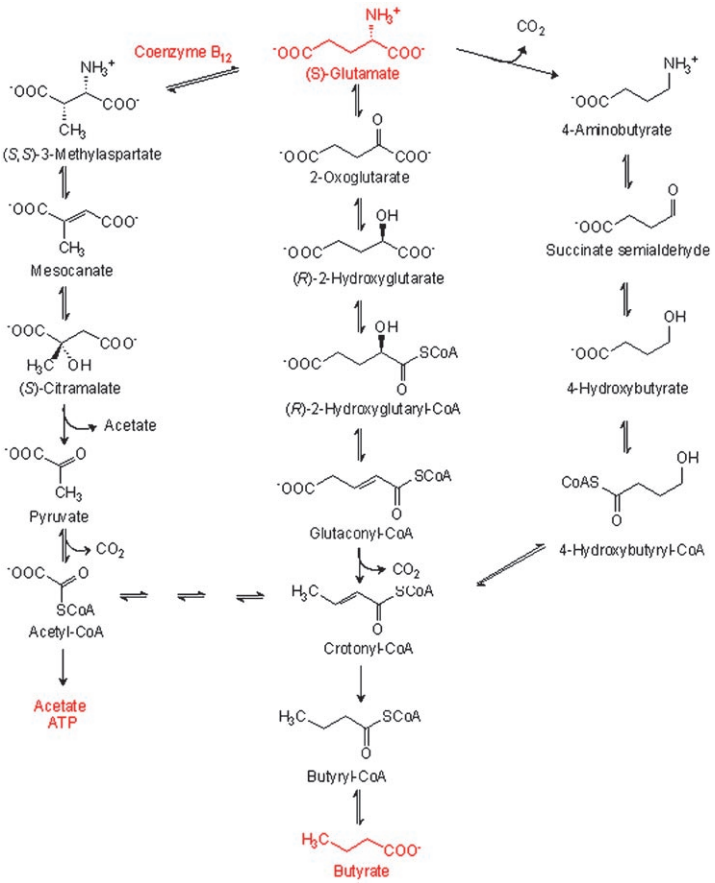
Scheme 4. Three pathways of propionate/pyruvate metabolism. Double half arrows indicate reversible reactions, full arrows irreversible reactions. Separate enzymes catalyse the irreversible carboxylation/decarboxylation reactions between propionyl-CoA/(*S*)-methylmalonyl-CoA and (*S*)-malate/pyruvate.

other anaerobes, such as *Clostridium propionicum*, pyruvate is reduced to lactate, which is activated to (*R*)-lactyl-CoA. Reversible dehydration to acryloyl-CoA ensues, followed by an irreversible NADH-dependent reduction to propionyl-CoA.[34] Notably the removal of a hydrogen atom from the methyl group of lactyl-CoA ($pK > 40$), during the dehydration to acryloyl-CoA, also requires radical chemistry. It has been proposed that the reaction proceeds via resonance-stabilised ketyl radicals (i.e., radical anions) rather than a much less stable methylene radical.[35,36]

The pathway via acryloyl-CoA has three drawbacks: it is irreversible in the direction of propionate formation,[34] acryloyl-CoA is toxic[37] and lactyl-CoA dehydratase is an extremely oxygen-sensitive enzyme.[38] Hence, aerobic bacteria such as enterobacteria and pseudomonads, as well as in fungi and probably plants, all of which lack coenzyme B_{12}, are forced to oxidise propionate by a different pathway. They utilise the methylcitrate cycle, which is also irreversible but in the opposite direction.[39] In this nonradical pathway the irreversible condensation of propionyl-CoA with the oxidising agent oxaloacetate to give (2*S*,3*S*)-methylcitrate results in an "Umpolung" of the α-carbon of propionate. After elimination of water from the methylcitrate to afford (*Z*)-2-methylaconitate, water returns but with the opposite regiochemistry to yield (2*R*,3*S*)-2-methylisocitrate.[40,41] In this way the propionate moiety of methylcitrate is oxidised to the pyruvate moiety of 2-methylisocitrate. The subsequent irreversible cleavage of this tricarboxylate leads to succinate and pyruvate. Finally succinate is oxidised via intermediates of the Krebs cycle to oxaloacetate.[42]

The reversible coenzyme B_{12} dependent pathway via methylmalonyl-CoA is employed for propionate formation as well as for its oxidation. Whereas propionibacteria use this pathway in order to dispose of reducing equivalents in glucose or glycerol fermentations, many aerobic organisms including animals and humans oxidise propionate to provide energy and to remove propionyl-CoA, an inhibitor of pyruvate dehydrogenase.[43] Why did humans acquire the methylmalonyl-CoA pathway rather than the methylcitrate cycle? We propose that the nervous system, with its high energy demand, could not have been developed in the presence of the methylcitrate cycle, because this would interfere with the Krebs cycle and diminish its rate of ATP synthesis or energy flow.[43] Notably both cycles share one enzyme, aconitase. In the Krebs cycle aconitase catalyses the dehydration of citrate, as well as the hydration of (*Z*)-aconitate to (2*R*,3*S*)-isocitrate. In the methylcitrate cycle this enzyme only mediates the hydration of (*Z*)-2-methylaconitate to (2*R*,3*S*)-2-methylisocitrate, whereas a different enzyme is required for the dehydration of (2*S*,3*S*)-methylcitrate.[40,41] Furthermore, (2*R*,3*S*)-2-methylisocitrate from the methylcitrate cycle is a potent inhibitor of the NADP-dependent isocitrate dehydrogenase of the Krebs cycle.[44]

