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Organometallic Chemistry of B₁₂ Coenzymes

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ABSTRACT: When coenzyme B₁₂ was identified as organometallic derivative of vitamin B₁₂, metal-carbon bonds were revealed to be relevant in life processes. Vitamin B₁₂, the “antipernicious anaemia factor” required for human health, was isolated earlier as a crystallizable cyano-Co(III)-complex. B₁₂ cofactors and other cobalt corrinoids play important roles not only in humans, but in the metabolism of archaea and other microorganisms, in particular. Indeed, the microorganisms are the only natural sources of the B₁₂ derivatives. For other B₁₂-requiring organisms the corrinoids are thus “vitamins”. However, vitamin B₁₂ also needs to be converted into organometallic B₁₂-forms, which are the typical coenzymes in metabolically important enzymes. One of these, methionine synthase, catalyzes the transfer of a methyl group and its corrinoid cofactor is methylcobalamin. Another one, methylmalonyl-CoA mutase uses a reversible radical process, and coenzyme B₁₂ (adenosylcobalamin) as its cofactor, to transform methylmalonyl-CoA into succinyl-CoA. In such enzymes, the bound B₁₂ derivatives engage (or are formed) in exceptional organometallic enzymatic reactions, which depend upon the organometallic reactivity of the B₁₂ cofactors. Clearly, organometallic B₁₂ derivatives hold an important position in life and have thus attracted particular interest from the medical sciences, biology, and chemistry. This chapter outlines the unique structures of B₁₂ derivatives and recapitulates their redox properties and their organometallic chemistry, relevant in the context of the metabolic transformation of B₁₂ derivatives into the relevant coenzyme forms and for their use in B₁₂-dependent enzymes.

KEYWORDS: cobalt-carbon bond · cobalt complex · coenzyme B₁₂ · electrochemistry · homolysis · methyl group transfer · organometallic bond · radical reaction · vitamin B₁₂

1. INTRODUCTION

The importance of a metal-carbon bond in enzymatic processes was first revealed in the early 1960s, when coenzyme B₁₂ was identified as organometallic derivative of vitamin B₁₂ [1]. Vitamin B₁₂ was discovered and isolated 60 years ago as a crystallizable, red complex [2,3], and was revealed to be a cobalt complex of the remarkable corrin ligand, a unique member of the natural tetrapyrroles [4]. Organometallic B₁₂ forms are the coenzymes in a variety of metabolically important enzymes. In humans, methionine synthase and methylmalonyl-CoA mutase use methylcobalamin and coenzyme B₁₂, respectively, as their B₁₂ cofactors [5–12].

B₁₂ coenzymes are now known to be required in the metabolism of a broad range of organisms. However, only microorganisms have the capacity

to biosynthesize B₁₂ and other natural corrinoids [13,14]. For other B₁₂-dependent organisms, such as humans, B₁₂ derivatives are thus vitamins [15]. Their functioning metabolism depends on the uptake and binding of B₁₂ derivatives [16], on their metabolic transformation into the relevant B₁₂ cofactors [17], the controlled transport of these [16] and the catalysis by B₁₂-dependent enzymes [18–23].

Perhaps B₁₂ coenzymes are Nature's physiologically most relevant organometallic cofactors [9–12]. Organometallic B₁₂ derivatives engage in protein-activated reactions in unique B₁₂-dependent enzymes. In these, B₁₂ cofactors (co)catalyze exceptional enzymatic reactions [18–23] that directly depend upon the reactivity of the cobalt-carbon bond [11,12,22]. Since the surprising identification of coenzyme B₁₂ as an organometallic compound, corrinoid cofactors have thus played central roles in the discovery and better understanding of biological organometallic processes [24–28].

Vitamin B₁₂ was identified as the “antipernicious anemia factor” over 60 years ago. It was isolated as the red cyanide-containing cobalt complex cyanocob(III)alamin (**1**, CNCbl), which crystallized readily and was revealed to be a relatively inert Co(III) complex [2,3]. It is the most important commercially available form of the naturally occurring B₁₂ derivatives, but it appears to have no physiological function itself [15]. The physiologically relevant vitamin B₁₂ derivatives are the highly light-sensitive and chemically more labile organometallic coenzymes, coenzyme B₁₂ (**2**, 5'-deoxy-5'-adenosylcobalamin, AdoCbl) and methylcobalamin (**3**, MeCbl), as well as the “inorganic” and easily reducible B₁₂ derivatives aquacob(III)alamin (**4**⁺, H₂OCbl⁺) and hydroxocob(III)alamin (**5**, HOCbl) [6,29,30] (Figure 1).

During the last six decades, remarkable scientific advances towards the solution of some of the major “B₁₂-mysteries” have been achieved. Five European Symposia on “Vitamin B₁₂ and B₁₂-Proteins” were dedicated to this subject, the first two of which took place in Hamburg (1956 and 1961, Germany), followed by one in Zürich (1979, Switzerland) [7], in Innsbruck (1996, Austria) [9], and in Marburg (2000, Germany). Among the top achievements in the B₁₂ field the elucidation of the structure of vitamin B₁₂ [4,31] and of coenzyme B₁₂ [1,32] are to be highlighted, the synthetic conquest of the vitamin B₁₂ structure [33–35], the elucidation of the biosynthetic pathways to B₁₂ [13,14], as well as crystal structures [36–44] and a solution structure [45] of a variety of B₁₂-binding proteins and B₁₂-dependent enzymes.

Several concise books on B₁₂ have been written, with earlier ones by Pratt [5] and by Friedrich [6]. More recent ones on “B₁₂” [7], “Vitamin B₁₂ and B₁₂-Proteins” [9] and on “Chemistry and Biochemistry of B₁₂” [10] and extensive reviews [11,12] describe the more recent findings on the chemistry of B₁₂ and on the biological roles of the B₁₂ derivatives.

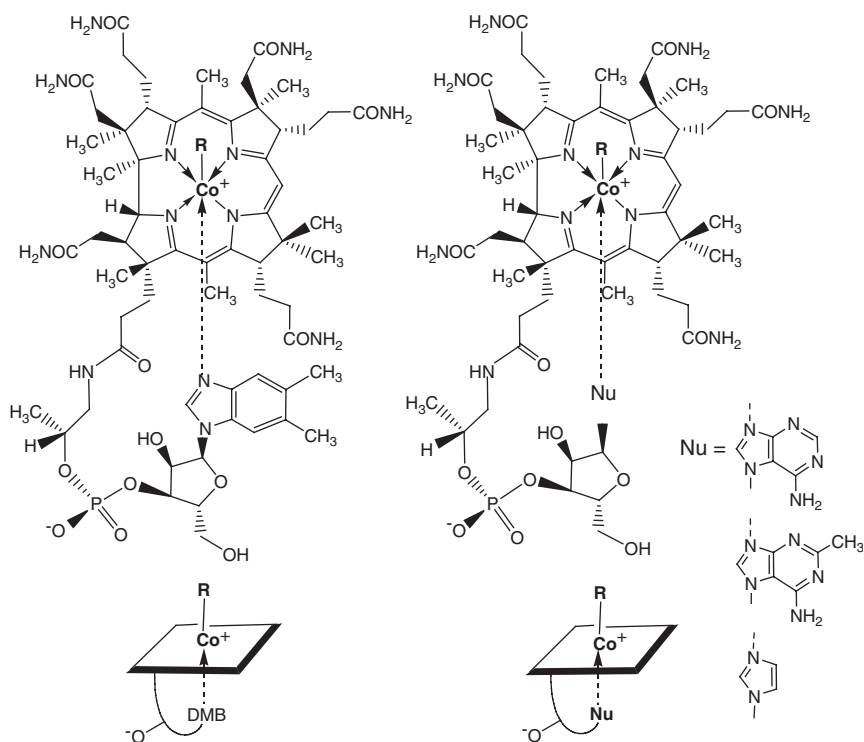


Figure 1. General structural formula. **Left:** cobalamins (Cbls = DMB-cobamides, Ado = adenosyl). Vitamin B₁₂ (**1**, CNCbl, R = CN), coenzyme B₁₂ (**2**, R = 5'-deoxy-5'-ado), methylcobalamin (**3**, MeCbl, R = CH₃), aquacobalamin (**4**⁺, R = H₂O⁺), hydroxocobalamin (**5**, HOCbl, R = HO), cob(II)alamin (**6**, B_{12r}, R = e⁻), chlorocobalamin (**18**, R = Cl), nitroxylobalamin (**19**, R = NO), 2,3-dihydroxypropyl-Cbl (**21**, R = 2,3-dihydroxy-propyl), α -adenosyl-Cbl (**22**, R = 5'-deoxy-5'- α -Ado), adenylpropyl-Cbl (**23**, R = 3-adeninyl-propyl), homocoenzyme B₁₂ (**24**, R = 5'-deoxy-5'-Ado-methyl), trifluoromethyl-Cbl (**25**, R = CF₃), difluoromethyl-Cbl (**26**, R = CHF₂), vinylcobalamin (**28**, R = CH=CH₂), *cis*-chlorovinyl-Cbl (**29**, R = CH=CHCl), bishomocoenzyme B₁₂ (**33**, R = 2-[5'-deoxy-5'-Ado]-ethyl), 2'-deoxycoenzyme B₁₂ (**48**, R = 2',5'-dideoxy-5'-Ado), 2',3'-dideoxycoenzyme B₁₂ (**49**, R = 2',3',5'-trideoxy-5'-Ado). **Right:** Structural formulae of other naturally occurring "complete" corrinoids (cobamides with other nucleotide functions "Nu" [6,11]: Co _{β} -cyano-imidazolylcobamide (**14**, R = CN, Nu = imidazole), Co _{β} -methyl-imidazolylcobamide (**27**, R = CH₃, Nu = imidazole); pseudovitamin B₁₂ (Co _{β} -cyano-7''-adeninylcobamide, **16**, R = CN, Nu = adenine), factor A (Co _{β} -cyano-7''-[2'-methyl]-adeninyl-cobamide, **17**, R = CN, Nu = 2-methyl-adenine). **Bottom:** symbols used.

2. STRUCTURE OF B₁₂ DERIVATIVES IN THE CRYSTAL AND IN SOLUTION

2.1. “Incomplete” Corrinoids

The structures of vitamin B₁₂ (**1**) and of coenzyme B₁₂ (**2**) were established largely through the pioneering X-ray crystallographic studies of Hodgkin et al. [4,31,32], who discovered the composition of the corrin core of **1** and the organometallic nature of **2** (see recent reviews [46–48]). These two cobalamins belong to the “complete” corrinoids, in which a pseudo-nucleotide function is attached via an amide linkage to the corrin moiety. The resulting combination is unique, as the B₁₂-nucleotide function may switch between the cobalt-coordinated “base-on” form and the de-coordinated (so called “base-off” form) – coenzyme B₁₂ thus can be considered to be a “molecular switch” [49].

Earlier important X-ray investigations also specifically relied on some crystallizable “incomplete” Co(III)-corrinoids, such as α -cyano- β -aqua cobyric acid (**7**) (see review [46]). Cobyric acid is the natural corrinoid moiety of the vitamin B₁₂ and it was the initial target for Eschenmoser and Woodward for the total synthesis of vitamin B₁₂ [33–35] (as it had already been shown how **7** could be converted to vitamin B₁₂). Since these times, crystallographic work with “incomplete” Co(III)-corrinoids has focused on obtaining detailed structural information. More recently analyzed structures include that of dicyano-heptamethyl-cobyrate (**8**, “cobester” [50]) [51,52] and of its analogues [53], including 15-norcobester (**9**) [54,55] (as reviewed in [47]). Very recently, a new type of B₁₂ dimer structure was found in the crystal of Co β -cyano-neocobyric acid (**10**) [56] (Figure 2). A similar dimer structure has not been observed in the “normal” corrinoids. However, in the “neo” corrinoid **10**, the configuration at the corrin ring C was inverted, apparently reducing the steric hindrance due to a propionate side chain, and making the dimer formation possible [56].

The crystal structure of heptamethyl-cob(II)yrinate (cob(II)ester (as the perchlorate complex **11**) gave the first detailed insights into the structure of a paramagnetic Co(II)-corrin [57]. It revealed a five-coordinated Co(II) center in the “incomplete” Co(II)-corrin **11**, to which a perchlorate ligand was coordinated via a long axial cobalt-oxygen bond (2.31 Å) [57]. The coordination of the axial ligand at the sterically less hindered “upper” β -face is in contrast with the preference seen in the “complete” cob(II)alamin (**6**, B_{12r}) [58,59] (see below).

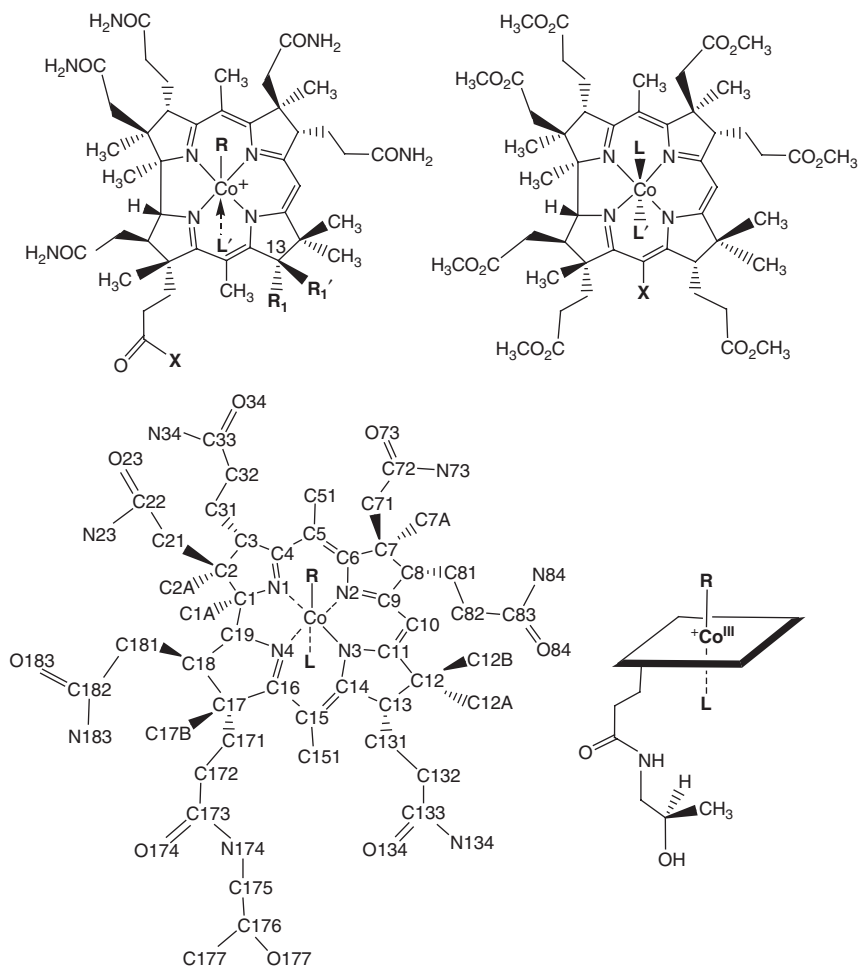


Figure 2. **Top left:** Structural formulae of “incomplete” corrinoids: Co_α -cyano- Co_β -aqua cobyric acid (**7**, $\text{R} = \text{H}_2\text{O}$, $\text{R}_1 = \text{propionamide}$, $\text{R}'_1 = \text{H}$, $\text{L}' = \text{CN}$, $\text{X} = \text{OH}$); Co_α -aqua- Co_β -cyano-13-epicobyric acid (**10**, $\text{R} = \text{CN}$, $\text{R}'_1 = \text{propionamide}$, $\text{R}_1 = \text{H}$, $\text{L}' = \text{H}_2\text{O}$, $\text{X} = \text{OH}$), Co_β -5'-deoxy-5'-adenosylcobinamide (**32**, $\text{R} = 5'$ -deoxy-5'-Ado, $\text{R}_1 = \text{propionamide}$, $\text{R}'_1 = \text{H}$, $\text{L}' = \text{H}_2\text{O}$, $\text{X} = \text{NH-CH}_2\text{-CHOH-CH}_3$). **Top right:** Structural formulae of heptamethyl-cobyrinates: cobester (**8**, $\text{L} = \text{L}' = \text{CN}$, $\text{X} = \text{CH}_3$), 15-nor-cobester (**9**, $\text{L} = \text{L}' = \text{CN}$, $\text{X} = \text{H}$); perchlorato-heptamethylcob(II)yrinate (**11**, $\text{L} = \text{ClO}_4$, $\text{L}' = \text{vacant}$, $\text{X} = \text{CH}_3$). **Bottom left:** Atom numbering for cobinamide [60]. **Bottom right:** general symbol used for a cob(III)inamide.

2.2. “Complete” Corrinoids

The “complete” corrinoid vitamin B₁₂ (**1**, CNCbl) is called a “cobalamin” (or a 5',6'-dimethylbenzimidazolyl-cobamide = DMB-cobamide), in which a cyanide ligand is bound at the “upper” axial coordination site (on the β-face, see e.g., [60]). In other “cobalamins”, the cyanide group at the β-face of CNCbl is replaced by a different ligand, e.g., an organometallic group, as in coenzyme B₁₂ (AdoCbl, **2**). Purinyl-cobamides are another important class of naturally occurring “complete” corrinoids, in which a purine base is part of the nucleotide function (see Figure 1) [6,11]. The systematic atom numbering used in this chapter for vitamin B₁₂ derivatives [60] builds on the convention that atom numbers of the heavy atoms of a substituent reflect the number of the points of attachment to the corrin ligand and are indexed consecutively [61] (see Figure 2); however, it deviates from that introduced by Hodgkin et al. [31,32,46] and still used by some authors [12].

2.2.1. “Complete” Corrinoids with an “Inorganic” β-Ligand

Only “base-on” cobalamins, where the nucleotide functionality coordinates in an intramolecular mode, have been analyzed by X-ray crystallography [46–48]. The “old” structure of vitamin B₁₂ [7] was re-analyzed using modern cryo-crystallography techniques [47,62], which showed the molecular geometry of the B₁₂ moiety to agree within experimental error of Hodgkin's original result. Neovitamin B₁₂ (**12**) was also studied by crystallography and was revealed to be the cyano-13-epicobalamin, a derivative of vitamin B₁₂ where the propionamide chain at the C13 position is in the β-configuration [63,64]. A notable difference between the two structures is an increased “non-planarity” in the corrin ring of the neo-derivative (expressed as a 6° larger fold angle, 23.7° versus 17.9°). The C8 epimer of vitamin B₁₂, cyano-8-epicobalamin (**13**), has an even larger fold angle of the corrin core (23.8°) [65] (Figure 3).

The discovery of the replacement of the cobalt-coordinated 5,6-dimethylimidazole base by a protein-derived imidazole in several B₁₂-dependent enzymes [36–38]), gave the analysis of Co_β-cyano-imidazolylcobamide (**14**) [62] particular interest. The less bulky and more nucleophilic imidazole base of **14** caused a number of structural differences. The corrin ring fold angle of **14** decreased to 11.3° and the axial (Co–N) bond shortened (from 2.011 Å in **1** to 1.968 Å in **14**). In addition, the “base tilt” of **12** (i.e., half the difference between the two Co–N–C angles to the coordinating base) decreased to near zero, within experimental error. In all crystal structures of 5',6'-dimethylbenzimidazolyl-cobamides a “tilt” of about 5° is