Other examples of pathways alternative to those utilising coenzyme B_{12} occur in the fermentation of glutamate to ammonia, CO_2, acetate and butyrate by several anaerobic bacteria, all of which belong to the orders *Clostridiales* and *Fusobacteriales* (Scheme 5).[36] Like the dehydration of lactyl-CoA, a hypothetical β-elimination of ammonia from glutamate would require the removal of a proton from a nonacidic (C-3) methylene group. In *Clostridium tetani*, *C. tetanomorphum* and *C. cochlearium*, this problem is elegantly solved by using a coenzyme B_{12} dependent glutamate mutase, which catalyses the rearrangement of (*S*)-glutamate to (2*S*,3*S*)-3-methylaspartate. Now the β-elimination proceeds smoothly, because the hydrogen atom to be removed from 3-methylaspartate is adjacent to a carboxylate group. The resulting mesaconate (methylfumarate) is easily converted to acetate and pyruvate, which is oxidised to CO_2 and acetyl-CoA. In order to balance the reducing equivalents, some hydrogen is released, but most of the acetyl-CoA is reduced to butyrate.[45] In the alternative coenzyme B_{12} independent pathway occurring, inter alia, in *Acidaminococcus fermentans* (order *Clostridiales*) and *Fusobacterium nucleatum*, glutamate is oxidised to 2-oxoglutarate and reduced to (*R*)-2-hydroxyglutarate. Activation affords (*R*)-2-hydroxyglutaryl-CoA, which is dehydrated to (*E*)-glutaconyl-CoA in a similar manner to the conversion of (*R*)-lactyl-CoA to acryloyl-CoA. Hence, these reversible dehydratases

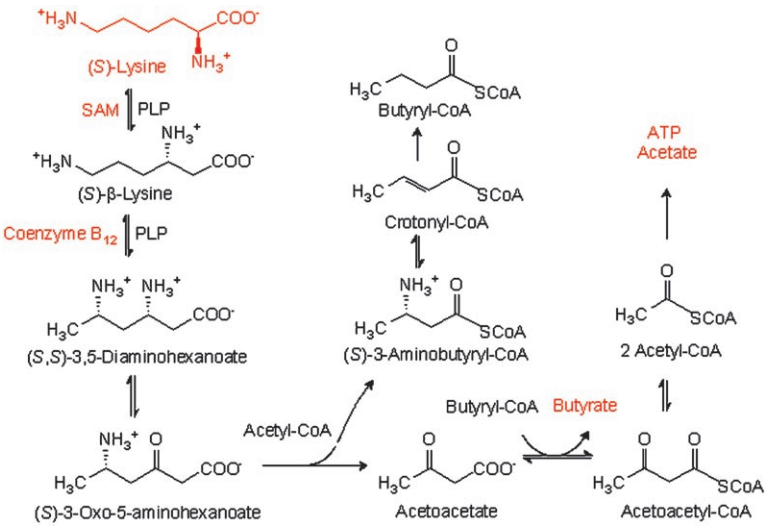


Scheme 5. Three pathways of glutamate fermentation to acetate and butyrate.

can be regarded as the mechanistic alternatives to the coenzyme B_{12} dependent carbon-skeleton mutases.[36] The further fate of (*E*)-glutaconyl-CoA involves a sodium-ion-dependent, energy-conserving, irreversible decarboxylation to crotonyl-CoA,[46,47] which disproportionates to acetate, butyrate and hydrogen.

A second coenzyme B_{12} independent pathway of glutamate degradation is initiated by its decarboxylation to 4-aminobutyrate (GABA) followed by the fermentation of this γ-amino acid to ammonia, acetate and butyrate by *Clostridium aminobutyricum*. Again oxidation and reduction exchanges the amino group with a hydroxyl group. The resulting 4-hydroxybutyrate is activated to 4-hydroxybutyryl-CoA and dehydrated to crotonyl-CoA, which disproportionates to acetate and butyrate.[45,48] Similar to the dehydrations of (*R*)-2-hydroxyacyl-CoAs, the β-*Si*-hydrogen atom ($pK \geq 40$) of 4-hydroxybutyryl-CoA has to be activated by radical anion chemistry.[49,50] The coenzyme B_{12} independent fermentation of 4-aminobutyrate can also be regarded as an alternative to the coenzyme B_{12} dependent fermentation of lysine in *Clostridium subterminale* (Scheme 6). In this pathway the coenzyme B_{12} dependent β-lysine-5,6-aminomutase, also called D-lysine-5,6-aminomutase,[51,52] is responsible for the downstream formation of 3-aminobutyryl-CoA, which is readily deaminated to crotonyl-CoA.[53] The absence of this mutase would result in the conversion of β-lysine to 4-aminobutyryl-CoA, which could also be processed to crotonyl-CoA through the dehydration of 4-hydroxybutyryl-CoA.