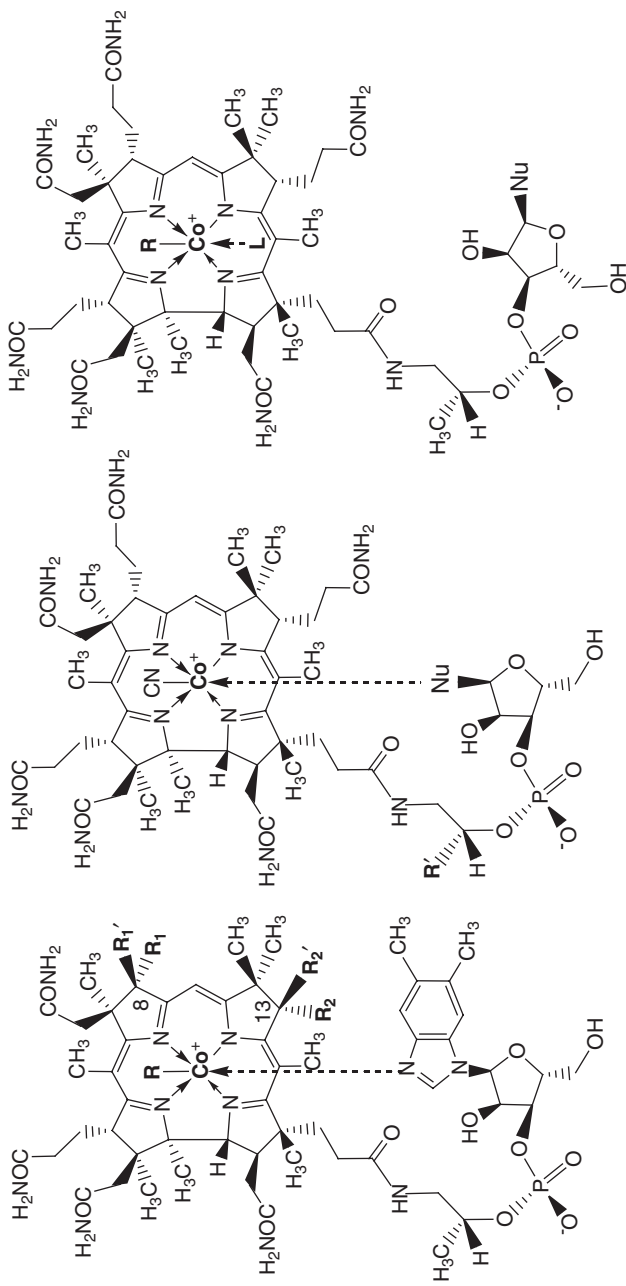


Figure 3. Left: Structural formulae of neovitamin B₁₂ (12, cyano-13-epicobalamin, R = CN, R' = R₂ = H, R₁ = R₂' = propionamide), cyano-8-epi-cobalamin (13, R = CN, R₁ = R₂' = H, R₂ = R₁' = propionamide), neocoenzyme B₁₂ (36, R = 5'-deoxy-5'-adenosyl, R₁' = R₂ = H, R₁ = R₂' = propionamide). Center: Structural formulae of pseudovitamin B₁₂ (16, R' = CH₃; Nu = adenine), norpseudovitamin B₁₂ (15, R' = H; Nu = adenine), norvitamin B₁₂ (37, R' = H; Nu = DMB). Right: Structural formulae of base-off cobalamides: pseudocoenzyme B₁₂ (34, R = 5'-deoxy-5'-adenosyl; L = H₂O; Nu = adenine), adenosyl-factor A (35, R = 5'-deoxy-5'-adenosyl, L = H₂O, Nu = 2-methyl-adenine), cob(D)alamin⁻ (B₁₂^{3a}, 39⁻, R = L = absent, Nu = DMB).

found [47,62], which appears to be an inherent property of the cobalt-coordinated DMB.

Norpseudovitamin B₁₂ (Co_β-cyano-7''-adeninyl-176-norcobamide) (**15**) represented the first natural example of a "complete" B₁₂ derivative that lacked one of the methyl groups (of C176) of the cobamide moiety [66]. X-ray crystal structures were determined for **15** and for the analogues, pseudovitamin B₁₂ (**16**) and factor A (Co_β-cyano-7''-[2-methyl] adeninylcobamide) (**17**) [66]. These first accurate crystal structures of complete corrinoids with an adeninyl pseudo-nucleotide confirmed the expected coordination properties around Co and corroborated the virtual conformational identity of the nucleotide moieties of **15** and its two homologues. For **16**, an axial (Co–N) bond of 2.026 Å and a fold angle of 16.9° were determined [66], for **15** and **17**, the axial (Co–N) bonds were 2.035 Å and 2.021 Å respectively, and both had a fold angle of 19.6°. The observed structural changes from the replacement of DMB by adenine or 2-methyladenine in cyano-Co(III) cobamides are hardly significant. In line with this structural point of view, purinyl-cobamides, such as **16** and **17**, have been shown recently to belong to a broadly occurring and naturally biosynthesized type of "complete" corrinoids [67].

For the crystal structure of aquacobalamin perchlorate (**4**⁺-ClO₄⁻) [68] the shortest known axial (Co_α-N) bond of a vitamin B₁₂ derivative was observed (1.925 Å). Together with the large upwards folding angle of 18.7°, the conclusion stated was, that steric repulsion between the DMB base and corrin core led to a flexing of the corrin ring [47,68]. The short axial Co_α-N bond for the **4**⁺-ion was consistent with the weak donor ability of the (*trans*-axial) Co_β-aqua ligand. Crystal structures of various other "inorganic" B₁₂ derivatives have been solved and previously reviewed elsewhere [46–48]. For several other "inorganic" B₁₂ derivatives crystal structures were obtained recently, including chlorocobalamin (**18**) [69] and NO–Cbl (**19**), which is best described a nitroxyl-cob(III)alamin [70].

Crystal structures of a cobalamin derivative, where the cyanide ligand of vitamin B₁₂ acts as bridging ligand between the rhenium carbonyl compounds (i.e., with a central Co–CN–Re feature) have been analyzed [71]. The attachment of diagnostically active ligands to a cobalamin has been explored as a way of using vitamin B₁₂ derivatives (as a "Trojan Horse") for introducing fluorescent [72] and radio-labeled compounds [73], or other bioactive molecules [74], into cells and living animals.

Information on cob(II)alamin (**6**, B_{12r}) was of particular interest [58], as (in a formal sense) it is the product of (Co–C) bond homolysis of coenzyme B₁₂ (**2**), and occurs during the catalytic cycle of coenzyme B₁₂-dependent enzymes. The crystal structure of cob(II)alamin showed that the corrin moiety of **6** and **2** were very similar [58]. The fold angle of the corrin ring in **6** is 16.3° compared to 13.3° in **2**. The axial cobalt-nitrogen bond is even

slightly shorter at the five coordinate Co(II) ion of cob(II)alamin (2.13 Å) than in the six-coordinated case in coenzyme B₁₂ (2.24 Å). However, the distance between the corrin ring and the coordinated DMB base is almost the same, due to a “downward” displacement of the cobalt atom from the plane of the corrin ligand in **6**. It was expected that the reduced Co(II) ion would have a longer bond than the Co(III) species. In view of this result, in **2** and related organocobalamins, the “structural *trans* effect” of the organic ligand appears to increase the axial Co(III)–N bond which compensates for the larger covalent radius of Co(II) compared to Co(III). From these observations the conclusion was made that the interactions (apoenzyme/coenzyme) at the corrin moiety of the coenzyme appear to be inadequate to provide the major means for a protein-induced activation of the bound coenzyme toward homolysis of its (Co–C) bond. Instead, the organometallic bond may be labilized by way of apoenzyme (and substrate) induced separation of the homolysis fragments, made possible by strong binding of the separated components to the protein [58]. Cob(II)alamin (**6**) has also been recently studied using neutron Laue diffraction studies, which came to the same conclusions regarding its structure [59]. In single crystals, cob(II)alamin (**6**) can be loaded with molecular oxygen at low temperature, to give a well ordered crystalline oxygenated complex (investigated earlier by ESR spectroscopy [75]) and best described as superoxo-cob(III)alamin (**20**), according to the X-ray analysis of the crystal structure [76]. In contrast, in (aqueous) solution, B_{12r} (**6**) is readily oxidized by air to aqua-cob(III)alamin (**4**).

2.2.2. “Complete” Corrinoids with an “Organic” β -Ligand

The original crystal structure of coenzyme B₁₂ (**2**) primarily helped to reveal the organometallic nature of **2** [1,32,77]. The organometallic adenosyl moiety was observed to be bound in an *anti* conformation and the adenine ring was found to be above ring C of the corrin ligand. However, this and more extensive recent studies by X-ray and neutron crystallography [78,79] also showed both axial (Co–C) (2.030 Å) and (Co–N) (2.237 Å) bonds to be relatively long [46,47]. In addition, the organometallic group in **2** exhibited a strikingly large Co–C_{5'}–C_{4'} bond angle of 125.4° [46–48,77].

To investigate if the large Co–C–C bond angle of AdoCbl (**2**) is typical for organocobalamins, the crystal structures of the 2,3-dihydroxypropylcobalamins (the diastereomeric *R*- and *S*-isomers **21R** and **21S**) were examined earlier [80]. The (Co–C) distances (2.00 and 2.08 Å for **21R** and **21S**, respectively) were similar to AdoCbl (2.03 Å) but the bond angles were smaller (119.6° for **21R** and 113.6° for **21S**). The value for **21S** should be

considered the “normal” angle, with little interactions between the corrin ring and β -substituent.

In α -adenosylcobalamin (**22**), a stereoisomer of AdoCbl (**2**), the organometallic adenine base is attached at the ribose moiety in an α -configuration. The crystal structure of **22** showed the lengths of the axial (Co–C) (2.02 Å) and (Co–N) (2.24 Å) bonds to be similar to **2** but the corrin ring was flatter (fold angle = 11.7° versus 13.3° in **2**) [81]. As in **2**, the adenosyl ligand was placed over the south-east quadrant (ring C), but the position of the adenine moiety relative to the ribose unit of the organometallic ligand was disordered due to different conformations of the adenine heterocycle.

Adenylalkylcobalamins, where a methylene chain connects the adenine with the cobalt center [82], inhibit various AdoCbl-dependent enzymes depending upon the length of the alkyl chain [83]. 3-Adenylpropylcobalamin (**23**) has been studied in solution as well as in the crystal [84]. The structure of the corrin ring and the lower nucleotide loop closely resembled that of **2**. However, the adenine group of **23** is oriented almost parallel to the corrin plane and is positioned over ring D of the corrin ligand, i.e., about 120° clockwise from its position in coenzyme B₁₂.

The homologue of coenzyme B₁₂ “homocoenzyme B₁₂” (**24**, Co β -(5'-deoxy-5'-adenosylmethyl)-cob(III)alamin) has been recently prepared, as it has been suggested to function as a covalent structural mimic of the hypothetical enzyme bound “activated” state of the B₁₂ cofactor [85]. In the crystal structure of **24** the cobalt center was observed to be at a distance of 2.99 Å from C5' of the homoadenosine moiety and the latter to be in the unusual *syn* conformation. In **24** the crucial distance from the corrin-bound cobalt center to the C5' of the homoadenosine moiety is, thus, roughly the same as found in one of the two “activated” forms of coenzyme B₁₂ in the crystal structure of glutamate [86]. Indeed, “homocoenzyme B₁₂” (**24**) is bound intact to glutamate mutase and does not function as cofactor [87]. In contrast, dioldehydratase and ethanolamine lyase, when reconstituted with **22**, still show weak activity [88].

The crystal structure of methylcobalamin (MeCbl, **3**), the simplest organometallic B₁₂-derivative, was analyzed in 1985 [89]. Interestingly, the solution structure of MeCbl, as derived by NMR spectroscopy [90], deviated from the earlier crystal with respect to the conformation of the nucleotide loop. The structures of crystals of MeCbl (from various solvent compositions) were thus re-investigated more recently, to provide a more accurate picture [91]. The structures confirmed the folding of the corrin core of **3** to be similar to that of coenzyme B₁₂ (AdoCbl, **2**) (fold angle in **3** = 14.7° [91]). This proved that the bulkiness of the 5'-deoxyadenosyl ligand in **2** was not a main contributor to the conformation of the corrin ligand of AdoCbl. The lengths of the axial (Co–C) (1.979 Å) and (Co–N) (2.162 Å) bonds are slightly shorter in MeCbl when compared to AdoCbl. The shorter axial bond

to the DMB base is consistent with the stronger nucleotide coordination in **3**. The crystal structures of the fluorinated analogues of MeCbl, of CF₃-cobalamin (**25**) [92] and CF₂H-cobalamin (**26**) were analyzed likewise [93].

Co_β-methyl-imidazolylcobamide (**27**) was prepared as a model for methyl-corrinoid cofactors in a “base-off/His-on” form and its crystal structure was analyzed [94]. The substitution by a less bulky and more nucleophilic imidazole base had the expected structural effects. The axial (Co–C) (1.97 Å) and (Co–N) (2.09 Å) bonds are shorter in **27** than in methylcobalamin and the fold angle of the corrin ligand was reduced by over 2° to 12.5°.

The structures of vinylcobalamin (**28**) [95] and *cis*-chlorovinylcobalamin (**29**) [96] were analyzed in the crystal as the first examples of organo-cobalamins with sp²-hybridized carbon ligands. The cobalamin **29** is considered a model for a putative intermediate in the reductive degradation of chlorinated ethylenes [97]. As expected for a vinyl ligand, the Co–C bond length (1.912 Å for **28** and 1.952 Å for **29**) is shorter than in adenosylcobalamin (**2**). Steric repulsion presumably causes the significantly longer (Co–C) bond in **29**. The axial (Co–N) bond lengths of 2.166 Å for **28** and 2.144 Å for **29** are also slightly shorter than in **2** and provide a good example of the “inverse” *trans* effect.

Crystallography has also contributed to the elucidation of the mutual arrangement of the two closely placed corrinoid moieties in the tetramethylene-bridged organometallic B₁₂-dimer tetramethylene-1,4-di-Co_β-cobalamin (**30**, see Figure 9 in Section 4.1) [98]. Likewise, the mode of bonding in the sterically strained conjugate (**31**) of MeCbl (**3**) and thymidine was revealed by the crystal structure of the remarkable sodium complex (**31**) of the “base-on” form of this organometallic “complete” corrinoid [49].

The “thermodynamic” and “structural” *trans* effects of B₁₂ derivatives are the effect of one cobalt-coordinated axial ligand on chemical equilibria and coordination properties of an axial ligand *trans* to the first one [99]. An increasing σ-donor power of the Co_β ligand X was found to correlate with the size of the thermodynamic *trans* effect in B₁₂ derivatives. The length of the axial (Co_α–N) bond to the DMB base in cobalamins generally increases with the σ-donor property of the Co_β ligand [46–48]. In the same sequence, the σ-ligand influences the base-on/base-off equilibria. A linear correlation thus exists between free enthalpy of the base-on/base-off equilibria in aqueous solution and the length of the (Co–N) bond [64]. However in B₁₂ derivatives, both axial bonds lengthen simultaneously with increasing σ-donor character of the axial ligands [46–48], a result of an “inverse” *trans* effect [100].

The saturated and direct *trans* junction between two of its four five-membered rings is the main cause of the non-planar nature of the corrin core in B₁₂ derivatives. The characteristic “ligand folding” is a main factor to the variability in the conformation of the corrin ligand [101]. The fold has

always been found as “upwards” (towards the β -face), about the C10–Co axis, and the “fold angle” is defined as the angle between the best planes through N1–C4–C5–C6–N2–C9–C10 and C10–C11–N3–C14–C15–C16–N4 [46]. Fold angles are mostly smaller in “incomplete” corrinoids with a typical observed value of 7.5° in dicyano-heptamethyl-cob(III)yrinate [52], when compared to “complete” corrinoids, where a value of 17.9° has been found in CNCbl (**1**, [47]). However, in epi-corrinoids the fold angle was larger both in the “complete” corrinoid cyano-8-epicobalamin (**13**) (23.8°) [65], and in the “incomplete” cyano-aqua-neo-cobyric acid (**10**) (22.5°) [56].

In cobalamins, the bulky DMB base was thus suggested to be a relevant contributor to the upwards folding of corrins [46,47]. This possible effect of the intramolecular coordination of the DMB base on the folding of the corrin in Cob(III)alamins has been examined in several model situations [47,62]. Both “inorganic” and “organometallic” cob(III)alamins have been compared and the conclusion is that longer (Co _{α} –N) bonds correlate with smaller “fold” angles (and *vice versa*) [47]; for example, aquacobalamin perchlorate (**4**⁺-ClO₄⁻, Co _{α} –N = 1.925 Å, fold angle = 18.7°) and coenzyme B₁₂ (**2**, Co _{α} –N = 2.237 Å, fold angle = 13.3°). In contrast, the folding of the corrin ligand in Co _{β} -cyano-imidazolylcobamide (**14**) (11.3°) is less than half of that of vitamin B₁₂ (**1**) regardless of the shorter (Co _{α} –N) bond (1.97 Å versus 2.01 Å) [62]. Accordingly, “folding” is more apparent in cob(III)-alamins with short (Co _{α} –N) bonds (near 2.0 Å or less), to which the known “inorganic” B₁₂ derivatives belong to. In organyl-cobalamins (such as methylcobalamin and coenzyme B₁₂) the length of the (Co _{α} –N) bond is close to or greater than 2.2 Å, so there is less steric interaction of the nucleotide base with corrin ligand.

2.2.3. Spectroscopic Studies of the Solution Structure of B₁₂ Derivatives

Nuclear magnetic resonance (NMR) spectroscopy has had a strong influence in the development of B₁₂ chemistry. A major feature of NMR spectroscopy is that the structures of non-crystalline B₁₂ derivatives can be characterized in solution. The early NMR spectroscopic studies thus established the nature of many non-crystalline B₁₂ derivatives, mostly in their Co _{β} -cyano forms, using one-dimensional analyses [102]. These studies were based on the ¹H and ¹³C chemical shift values from spectra of several already well-characterized B₁₂ derivatives and used to identify and describe the structure of synthetic and natural analogues of vitamin B₁₂ [66,103]. Along these lines, the natural corrinoids from a range of bacteria were first characterized by NMR [104,105].

Earlier assignment problems regarding B₁₂ derivatives in aqueous or non-aqueous solutions have now been eliminated by the use of heteronuclear NMR spectroscopy [103,106]. Following on from the pioneering studies of coenzyme B₁₂ (**2**) [107,108] and the non-crystalline B₁₂ derivative Co_β-5'-deoxy-5'-adenosylcobinamide (**32**) [109], NMR-studies in solution have begun to complement (and in certain aspects rival) X-ray analytical studies of B₁₂ derivatives in the solid state. By applying a selection of, now well-established, homo- and heteronuclear 2D experiments, the assignment of signals in ¹H, ¹³C, and ¹⁵N spectra provide a reliable basis for detailed structure and dynamic information of B₁₂ derivatives. Techniques for suppression of the solvent (water) signal allow the recording of spectra from an aqueous solution with little or no loss of information [106]. Characteristic chemical shift values from ¹H, ¹³C, ¹⁵N, and ³¹P spectra provide important information on the constitution and conformation of “complete” B₁₂ derivatives [103,106]. The coordination of the DMB base, in “base-on” compounds, induces a high-field shift of the ¹H NMR signal of HC10, due mainly to an increase in the electron density of the corrin ligand by the axial coordination of the base. This characteristic has been used to determine the temperature-dependent “base-on/base-off” equilibria (in aqueous solutions) of organometallic B₁₂ derivatives (e.g., methylcobalamin **3**). In the ¹H NMR spectrum of, e.g., **3**, the anisotropic shielding effect of the coordinated DMB base also induces high-field shift of protons located nearby, such as of the methyl group H₃C1A and methylene groups H₂C81 and H₂C82 [90]. Shielding by the cobalt-corrin in the axial direction leads to high-field shifts of the DMB protons closest to the cobalt-corrin, HC2N and HC4N. Likewise protons of organometallic ligands are characteristically up-field as seen in the ¹H NMR spectra of homocoenzyme B₁₂ (**24**) and bishomocoenzyme B₁₂ (**33**) [85]. Significant conformational differences between the solution and crystal structure were revealed in some cases, such as in the studies of AdoCbl (**2**) [107] and MeCbl (**3**) [90].

One of the main examples of this is the natural “complete” but “base-off” protonated form of coenzyme B₁₂ (**2-H⁺**) [108]. More recently, the solution structures of the organometallic derivatives pseudocoenzyme B₁₂ (**34**), adenosyl-factor A (**35**) [110], and neocoenzyme B₁₂ (**36**) [111] could be analyzed in great detail (see Figure 3). The structures and “base-on/base-off” equilibria of a range of “complete” cobamides have also been studied in aqueous solution [103,106].

From NOE measurements a reliable assignment (“upper”/Co_β or “lower”/Co_α) of the cobalt-bound methyl group in non-crystalline methyl-cob(III)yr-inates was achieved [112]. Also from NOE data and three bond coupling constants, detailed and important information on the conformational properties of the nucleotide moiety, the organometallic group, and of other

peripheral side chains was extracted [103,106]. Such studies resulted in the detection of significant conformational dynamics of the organometallic 5'-deoxy-5'-adenosyl moiety in the pioneering study of coenzyme B₁₂ (**2**) [107,108]. In a related context extensive conformational dynamics of the organometallic adenosyl ligand and the unusual *syn* orientation of the adenine heterocycle were observed in a series of coenzyme B₁₂ analogues, such as homo- and bishomocoenzyme B₁₂ (**24** and **33**) [85], pseudocoenzyme B₁₂ (**34**) [110], neocoenzyme B₁₂ (**36**) [111], and other adenosyl-cobamides [106]. In the solution structures of adeninyl-alkyl-cobamides, significant conformational flexibility of the organometallic ligand was also discovered [82,84].