A direct alternative to the coenzyme B_{12} dependent β-lysine-5,6-aminomutase appears to be the SAM-dependent α-lysine-2,3-aminomutase,[8,54,55] which is also present in *C. subterminale* (Scheme 6). In both reversible reactions starting from β-lysine or α-lysine, substrate-derived methine radicals are formed by hydrogen-atom abstraction mediated by the 5′-deoxyadenosyl radical.[56] The distinguishing feature of these two mutase reactions is the different stabilities of the product-related radicals obtained after the migration of the amino group, which is bound to pyridoxal-5′-phosphate (PLP) as a Schiff's base. Both reactions probably occur via an aza-cyclopropylmethine radical that is resonance stabilised by the adjacent pyridoxalimine moiety. The product radical in the coenzyme B_{12} dependent reaction is a methylene radical, whereas the SAM-dependent reaction leads to a stabilised α-carboxyl radical, which can be detected by EPR spectroscopy.[56] We propose that the reversible generation of the methylene radical from the substrate-derived radical requires coenzyme B_{12} to stabilise it and to lower the transition-state energy leading to this radical. Like β-lysine-5,6-aminomutase the coenzyme B_{12} dependent ornithine-4,5-aminomutase reaction[57] also involves an intermediate methylene radical. Furthermore, all four coenzyme B_{12} dependent carbon-skeleton mutases proceed via reversibly formed methylene radicals.



Scheme 6. Fermentation of lysine to butyrate and acetate by *Clostridium subterminale* and *Fusobacterium nucleatum*; PLP = pyridoxal-5′-phosphate.

Mechanisms of Ethanolamine Ammonia Lyase and Diol Dehydratase

The reactions mediated by ethanolamine ammonia lyase and diol dehydratase (with ethane-1,2-diol as substrate) also involve methylene radicals as precursors of the methyl group of the common product acetaldehyde [Eqs. (1) and (2)].[58] In both cases the methylene radicals are formed by rearrangement of the substrate-derived radicals and are irreversibly trapped by hydrogen-atom transfer from 5′-deoxyadenosine or a thiol group (the latter only in SAM-dependent glycerol dehydratase). Considering the detailed reaction pathways of the diol dehydratases and the mutases leads us to suggest that the intermediate methylene radicals of the mutases are stabilised by cob(II)alamin.

The eliminases catalyse the irreversible replacement of a leaving group X (OH or NH_3^+) by hydrogen in substrates with a primary or secondary hydroxyl group on the adjacent carbon atom. Initially a thiyl or 5′-deoxyadenosyl radical abstracts the hydrogen atom on this carbon atom leading to a carbon-centred radical, which lowers the p*K* of the attached hydroxyl group by about 5 units and thus facilitates its deprotonation.[10] This is relevant to the "push–pull" mechanism for diol dehydratase, which emerged from advanced ab initio molecular orbital calculations.[59,60] The substrate-derived radical (e.g., 1,2-dihydroxyethyl), held at the active site by coordination to a potassium ion and by interactions with four protein residues including glutamate

(Gluα170) and histidine (Hisα143), is partially deprotonated at the nonmigrating hydroxyl group by the glutamate and simultaneously partially protonated at the migrating hydroxyl group by the histidine imidazole. This arrangement provides a low-energy reaction pathway via a bridged transition state that lies only 7.5 kJ mol^{-1} above the substrate radical and 2.7 kJ mol^{-1} above the product radical (e.g., 2,2-dihydroxyethyl, according to calculations performed with the model acid–base pair of ammonium and ammonia).[59] In the diol dehydratases and ethanolamine ammonia lyase it is important that the product radical is a methylene radical, because this is able to remove a hydrogen atom from the transiently formed 5′-deoxyadenosine, whereby the starting 5′-deoxyadenosyl radical is regenerated. In a variant of this mechanism,[61] applied to ribonucleotide reductases, formation of a radical at C-3 leads to a ketyl radical anion by deprotonation of the C-3 hydroxyl group. This could act as a nucleophile initiating the elimination of the adjacent C-2 hydroxyl group. Two-electron reduction of the enoxy radical thus formed leads to a product-related ketyl radical anion, to which the initially abstracted proton and hydrogen atom are returned.

To generate substrate-derived radicals in the eliminase diol dehydratase, the carbon–cobalt bond of coenzyme B_{12} homolyses, the ribose moiety of the 5′-deoxyadenosyl radical flips over by rotation around the *N*-glycosidic bond and moves towards the substrate diol bound approximately 10 Å from the cobalt atom of cob(II)alamin.[62] The 5′-deoxyadenosyl radical can now abstract a hydrogen atom from the substrate. Any significant interaction of Co^{II} with the substrate-derived or product-related radicals is thus impossible and in any case is not necessary to drive the rearrangement. The function of cob(II)alamin is that of a mere spectator. There is no reason why the 5′-deoxyadenosyl radical cannot be derived from another source, like SAM, or be replaced by other radicals, such as the thiyl radical found in ribonucleotide reductase and the glycyl radical enzymes.