NMR spectroscopy has proven to be a versatile method in the detection of intra- and inter-molecular H bonding. The water ligand of H₂OCl-perchlorate (**4**⁺-ClO₄⁻), which from the crystal structure forms an H bond to an acetamide side chain, was shown by NMR to still form a similar H bond in aqueous solution [68]. Pseudo-intramolecular H bonding of a specific "external" water molecule to the nucleotide portion of methylcobalamin (**3**) was characterized by NMR spectroscopy [90], which is accompanied by a remarkable adjustment in the conformation of the nucleotide moiety [106]. In this way first insights into the hydration behavior of B₁₂ derivatives in aqueous solution were gained. Further exploratory studies, in which B₁₂ derivatives were investigated in greater detail in their solvent environment, complement other recent results obtained from studies on the structure of the water networks in crystals of B₁₂ derivatives [68,79]. The aqueous solution environments of **3** and the protonated "base-off" form (**3-H**⁺) of **3** have been investigated in such a way, by measuring NOEs between the solvent. Initial results also support the presence of a water molecule as the Co_α-axial ligand, thus providing the first experimental evidence for a hexacoordinated cobalt center in the (solution) structure of an organometallic cobyrinic acid derivative [113].

Electron spin resonance spectroscopy has likewise provided important information on the coordination environment of Co(II)-corrins, where DMB coordination in cob(II)alamin (B_{12r}, **6**) can be detected well by ESR [114]. This technique actually also gave the first hints that Co(II) coordination of the endogenous DMB base may not be typical of enzyme-bound cob(II)alamin (**6**); instead, in some B₁₂-dependent organisms histidine binding was indicated by ESR spectroscopy [115] as was found by crystallography in various B₁₂-dependent enzymes [36,37,116]. Modern, 2D-ESR techniques have also allowed the detection of external ligands to "incomplete" Co(II)-corrins in (frozen) solution, such as Co(II)cobester (**11**) [117], and in the protonated, base-off-form of cob(II)alamin (**6-H**⁺) [118]. The application of modern methods of absorbance spectroscopy, combined with theory-based interpretation of the data has also opened a new look at

important open questions on the coordination properties of (enzyme-bound) corrinoids (see e.g., [119,120] and Chapter 12 of this book).

2.3. The “Base-On/Base-Off” Constitutional Switch of “Complete” Corrinoids

The typical functional B₁₂ cofactors are “complete” corrinoids, and are unique conjugates of natural cobyrinates with unusual α -nucleotide function [6,11,12]. The constitution of the “nucleotide base” can vary in a remarkable way in the natural “complete” corrinoids. Not only benzimidazoles (such as the 5,6-dimethylbenzimidazole of the cobalamins) are among the known nucleotide functionalities, but also purines (such as adenine and 2-methyladenine in pseudovitamin B₁₂ (**16**) and factor A (**17**), respectively) are found abundant [121]. This variety in the structure of the α (pseudo)-nucleotide unit appears to be largely a consequence of the particular biosynthetic availability in the various microorganisms [67]. The known purine bases of “complete” corrinoids are mostly adenine derivatives or related heterocycles [105] as also found in RNA [122]. While the DMB base is directly accessible from degradation of riboflavine in aerobes [121,123], anaerobes have evolved an unrelated and still incompletely elucidated, complex biosynthetic path to DMB and to a variety of related benzimidazoles [121].

In cobalamins, the DMB nucleotide function is structured in such a way that relatively strong intramolecular cobalt coordination is possible, and occurs with little build-up of strain [124]. However, most cobalamins and other “complete” corrinoids are still observed in two forms: they either have their nucleotide appendage cobalt-coordinated (in a “base-on” form), or de-coordinated, i.e., in a “base-off” form (see Figure 4) [22]. Depending upon their availability in either “base-off” or “base-on” forms, the reactivity of B₁₂ derivatives in biologically relevant organometallic reactions is modified (as a consequence of the coordinating nucleotide function), as well as the face-selectivity at the corrin-bound cobalt center [104,125]: The DMB nucleotide function may be effective in directing alkylation (and other ligation) reactions (in cobalamins) to the “upper” (or β -face) in a thermodynamic sense, and it may stabilize the (organo)-B₁₂ derivative in its “base-on” form [126,127]. As discussed below, this is particularly relevant in methyl-corrinoids, such as MeCbl (**3**) [126].

“Base-on” and “base-off” forms of the “complete” corrinoids represent constitutional isomers, that may (or may not) be well structured for binding by specific B₁₂ apoenzymes: some B₁₂-dependent enzymes, such as the methyltransferases, typically bind a “base-off” form of the B₁₂ cofactor [36,128]. However, coenzyme B₁₂-dependent enzymes come in two classes,

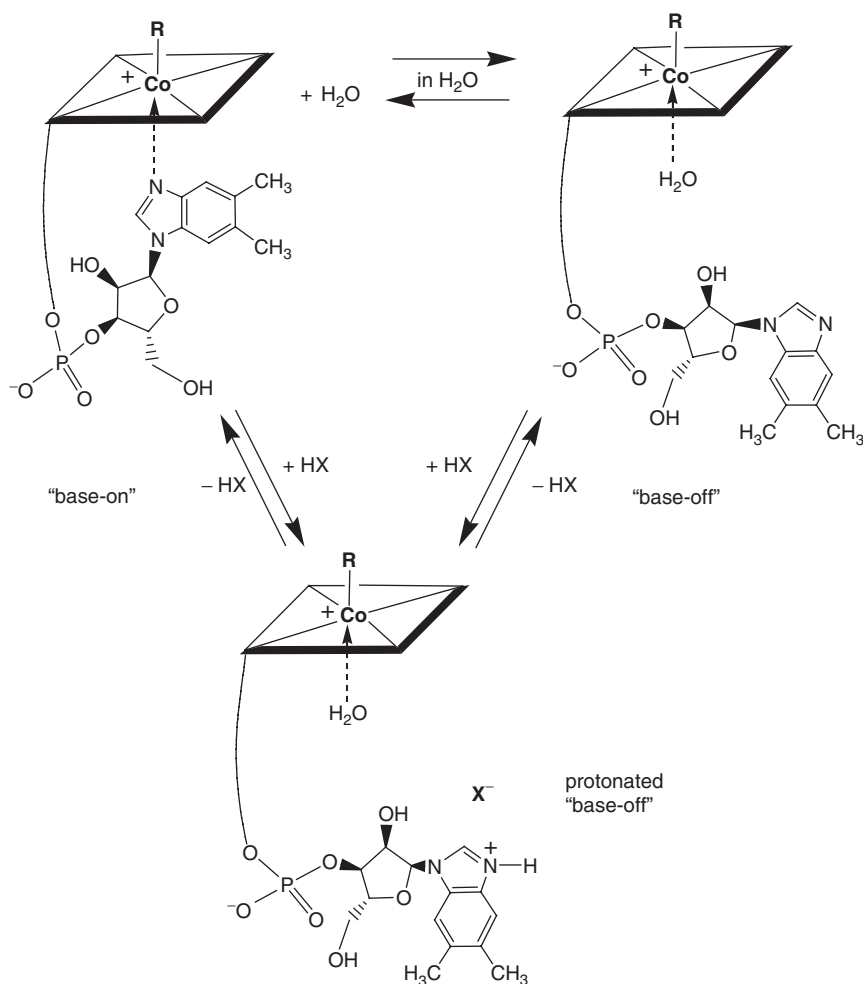


Figure 4. B₁₂ – a constitutional “molecular switch”: The nucleotide base of B₁₂ cofactors is cobalt-coordinated in the “base-on” form or de-coordinated in the “base-off” form; the two forms represent “constitutional” isomers and differ by their (bond) connectivity; protonation of the de-coordinated nucleotide base stabilizes the “complete” corrinoids in their “base-off” form.

one of them with a base-on B₁₂ cofactor, the other with the B₁₂ cofactor bound in a “base-off” (and “His-on”) form (a still puzzling observation, see e.g., [129]).

The unusual α -configuration of the “nucleotide moiety” is a common (stereochemical) feature of all known “complete” corrinoids. It allows, first

of all, for the intramolecular coordination of the heterocyclic nucleotide base to the “lower” α -axial coordination site of the corrin-bound cobalt center [11,12,32]. However, in some natural corrinoids non-coordinating phenols are found in their α -pseudonucleotide, such as *p*-cresol in *p*-cresolylcobamide (**38**) [11,130,131]. These remarkable findings may call for an additional “functional” rationalization of the differing properties of the “complete” corrinoids.

The complete structure of the nucleotide moiety clearly is very important for the selective and tight binding by B_{12} -binding proteins [132]. This is considered as the relevant basis for discriminating between the natural B_{12} derivatives by the human B_{12} uptake and transport system [16,133], which recognizes and binds its B_{12} load in the “base-on” form [132]. When binding “base-off analogues”, such as pseudo-coenzyme B_{12} (**34**) or adenosyl-factor A (**35**), mammalian B_{12} transporters may even restructure them into their “base-on” constitution, which is the less stable form in aqueous solution [133].

A protein environment may thus bind and switch the bound B_{12} cofactors from “base-on” to “base-off” or *vice versa* [22,49]. In an artificially developed B_{12} -binding protein, an antibody raised against coenzyme B_{12} (AdoCbl, **2**), the bound analogues **34** and **35** were indeed found restructured into their base-on forms [134]. Similar phenomena may also arise in a(n oligo-) nucleotide environment [49]. Indeed, an interaction in the reverse sense was recently discovered in a particular type of mRNA, for which coenzyme B_{12} (AdoCbl, **2**) is a strongly bound ligand: this part of mRNA in its untranslated region (a “ B_{12} riboswitch”) not only is able to bind coenzyme B_{12} (AdoCbl, **2**) strongly, but it is also switched upon binding of the B_{12} ligand, so as to inhibit the expression of their gene product [135].

The cobalamins and related “complete” corrinoids thus have the capacity to switch their constitution between the “base-on” and the “base-off” forms and thus represent natural “molecular switches” [49]. The “base-on” to “base-off” switch can also be achieved by protonation of the nucleotide base and decoordination from the corrin-bound cobalt ion [11,12]. The proton-assisted decoordination is inhibited by strong cobalt coordination, as is typical for **1** and other cyano-Co(III)-corrins, and is achieved readily in organic B_{12} derivatives, such as AdoCbl (**2**). The associated acidity of the protonated base-off form (as expressed by its pK_a) thus informs quantitatively on the strength of the intramolecular coordination of the nucleotide base [11,12].

3. REDOX CHEMISTRY OF B_{12} DERIVATIVES

Under physiological conditions vitamin B_{12} derivatives exist in three different oxidation states – Co(III), Co(II), and Co(I) – each possessing

different coordination properties and characteristically differing reactivities [5–12,22]. Oxidation-reduction processes are, therefore, of key importance in the chemistry and biology of B₁₂. Electrochemical methods have been used for measuring the crucial redox potentials, for the purpose of generating reduced forms of protein-bound B₁₂ derivatives [136] and electrode-bound B₁₂ derivatives for analytical applications [137], as well as in the synthesis of organometallic B₁₂ derivatives [138].

Axial coordination to the corrin-bound cobalt center depends on the formal oxidation state of the cobalt ion [11,12,138,139]: As a rule, the number of axial ligands decreases with the cobalt oxidation state. In the thermodynamically predominating forms of cobalt corrins, the diamagnetic Co(III) has two axial ligands bound (coordination number 6), the paramagnetic (low spin) Co(II) has one axial ligand bound (coordination number 5), and for the diamagnetic Co(I) no axial ligands are bound (coordination number 4), or only very weakly. Electron transfer reactions involving B₁₂ derivatives are, therefore, accompanied by a change in the number of axial ligands, which, in reverse, heavily influence the thermodynamic and kinetic features of the electrochemistry of cobalt corrins [138,139].

In Co(III)-corrins, such as vitamin B₁₂ (**1**) and hydroxocobalamin (**5**), the corrin-bound cobalt center binds two axial ligands (one of them the DMB base in the “base-on” cobalamins). In contrast, the metal center in Co(I)-corrins, such as cob(I)alamin (**39**[−], B_{12s}), is highly nucleophilic [140] and carries no axial ligand [139] (Figure 5). The intermediate oxidation state of Co(II)-corrins, such as in cob(II)alamin (**6**, B_{12r}), provides a highly reactive metal-centered radicaloid species [58,141]. The use of electrochemistry thus provides an excellent means for generating, under controlled conditions, B₁₂ derivatives of specific redox reactivity, as well as investigating the redox

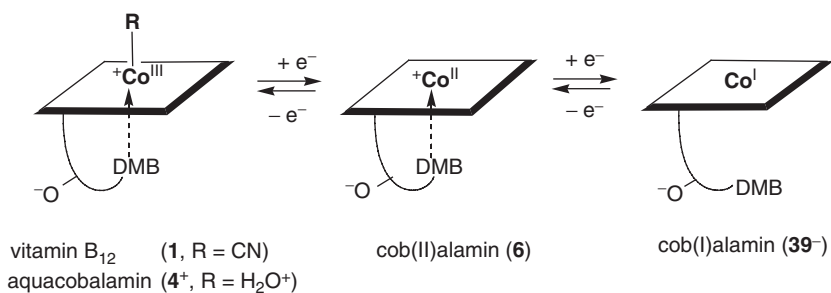


Figure 5. Outline of the redox transitions between cob(III)alamins (e.g. vitamin B₁₂ (CNCbl, **1**) or aquacobalamin (H₂OCbl, **4**⁺), cob(II)alamin (**6**), and cob(I)alamin (**39**[−]).

processes in their interconversion between oxidation states, as studied and reviewed by Lexa and Savéant [139].

3.1. Thermodynamics of Redox Processes

The electrochemistry of the B_{12} derivative aquacobalamin (4^+) has been particularly well studied [139]: one-electron reduction of 4^+ first gives B_{12r} (**6**) and then B_{12s} (39^-) (see Figure 5). Typically, electrochemical studies of aquacobalamin (4^+) were carried out in aqueous solution. A complete standard potential versus pH diagram has been established that correlates the thermodynamics of the aquacobalamin (4^+)- B_{12r} (**6**)- B_{12s} (39^-) system [139]. The interconversion between the different oxidation states of B_{12} derivatives can usually be seen with the eye or monitored effectively by UV-Vis spectroscopy. The redox data from potentiostatic measurements can thus be critically supported by UV-Vis spectroscopy [139,142]. Within the pH range -1 to 11 and applied potentials $E = 0.5$ V and -1.2 V versus SCE, seven solution cobalamins predominate thermodynamically, spanning a range of the three formal oxidation states of B_{12} [138,139].

Aquacobalamin (4^+) and HOCbl (**5**) differ by protonation of the “upper” (β) axial ligand with $pK_a(4^+) = 7.8$ [139]. The Co(II)-corrin B_{12r} (**6**) represents the “base-on” form of the Co(II) oxidation level (i.e., the nucleotide loop is coordinated intramolecularly), this is converted into the “base-off” ($6-H^+$) by protonation of the DMB base, with $pK_a(6-H^+) = 2.9$ [139]. At the Co(I) level, cob(I)alamin B_{12s} (39^-) is first protonated at the nucleotide base to give $39-H$. For the pK_a of $39-H$, an original value of 4.7 was determined [139,143], but more recently this has been estimated to be 5.6 [144]. A second protonation then occurs at the Co(I) center to give the “Co(III)-hydride” [145] $39-H_2^+$, with $pK_a(39-H_2^+) = 1$ [139,146].

In the pH range 2.9 to 7.8 , 4^+ and (base-on) B_{12r} (**6**) represent the predominant Co(III)/Co(II) redox couple, with a standard potential of -0.04 V. For the Co(II)/Co(I) redox system there are two pH-independent standard potentials [139]: at a pH less than 5.6 the Co(II)/Co(I) couple (base-off) $6-H^+/39-H$ predominates at a standard potential of -0.74 V, but for the redox couple (base-on) B_{12r} (**6**)/ B_{12s} (39^-) a more negative standard potential of -0.85 V [139] is required.

This shift by about 110 – 140 mV to a more negative potential for the reduction of (base-on) B_{12r} (**6**) when compared to that of the protonated base-off form $6-H^+$, reflects the selective stabilization of the Co(II)-corrin **6** by intramolecular nucleotide coordination [126,139]. A dependence of the standard potentials of the Co(III)/Co(II) redox couples occurs at approximately 60 mV per pH unit, at pH > 7.8 for HOCbl (**5**)/ B_{12r} (**6**) and at pH

<2.9 for $4^+/6\text{-H}^+$ and this reflects the effect of the removal by protonation of one axial ligand. An analogous dependence of the potential occurs between pH 2.9 and ca. 5.6 for $6/6\text{-H}^+$ as well as below pH 1 for the Co(II)/Co(I) redox couple $6\text{-H}^+/39\text{-H}_2^+$. At all pH values the disproportionation of Co(II)-corrins to Co(III)- and Co(I)-corrins is thermodynamically disfavored (the disproportionation equilibrium constant is below 10^{-10}) [139].

A complex interplay between the thermodynamic and kinetic factors of electron transfer reactions occurs in the analogous studies of vitamin B₁₂ (**1**), due to the strongly coordinating cyano ligand [139]. Coordination of (one or two) cyanide ligands to the Co(III) center stabilizes it against reduction and the Co(III)/Co(II) standard potentials are shifted to more negative values [139,147]. Cyanide ions transform **1** into (base-off) dicyano-cob(III)alamin (**1-CN⁻**) with an equilibrium constant of about 10^4 M^{-1} [147].

Electrochemical studies of the “incomplete” diaquacobinamide (**40²⁺**) (Figure 6) gave a standard electrochemical potential for the diaquacob(III)inamide (**40²⁺**)/aqua-cob(II)inamide (**41⁺**) couple of +0.27 V [139,148]. This corresponds to the extrapolated value for the highly acidic protonated base-off form (**4-H²⁺**) of aquacob(III)alamin (**4⁺**), with $\text{p}K_{\text{a}}(\text{4-H}^{2+}) = \text{ca. } -2.4$ [139]. The potential of the corresponding aquacob(II)inamide (**41⁺**)/cob(I)inamide (**42**) couple was determined as -0.73 V [139]. The standard potential of the redox couple between **41⁺** and **42** is thus indistinguishable from that of the base-off cobalamins B_{12r}-H⁺ (**6-H⁺**)/B_{12s}-H (**39-H**).

Electrochemical studies of organometallic B₁₂ derivatives are complicated due to the rapid and irreversible loss of the organic ligand upon reduction [139]. Low temperature conditions are therefore required to obtain pertinent thermodynamic data of organometallic B₁₂ derivatives [149]. The standard

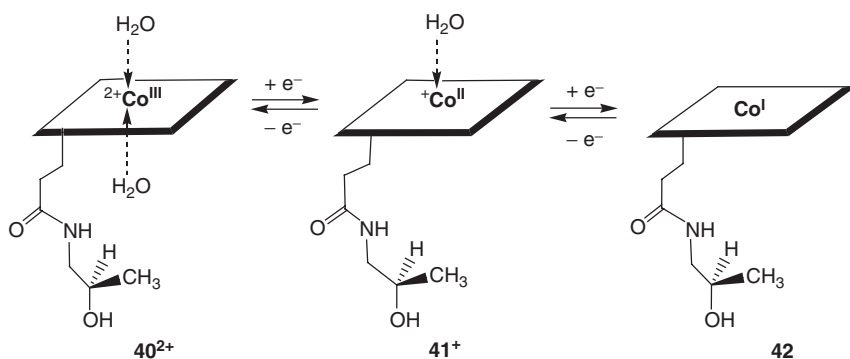


Figure 6. Outline of the redox transitions between cob(III)inamide (**40²⁺**), cob(II)inamide (**41⁺**) and cob(I)inamide (**42**).

potential (at $-30\text{ }^{\circ}\text{C}$) for the methylcob(III)alamin (**3**)/methylcob(II)alamin redox couple was estimated as -1.60 V versus SCE [139,149] similar to the value obtained for the coenzyme $\text{B}_{12}/5'$ -deoxy-5'-adenosyl-cob(II)-alamin pair [144]. The standard potential of the typical Co(III)/Co(II) redox pair of organometallic B_{12} derivatives is significantly more negative than that of B_{12r} (**6**)/ B_{12s} (**39**[−]) and out of the reach of biological reductants. However, upon one-electron reduction of **3** fast decoordination of the nucleotide base occurs, followed by rapid decomposition to a methyl radical and cob(I)alamin (**39**[−]).