Mechanism of the Coenzyme B_{12} Dependent Mutases

All six known mutases have in common that a hydrogen atom has to be *reversibly* abstracted from an unactivated methyl group to yield a methylene radical, which is not stabilised by any adjacent group. In the case of glutamate mutase both partners of the interconversion, (*S*)-glutamate and (2*S*,3*S*)-3-methylaspartate, are held in the active site by three arginine residues and one glutamate, approximately 6 Å apart from the cobalt atom;[18] this arrangement has been termed the "arginine claw".[63] Abstraction of a hydrogen atom from the 4-*Si* position of glutamate by the 5′-deoxyadenosyl radical yields the 4-glutamyl radical, which fragments to acrylate and a glycyl radical still fixed in the arginine claw.[64,65] A hand-over of the acrylate bound at one arginine to another[66] brings the two partners into a configuration favouring recombination to the 3-methyleneaspartate radical, which finally re-abstracts a hydrogen atom from the methyl group of 5′-deoxyadenosine to afford 3-methylaspartate. Considering the substrate-derived and product-related radicals in this mechanism, only the 4-glutamyl radical can be stabilised by the neighbouring carboxylate and is observed by EPR spectroscopy.[67,68]

There are several model reactions for coenzyme B_{12} dependent mutases, which demonstrate the intrinsic ability of free methylene radicals to rearrange to methine radicals in the absence of the coenzyme. As a model for methylmalonyl-CoA mutase the photochemically generated 3-ethoxy-2-(ethoxycarbonyl)-3-oxopropyl radical rearranged to the corresponding succinyl derivative. Performing the reaction in the presence of zeolite mimicked the active cavity of an enzyme and dramatically increased the yield from 2.2 to 8.1 %.[69] This important result apparently contradicts our postulate for the requirement of coenzyme B_{12} for the rearrangement of methylene radicals. This model reaction, however, is a completely irreversible process and therefore cannot be compared with the reversible enzyme-catalysed formation and rearrangement of a methylene radical.

In contrast to the diol dehydratases, the problem faced by glutamate mutase is how to surmount the two relatively high transition-state energy barriers (calculated as $\Delta H = +59.9$ and $+66.5$ kJ mol^{-1}[70]) connecting the 4-glutamyl radical via acrylate and a glycyl radical (see above) to the 3-methylene-aspartate radical, which is significantly less stable than the resonance-stabilised 4-glutamyl radical ($\Delta H = +20.3$ kJ mol^{-1}[70]). Similarly, with 2-methyleneglutarate mutase[71] and methylmalonyl-CoA mutase,[72] the radicals are interconnected via relatively high-energy transition states and the methylene radicals are much less stable than the methine radicals adjacent to the carboxylates.

From high-resolution X-ray crystallography it can be deduced that the formation of the radicals in glutamate mutase follows three steps.[73] Binding of the coenzyme to the enzyme is achieved by base-exchange "base-off, his-on" without affecting the cobalt–carbon bond length of 2.0 Å.[74,75] Addition of the substrate (*S*)-glutamate or (2*S*,3*S*)-methylaspartate leads to the opening of a cavity into which the adenosyl moiety of the coenzyme fits very well. The attraction of the adenosyl moiety into the cavity elongates the cobalt–carbon bond to 3.2 Å (Figure 2, left). Pseudorotation of the ribose moiety of the 5′-deoxyadenosyl radical shifts the 5′-carbon atom towards the substrate with a further elongation of the Co–C σ bond to 4.2 Å (Figure 2, right). Hence, there seems at this point to be no *complete* homolysis of the Co–C σ bond in contrast to the situation with the eliminase diol dehydratase (see above). Probably, it is now that hydrogen abstraction occurs from C-4 of glutamate (H-*Si*) to yield the 4-glutamyl radical or from the methyl group of 3-methylaspartate leading to the 3-methyleneaspartate radical as shown in Figure 3. A critical question is the extent to which there is an interaction between the cobalt atom, the 5′-carbon of the adenosyl residue and the developing radical at C-4 of glutamate or at the methyl group of 3-methylasparatate. A possible interaction is shown

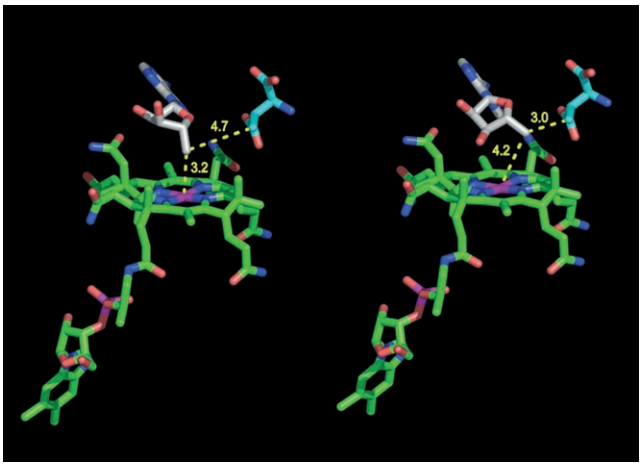


Figure 2. Crystal structure of glutamate mutase in action showing only the coenzyme B_{12} in the base-off form and (2*S*,3*S*)-3-methylaspartate. The carbon atoms of cobalamin are green, those of the 5′-deoxyadenosine radical are in grey and those of 3-methylaspartate are in light blue; all nitrogen atoms are in dark blue; oxygen atoms are in red; the phosphorus and the cobalt atoms are in magenta. Left: By substrate-induced conformational change a pocket of the protein is opened, into which the adenine moiety of the 5′-deoxyadenosine residue is moved thus causing the elongation of the carbon–cobalt bond from 2.0 to 3.2 A. Right: Pseudorotation of the ribose ring brings the 5′-carbon atom closer to the methyl group of 3-methylaspartate, but still in orbital overlap with the cobalt. Note that the adenine moiety retains its position in the pocket.