The thermodynamic features of B_{12} -redox systems can be summarized as:

- (i) Intramolecular coordination of the nucleotide base or strong coordinating or nucleophilic ligands (such as cyanide ions) stabilize the corrin-bound cobalt center against one-electron reduction and shift the Co(III)/Co(II) redox couples to more negative potentials.
- (ii) The one-electron reduction of organometallic Co(III)-corrins typically occurs at more negative potentials than the Co(II)/Co(I) redox couple $\text{B}_{12r}/\text{B}_{12s}$ [139]. Exceptions to this are provided by organometallic B_{12} derivatives with electron withdrawing substituents on the organometallic group, such as methoxycarbonylmethyl-cob(III)-alamin [150].

3.2. Kinetics of the Redox Processes

One-electron transfer reactions of cobalt corrinoids are accompanied by either cleavage or formation of a bond to an axial ligand. Typically, a reduction is accompanied by an expulsion, and an oxidation by the coordination, of the ligand [139]. The electron transfer step accordingly takes place either in a concerted fashion or in a rapid associated step with coordination or dissociation of the axial ligand.

Electron transfer in the protonated Co(II)/Co(I) couple $\text{B}_{12r}\text{-H}^+$ (**6-H**⁺)/ $\text{B}_{12s}\text{-H}$ (**39-H**) is fast in aqueous solution ($k_s^{\text{app}} > 0.1\text{ cm s}^{-1}$) as the presumed axial water ligand is only kinetically weakly bound in the base-off Co(II)-corrin **6-H**⁺ [139,149]. However, when the aqua ligand in **6-H**⁺ is substituted by a stronger axial ligand, e.g., by the nucleotide base as in base-on B_{12r} , the electron transfer is slowed down sufficiently so that its kinetics can be conveniently measured by cyclic voltammetry [139,151,152]. For example, in the Co(II)/Co(I) redox couple **6/39**[−] $k_s^{\text{app}} = 0.0002\text{ cm s}^{-1}$ [139], the electron transfer is at least a thousand times slower than in the base-off forms **6-H**⁺/**39-H**.

The trend in kinetics for Co(III)/Co(II) couples follows the same trend as those for the corresponding Co(II)-/Co(I)-couples, albeit much slower. The Co(III)/Co(II) couple aquacob(III)alamin (4^+)/B_{12r} (**6**) has a rate constant for heterogeneous electron transfer of about $10^{-5} \text{ cm s}^{-1}$ [139]. The electron transfer steps for the cyano-cob(III)- and cyano-cob(II)alamins **1-CN⁻** and **23-CN⁻** are slower still [139,147].

There is an approximate linear correlation between the equilibrium constant for the coordination of the axial ligand and the standard apparent rate constant for electron transfer [139]. This correlation has been rationalized by a model, in which stretching of the bond between the cobalt ion and the axial ligand represents the main factor of the kinetics of the electron transfer. As a consequence, kinetic and thermodynamic dependence of the electron transfer on the strength of the complexation of the axial ligands both add up, resulting in more negative reduction potentials as the strength of the ligand increases.

Organocobalamins, such as coenzyme B₁₂ (**2**) and MeCbl (**3**), have a different kinetic behavior from CNCbl (**1**) and other Co(III)-corrins with strong axial ligands [139,153,154]. Whereas the Co(III)/Co(II) reduction potentials are quite negative, the kinetics of electron transfer are fast. The one-electron reduction of **3** to the unstable methylcob(II)alamin anion (43^-) was estimated to have a rate constant of 1200 s^{-1} at 30 °C. However, the product of the one-electron reduction of methylcobinamide (44^+), methylcob(II)inamide (**45**), has a half life of only about 0.1 s at -20 °C and decomposes into a methyl radical and cob(I)inamide (**42**) (see Figure 7). An Arrhenius plot of the kinetics of the decomposition of **45** gave the activation

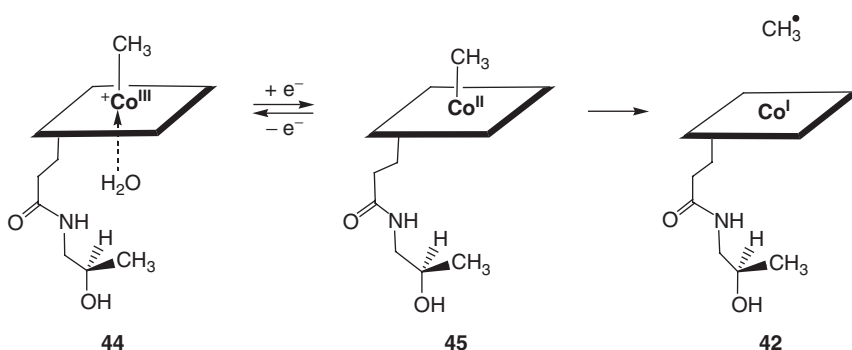


Figure 7. One-electron reduction of methylcob(III)inamide (44^+) gives methylcob(II)inamide (**45**), which rapidly decomposes into cob(I)inamide (**42**) and a methyl radical.

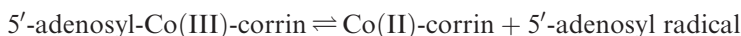
energy to be 19 kcal/mol and a pre-exponential factor $A = 10^{17.6} \text{ s}^{-1}$ [153]. From the values of the (Co–C) bond dissociation energy (37 kcal/mol) of MeCbl (**3**) [155] and the kinetics of the decomposition of the intermediate 43^- , the one-electron reduction is suggested to reduce the strength of the (Co–C) bond of **3** (by about 12 kcal/mol) to “half” of its value [139,155].

4. REACTIVITY OF B_{12} DERIVATIVES IN ORGANOMETALLIC REACTIONS

Formation and cleavage of the (Co–C) bond in organometallic B_{12} cofactors are crucial steps not only in the reactions catalyzed by B_{12} -dependent enzymes, but also for the chemistry of B_{12} derivatives in solution [9–12,22,25,156–159]. The reactivity of B_{12} derivatives in organometallic reactions thus holds the key to the understanding of the biological activity of the B_{12} -dependent enzymes. The most common methods for the preparation of such organometallic B_{12} derivatives typically rely on the efficient alkylation of Co(I)-corrins. One practical method is an electrochemical approach, as also described below.

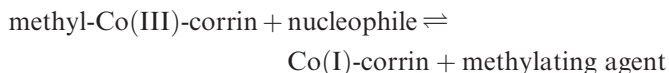
In solution cleavage and formation of the (Co–C) bond have been observed to occur in all three basic oxidation levels of the corrin-bound cobalt center [22,156–159]. So far, two main paths for these organometallic reactions have also been found to be relevant in enzymes:

- (i) the homolytic mode is typical of the reactivity of coenzyme B_{12} (AdoCbl, **2**):



Formally it is a one-electron reduction/oxidation of the corrin-bound cobalt center and it results in the cleavage or formation of a single axial bond [11,22,158–160].

- (ii) the nucleophile-induced, heterolytic mode is typical of the reactivity of MeCbl (**3**):



Formally, it involves a two-electron reduction/oxidation of the corrin-bound cobalt center and the cleavage or formation of two (*trans*) axial bonds [11,22].

may be a penta-coordinate (or already solvated and effectively hexa-coordinate) Co_β -alkyl-Co(III)-corrin;

- (ii) in aqueous solution and at room temperature the “base-on” (hexa-coordinate) methylcob(III)alamin is more stable by about 4 kcal/mol than the “base-off” Co_α -aqua- Co_β -methylcob(III)alamin [126]. From NMR studies, the latter has been estimated to still be more stable in water, by around 7 kcal/mol, than the corresponding “base-off” and dehydrated form of Co_β -methylcob(III)alamin, which has a penta-coordinate Co_β -methyl-Co(III) center [165].

With “incomplete” cobalt-corrins the situation is again more complex, with two diastereoisomeric alkylation products often formed [11,12,112]. In specific cases, under suitable kinetic control, one of the alkyl-Co(III)-corrin diastereoisomers can form with high selectivity. For example with the Co(I) form of the lipophilic heptamethylcob(II)yrinate (**11**), the $\text{S}_\text{N}2$ -pathway can provide β -methylation with high diastereoselectivity (>96%), whilst the one-electron transfer mechanism permits the formation of the Co_α -methylated product with high diastereoselectivity (>98%) [22,112]. However, configurational equilibration via rapid methyl group transfer reactions (involving Co(I)-, Co(II)-, and unalkylated Co(III)-corrins as methyl group acceptors) may give another overall outcome [22,126].

Electrochemistry is an excellent method for the selective and controlled production of reduced B_{12} forms under potentiostatic control. As alkyl halides or alkyl tosylates react quickly and efficiently with Co(I)-corrins [138], which are cleanly generated at controlled electrode potentials near that of Co(II)/Co(I) couples, electrochemistry provides a suitable method for the synthesis of organometallic B_{12} derivatives [139]. Indeed, the one-electron reduction of organometallic Co(III)-corrins typically occurs at more negative potentials than the Co(II)/Co(I) redox couple $\text{B}_{12\text{r}}/\text{B}_{12\text{s}}$ [139]!

Using electrolysis at a controlled potential of -1.1 V versus SCE, coenzyme B_{12} (**2**) was prepared in 95% yield from vitamin B_{12} (**1**) or from aquacobalamin (4^+) by alkylating cob(I)alamin (39^-) with 5'-chloro-5'-deoxyadenosine [166]. Other organometallic B_{12} derivatives produced in an analogous method were, e.g., pseudocoenzyme B_{12} (**37**) (78% yield from pseudovitamin B_{12}) [110], neocoenzyme B_{12} (**39**) (89% from neovitamin B_{12}) [111] and homocoenzyme B_{12} (**24**) (99% from 4^+) [85]. Co_β -methyl-imidazolylcobamide (**31**) (90% yield from Co_β -cyano-imidazolylcobamide) [94] and methyl-13-epicob(III)alamin (**46**) (88% yield from neovitamin B_{12}) [111] were synthesized by alkylation with methyl iodide. Also, dimeric B_{12} derivatives, such as the Co_β -alkyl bridged and sterically crowded tetramethylene- Co_β -1,4-biscobalamin (**30**) [98], and a strained organometallic B_{12} -rotaxane [167], were synthesized by similar methods (see Figure 9).