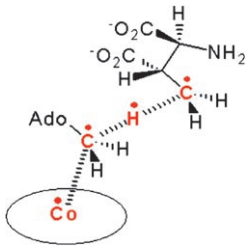


Figure 3. Schematic representation of the hydrogen abstraction step from 3-methylaspartate performed by the 5′-deoxyadenosyl radical (for simplicity only the cobalt ion of cob(II)alamin is shown). A similar arrangement with methylmalonyl-CoA mutase is depicted as structure 57 in a recent review.[2]

qualitatively in Figure 3. It is also necessary to consider how the intermediate state of the glycyl radical and acrylate could be stabilised. The contribution of cob(II)alamin is probably not restricted to the cobalt ion alone, but could involve the corrin π-system. It is notable that the X-ray structure of methylmalonyl-CoA mutase[17] indicates a very similar pattern to that described for glutamate mutase.

Reversible Formation and Stabilisation of Methylene Radicals

In the arrangement shown in Figure 3, a hydrogen atom moves between two carbon atoms still interacting with the d orbitals of Co^{II} and the π system of the corrin. Thus, cob(II)alamin could stabilise a transition state in which a hydrogen atom bridges the 5-deoxyadenosyl moiety and the substrate, intermediate or product radicals. This will be especially beneficial for the 3-methyleneaspartate radical, which is about 0.5 Å closer to the cobalt than the 4-glutamyl radical. An interaction of the substrate-derived radical with cob(II)alamin is clearly seen in the EPR spectrum of an incubation of glutamate mutase, coenzyme B_{12} and (*S*)-glutamate or (2*S*,3*S*)-3-methylaspartate stopped by freezing with liquid nitrogen during steady state. The main signal of cob(II)alamin at $g=2.3$ and of the organic radical at $g=2.0$ merge to a new signal at $g=2.1$.[68] Similar observations have been made with methylmalonyl-CoA mutase[76] and 2-methyleneglutarate mutase.[77] The organic radical partner in these spectra is always the most stable glutamate 4-yl radical and succinyl-CoA 3-yl or possibly 2-methyleneglutarate 4-yl radicals, respectively, whereas the corresponding methylene radicals cannot be seen, certainly due to their low steady-state concentrations. Probably ultra fast quenching techniques are required to detect these radicals.[78] That none of these radicals are "free" is also demonstrated by the relatively low oxygen sensitivity of the reactions catalysed by all three mutases.

The enthalpy of hydrogen abstraction by the 5′-deoxyadenosyl radical from the methyl group of 3-methylaspartate has been calculated as $\Delta H=0$ kJ mol^{-1}.[70] The barrier for this conversion was estimated as about 63 kJ mol^{-1} from the large isotope effect observed for the hydrogen transfer from 5′-deoxyadenosine to the product methylene radical in ethanolamine ammonia lyase.[79,80] With glutamate mutase, the overall primary deuterium isotope effects measured with [4-$^2H_2$]glutamate and 3-[*methyl*-$^2H_3$]methylaspartate were the same in both directions ($^DV=7\pm1$),[67,81] whereas hydrogen abstraction from an isolated methyl group should be more difficult than from a methylene group adjacent to a carboxylate moiety. This observation is consistent with the proposed stabilisation of the 3-methyleneaspartate radical by cob(II)alamin and may result in an activation energy significantly less than 63 kJ mol^{-1}. Recent experiments indicated that the primary isotope effect for the first deuterium transfer from [4-$^2H_2$]glutamate to yield 5′-deoxy[5′-$^2H_1$]adenosine was much smaller ($^DV=2.4\pm0.4$), whereas the higher overall isotope effect ($^DV=7\pm1$) was mainly attributed to a large secondary equilibrium isotope effect resulting from the labelling of the 5′-carbon atom of deoxyadenosine with deuterium during turnover.[82] This new finding supports our idea that not only hydrogen abstraction from the substrate but also homolysis of the cobalt-carbon-bond contributes to the rate-limiting step (Figure 3).

Thus, it is proposed that cob(II)alamin functions as a conductor in the mutases, an idea that has often been considered in B_{12} research,[83] although cob(II)alamin is now generally downgraded to mere spectator.[10,11] The proposals in this paper regarding hydrogen-atom bridging and cob(II)alamin are presented in order to stimulate further theoretical and experimental studies. In summary, other radical generators can substitute coenzyme B_{12} in ribonucleotide reductase, diol dehydratases and ethanolamine ammonia lyase. This is impossible for the coenzyme B_{12} dependent mutases and so alternative pathways exist leading to the same products as those with the mutases. Since the alternative pathways use the much simpler primordial cofactors, such as flavins and iron–sulfur clusters, they could have evolved before the more elaborate mutases, which manage to catalyse the

most difficult of all enzymatic reactions. The absence of coenzyme B_{12} in the biosynthetic pathways of all other cofactors—a possible exception could be the cobalamin-dependent isocyclic ring formation in anaerobic chlorophyll biosynthesis[84]—supports the idea of its more recent emergence during the origin of life (but see reference [85]). The biosyntheses of biotin and lipoic acid, which are both sulfur-containing cofactors, involve hydrogen-atom abstraction from unactivated methyl groups by 5′-deoxyadenosyl radicals derived from SAM. The reactions, however, lead to irreversible formation of stoichiometric amounts of 5′-deoxyadenosine and the resulting methylene radicals are trapped by insertion of sulfur from an iron–sulfur cluster of the protein.[86,87] Therefore, according to our hypothesis, stabilisation of the methylene radicals by interaction with cob(II)alamin is not required.

Despite all the ways for circumventing coenzyme B_{12}, it seems that human life without B_{12} is impossible because methylmalonyl-CoA mutase, a carbon-skeleton mutase, requires assistance from cob(II)alamin. Although the methylcitrate cycle provides a route, in principle, to circumvent coenzyme B_{12} dependent methylmalonyl-CoA mutase, this would have interfered with the Krebs cycle and probably resulted in a lower rate of energy production. Hence, cells using the methylcitrate cycle for propionate oxidation might have been unable to develop into high-energy-demanding neurons. These considerations highlight the bacterial coenzyme B_{12} as a prerequisite for human evolution.

Acknowledgements

The authors thank Leo Radom (University of Sydney, Australia) and David Smith (University of Zagreb, Croatia) for helpful discussions. This work was funded by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, the European Commission (Contract No HPRN-CT-2002-00195) and the Biotechnology Development Center, Cargill Incorporated, Minneapolis, MN (USA).

[1] R. Banerjee, *Chemistry and Biochemistry of B_{12}*, Wiley, New York, **1999**.
[2] K. L. Brown, *Chem. Rev.* **2005**, *105*, 2075.
[3] R. G. Finke, B. D. Martin, *J. Inorg. Biochem.* **1990**, *40*, 19.
[4] C. Raynaud, P. Sarcabal, I. Meynial-Salles, C. Croux, P. Soucaille, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5010.
[5] J. R. O'Brien, C. Raynaud, C. Croux, L. Girbal, P. Soucaille, W. N. Lanzilotta, *Biochemistry* **2004**, *43*, 4635.
[6] J. J. Baker, T. C. Stadtman, in *B_{12}, Vol. 2: Biochemistry and Medicine* (Ed.: D. Dolphin), Wiley-Interscience, New York, **1982**, p. 203.
[7] W. Buckel, B. T. Golding, *Chem. Soc. Rev.* **1996**, *25*, 329.
[8] P. A. Frey, C. H. Chang, in *Chemistry and Biochemistry of B_{12}* (Ed.: R. Banerjee), Wiley, New York, **1999**, p. 835.
[9] Swissprot, http://www.expasy.ch/enzyme/, **2005**.
[10] B. T. Golding, L. Radom, *J. Am. Chem. Soc.* **1976**, *98*, 6331.
[11] J. Halpern, *Science* **1985**, *227*, 869.
[12] C. C. Lawrence, G. J. Gerfen, V. Samano, R. Nitsche, M. J. Robins, J. Rétey, J. Stubbe, *J. Biol. Chem.* **1999**, *274*, 7039.
[13] N. Shibata, J. Masuda, T. Tobimatsu, T. Toraya, K. Suto, Y. Morimoto, N. Yasuoka, *Structure* **1999**, *7*, 997.
[14] A. Abend, V. Illich, J. Rétey, *Eur. J. Biochem.* **1997**, *249*, 180.
[15] E. N. Marsh, D. E. Holloway, *FEBS Lett.* **1992**, *310*, 167.
[16] O. Zelder, B. Beatrix, F. Kroll, W. Buckel, *FEBS Lett.* **1995**, *369*, 252.
[17] F. Mancia, N. H. Keep, A. Nakagawa, P. F. Leadlay, S. McSweeney, B. Rasmussen, P. Bosecke, O. Diat, P. R. Evans, *Structure* **1996**, *4*, 339.
[18] R. Reitzer, K. Gruber, G. Jogl, U. G. Wagner, H. Bothe, W. Buckel, C. Kratky, *Structure* **1999**, *7*, 891.
[19] L. Poppe, H. Bothe, G. Bröker, W. Buckel, E. Stupperich, J. Rétey, *J. Mol. Catal. B* **2000**, *10*, 345.
[20] F. Berkovitch, E. Behshad, K. H. Tang, E. A. Enns, P. A. Frey, C. L. Drennan, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15870.
[21] C. L. Drennan, S. Huang, J. T. Drummond, R. G. Matthews, M. L. Ludwig, *Science* **1994**, *266*, 1669.
[22] M. Vlasie, S. Chowdhury, R. Banerjee, *J. Biol. Chem.* **2002**, *277*, 18523.
[23] H. A. Barker, *Methods Enzymol.* **1969**, *13*, 319.
[24] M. Kolberg, K. R. Strand, P. Graff, K. K. Andersson, *Biochim. Biophys. Acta* **2004**, *1699*, 1.
[25] J. Stubbe, M. Ator, T. Krenitsky, *J. Biol. Chem.* **1983**, *258*, 1625.
[26] S. Licht, G. J. Gerfen, J. A. Stubbe, *Science* **1996**, *271*, 477.
[27] M. G. Hartmanis, T. C. Stadtman, *Arch. Biochem. Biophys.* **1986**, *245*, 144.
[28] M. G. Hartmanis, T. C. Stadtman, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 76.
[29] G. Speranza, W. Buckel, B. T. Golding, *J. Porphyrins Phthalocyanines* **2004**, *8*, 290.
[30] T. Toraya, in *Chemistry and Biochemistry of B_{12}* (Ed.: R. Banerjee), Wiley, New York, **1999**, p. 783.
[31] P.-H. Szu, X. He, L. Zhao, H.-W. Liu, *Angew. Chem.* **2005**, *117*, 6900; *Angew. Chem. Int. Ed.* **2005**, *44*, 6742.
[32] S. Ollagnier, E. Kervio, J. Rétey, *FEBS Lett.* **1998**, *437*, 309.
[33] R. Banerjee, *Chem. Rev.* **2003**, *103*, 2083.
[34] M. Hetzel, M. Brock, T. Selmer, A. J. Pierik, B. T. Golding, W. Buckel, *Eur. J. Biochem.* **2003**, *270*, 902.
[35] W. Buckel, M. Hetzel, J. Kim, *Curr. Opin. Chem. Biol.* **2004**, *8*, 462.
[36] J. Kim, M. Hetzel, C. D. Boiangiu, W. Buckel, *FEMS Microbiol. Rev.* **2004**, *28*, 455.
[37] G. Herrmann, T. Selmer, H. J. Jessen, R. R. Gokarn, O. Selifonova, S. J. Gort, W. Buckel, *FEBS Lett.* **2005**, *272*, 813.
[38] R. D. Kuchta, R. H. Abeles, *J. Biol. Chem.* **1985**, *260*, 13181.
[39] S. Textor, V. F. Wendisch, A. A. De Graaf, U. Müller, M. I. Linder, D. Linder, W. Buckel, *Arch. Microbiol.* **1997**, *168*, 428.
[40] A. R. Horswill, J. C. Escalante-Semerena, *Biochemistry* **2001**, *40*, 4703.
[41] M. Brock, C. Maerker, A. Schütz, U. Völker, W. Buckel, *Eur. J. Biochem.* **2002**, *269*, 6184.
[42] M. Brock, D. Darley, S. Textor, W. Buckel, *Eur. J. Biochem.* **2001**, *268*, 3577.
[43] M. Brock, W. Buckel, *Eur. J. Biochem.* **2004**, *271*, 3227.
[44] R. L. Beach, T. Aogaichi, G. W. Plaut, *J. Biol. Chem.* **1977**, *252*, 2702.
[45] W. Buckel, *Appl. Microbiol. Biotechnol.* **2001**, *57*, 263.
[46] W. Buckel, *Biochim. Biophys. Acta* **2001**, *1505*, 15.
[47] K. S. Wendt, I. Schall, R. Huber, W. Buckel, U. Jacob, *EMBO J.* **2003**, *22*, 3493.
[48] W. Buckel, B. M. Martins, A. Messerschmidt, B. T. Golding, *Biol. Chem.* **2005**, *386*, October.
[49] W. Buckel, B. T. Golding, *FEMS Microbiol. Rev.* **1999**, *22*, 523.
[50] B. M. Martins, H. Dobbek, I. Cinkaya, W. Buckel, A. Messerschmidt, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15645.
[51] T. C. Stadtman, L. Tsai, *Biochem. Biophys. Res. Commun.* **1967**, *28*, 920.
[52] C. H. Chang, P. A. Frey, *J. Biol. Chem.* **2000**, *275*, 106.
[53] I. M. Jeng, H. A. Barker, *J. Biol. Chem.* **1974**, *249*, 6578.
[54] T. P. Chirpich, V. Zappia, R. N. Costilow, H. A. Barker, *J. Biol. Chem.* **1970**, *245*, 1778.
[55] J. Baraniak, M. L. Moss, P. A. Frey, *J. Biol. Chem.* **1989**, *264*, 1357.
[56] P. A. Frey, S. J. Booker, *Adv Protein Chem.* **2001**, *58*, 1.
[57] Y. Tsuda, H. C. Friedmann, *J. Biol. Chem.* **1970**, *245*, 5914.

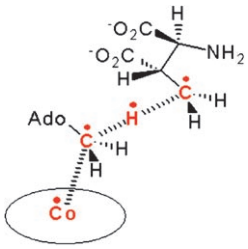
[58] T. J. Carty, B. M. Babior, R. H. Abeles, *J. Biol. Chem.* **1971**, *246*, 6313.
[59] D. M. Smith, B. T. Golding, L. Radom, *J. Am. Chem. Soc.* **2001**, *123*, 1664.
[60] T. Toraya, M. Eda, T. Kamachi, K. Yoshizawa, *J. Biochem.* **2001**, *130*, 865.
[61] W. Buckel, *FEBS Lett.* **1996**, *389*, 20.
[62] J. Masuda, N. Shibata, Y. Morimoto, T. Toraya, N. Yasuoka, *Structure* **2000**, *8*, 775.
[63] W. Buckel, G. Bröker, H. Bothe, A. J. Pierik, in *Chemistry and Biochemistry of B_{12}* (Ed.: R. Banerjee), Wiley, New York, **1999**, p. 757.
[64] B. Beatrix, O. Zelder, F. Kroll, G. Örlygsson, B. T. Golding, W. Buckel, *Angew. Chem.* **1995**, *107*, 2573; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2398.
[65] H. W. Chih, E. N. G. Marsh, *J. Am. Chem. Soc.* **2000**, *122*, 10732.
[66] A. J. Pierik, D. Ciceri, G. Bröker, C. H. Edwards, W. McFarlane, J. Winter, W. Buckel, B. T. Golding, *J. Am. Chem. Soc.* **2002**, *124*, 14039.
[67] O. Zelder, B. Beatrix, U. Leutbecher, W. Buckel, *Eur. J. Biochem.* **1994**, *226*, 577.
[68] H. Bothe, D. J. Darley, S. P. Albracht, G. J. Gerfen, B. T. Golding, W. Buckel, *Biochemistry* **1998**, *37*, 4105.
[69] Y. Chen, E. Kervio, J. Rétey, *Helv. Chim. Acta* **2002**, *85*, 552.
[70] S. D. Wetmore, D. M. Smith, B. T. Golding, L. Radom, *J. Am. Chem. Soc.* **2001**, *123*, 7963.
[71] D. M. Smith, B. T. Golding, L. Radom, *J. Am. Chem. Soc.* **1999**, *121*, 1037.
[72] D. M. Smith, B. T. Golding, L. Radom, *J. Am. Chem. Soc.* **1999**, *121*, 1383.
[73] K. Gruber, R. Reitzer, C. Kratky, *Angew. Chem.* **2001**, *113*, 3481; *Angew. Chem. Int. Ed.* **2001**, *40*, 3377.
[74] L. Ouyang, P. Rulis, W. Y. Ching, G. Nardin, L. Randaccio, *Inorg. Chem.* **2004**, *43*, 1235.
[75] K. P. Jensen, U. Ryde, *J. Am. Chem. Soc.* **2005**, *127*, 9117.
[76] S. O. Mansoorabadi, R. Padmakumar, N. Fazliddinova, M. Vlasie, R. Banerjee, G. H. Reed, *Biochemistry* **2005**, *44*, 3153.
[77] A. J. Pierik, D. Ciceri, R. F. Lopez, F. Kroll, G. Bröker, B. Beatrix, W. Buckel, B. T. Golding, *Biochemistry* **2005**, *44*, 10541.
[78] A. V. Cherepanov, S. De Vries, *Biochim. Biophys. Acta* **2004**, *1656*, 1.
[79] D. A. Weisblat, B. M. Babior, *J. Biol. Chem.* **1971**, *246*, 6064.
[80] M. Semialjac, H. Schwarz, *J. Org. Chem.* **2003**, *68*, 6967.
[81] R. G. Eagar, Jr., M. M. Herbst, H. A. Barker, J. H. Richards, *Biochemistry* **1972**, *11*, 253.
[82] M. C. Cheng, E. N. Marsh, *Biochemistry* **2005**, *44*, 2686.
[83] J. M. Pratt, in *Chemistry and Biochemistry of B_{12}* (Ed.: R. Banerjee), Wiley, New York, **1999**, p. 113.
[84] S. P. Gough, B. O. Petersen, J. Ø. Duus, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6908.
[85] A. Eschenmoser, *Angew. Chem.* **1988**, *100*, 5; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 5.
[86] A. Marquet, B. T. Bui, D. Florentin, *Vitam. Horm.* **2001**, *61*, 51.
[87] R. M. Cicchillo, S. J. Booker, *J. Am. Chem. Soc.* **2005**, *127*, 2860.
[88] J. J. Baker, I. Jeng, H. A. Barker, *J. Biol. Chem.* **1972**, *247*, 7724.

Published online: ■■ ▮▮, 2005

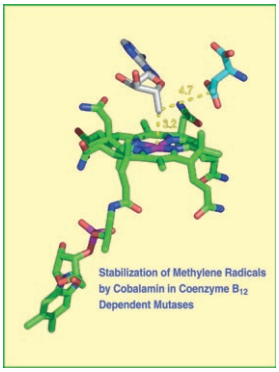
Biochemistry

W. Buckel, C. Kratky,*
*B. T. Golding** ..... ■■■■-■■■■

Stabilisation of Methylene Radicals by Cob(II)alamin in Coenzyme B_{12} Dependent Mutases



Cob(II)alamin may not only be a spectator in coenzyme B_{12} dependent reactions. Evidence is presented that for carbon skeleton rearrangements and amino group 1,2-migrations cob(II)alamin may stabilise transition states and intermediate methylene radicals in the manner as indicated in the figure.



Coenzyme B_{12}
Coenzyme B_{12} initiates radical chemistry in two types of enzymatic reactions, the irreversible eliminases and the reversible mutases. In their Concept on page ■■ ff., W. Buckel, B. T. Golding, and C. Kratky suggested that cob(II)alamin acts as a conductor by stabilising both the 5′-deoxyadenosyl radical and the product-related methylene radicals in coenzyme B_{12} dependent mutases.