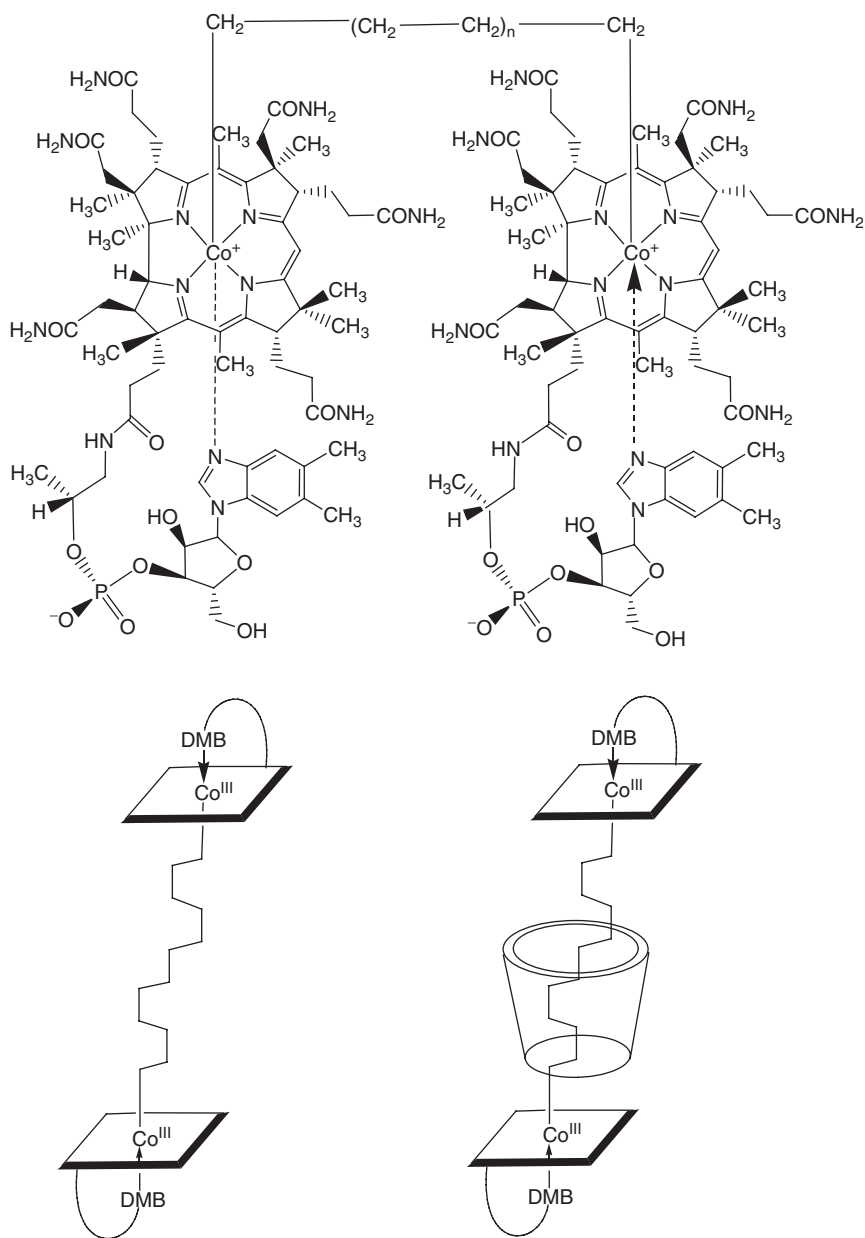


Figure 9. Electrochemistry as means for the preparation of alkyl-bridged biscorrioids. Structural formulae of tetramethylene-bridged biscobalamins (**30**, $n = 1$) [97] and of a dodecamethylene-bridged biscobalamins ($n = 5$); symbolic representations of alkyl-bridged biscobalamins and of a cyclodextrin-based B₁₂-rotaxane [166].

The high nucleophilicity of cob(I)alamin (39^-) towards alkylating agents makes it a versatile tool for the detection of toxicologically relevant electrophilic reagents. Such analytical methods are facilitating *in vitro* and *in vivo* studies of genotoxic compounds in cancer risk assessment. Many genotoxic compounds are directly (or indirectly) electrophilically reactive. The use of cob(I)alamin (39^-) as an analytical tool has been investigated in the trapping of oxiranes, metabolites of alkenes, to form alkyl-Cbls (Figure 10) [168,169]. It is presumed that the reaction proceeds according to an S_N2 reaction following attack at the least hindered carbon [170].

In the work of Fred et al. [168], the 1,2-epoxide metabolites (oxiranes) of 1,3-butadiene were studied. For each metabolite a specific alkyl-Cbl was formed and it was possible to discriminate between the products by HPLC-UV and by LC-MS. The cob(I)alamin (39^-), used in this study, had the advantage of reacting about 400,000 times faster than, e.g., nicotinamide, and therefore gave a better on-the-spot account [168]. Similar processes may also be relevant *in vivo*, as made likely by recent studies, which aimed to mimic the chemical reactions that could deplete vitamin B₁₂ as a result of human exposure to electrophilic xenobiotics (styrene, chloroprene, and 1,3-butadiene) [171].

The electrochemical methodology (see previous section) has been further developed as a method for the clean preparation of easily reduced

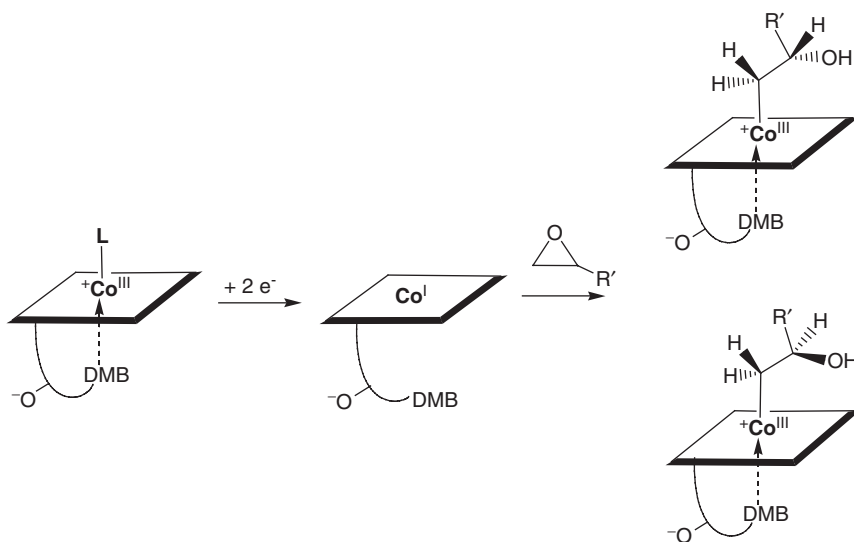


Figure 10. Illustration of the alkylation of a cob(I)alamin (39^-) by reaction with an oxirane [167].

organocorrinoids, such as Co_β-[(methoxycarbonyl)methyl]-cob(III)alamin (**47**) via the alkylation of cob(II)alamin (**6**) [172]. Easily reducible organo-cob(III)alamins are known to be cleaved by direct electrochemical reduction or by reduction with cob(I)alamin (**39**[−]) [150]. An acceptor-substituted C atom is directly bound to the Co center in **47** inducing it to be reduced at a peak potential of 0.90 V versus SCE (in DMF, room temperature) [173]. This value is close to that of the redox couple (base-on) B_{12r} (**6**)/B_{12s} (**39**[−]) (−0.85 V) and explains the difficulties encountered when preparing **47** via alkylation of **39**[−] [173]. However, when aquacob(III)alamin chloride (**4**⁺-Cl[−]) was submitted to a controlled potential of −0.45 V (versus 0.1 N CE) under potentiostatic conditions, it gave **6** cleanly. After the addition of an excess of methylbromoacetate and continued electrochemical reduction at −0.45 V, the crystalline alkyl cob(III)alamin was isolated in 75% yield [172]. The reaction was proposed to take place directly via **6** and radical intermediates [172]. The alkylation of “complete” Co(II)corrinsoids with sufficiently reactive alkylating agents (methyl iodide, methylbromoacetate, etc.) thus is an efficient and alternative method to the more established synthetic procedures via Co(I)corrinsoids for the synthesis of reduction-labile Co(III) organocorrinoids [172,174].

Organocobalamins are also accessible by the reaction between Co(II)-corrins and radicals. In particular, the radicaloid cob(II)alamin (B_{12r}, **6**) has a penta-coordinated Co(II) center and can be considered to fulfill all the structural criteria of a highly efficient “radical trap”, as revealed by the crystal structure of **6** [58]: the reactions of B_{12r} (**6**) with alkyl radicals are indicated to occur with negligible restructuring of the (DMB nucleotide coordinated) cobalt corrin moiety and to furnish coenzyme B₁₂ (**2**) and other organo-Cbls directly by the “homolytic” mode of formation of the (Co–C) bond [58]. From this it is understandable that the remarkably high reaction rate of **6** with alkyl radicals (such as the 5′-deoxy-5′-adenosyl radical), and the diastereospecificity for the reaction at the β-face, are both consistent and explainable due to the structure of cob(II)alamin (see Figure 11). The

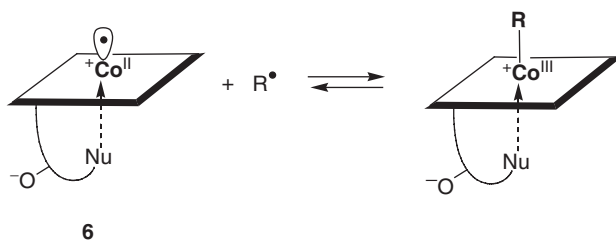


Figure 11. The “radical trap” cob(II)alamin (**6**) rapidly combines with radicals on the “upper” β-face.

coordination of the DMB-nucleotide in **6** controls the (α/β)-diastereoface selectivity (in both a kinetic and thermodynamic sense) in alkylation reactions at the Co(II) center, which give β -alkyl-CbIs directly [141,159].

The stereochemical situation is appreciably more complex in “incomplete” corrins, such as cob(II)ester (**11**) and “base-off”-forms of “complete” corrins [22]. The axial ligand at the corrin-bound Co(II) center is expected to direct the formation of the (Co–C) bond. In this way kinetic control can lead with high efficiency to the “rare” α -alkyl-Co(III)-corrins [112,175]. In such radical recombination reactions the axial ligand at the α - or β -side of the metal center will not only steer the diastereoselectivity of the alkylation but also may contribute to significant altering of the cage effects [160,176].

The two most relevant modes of formation (and cleavage) of the (Co–C) bond of the cobalt center differ significantly in their structural requirements (see Figure 12):

- The heterolytic mode of formation (and cleavage) of the (Co–C) bond, in which significant reorganizations at both faces of the corrin-bound cobalt center occur;
- The homolytic mode of formation (and cleavage), in which the cobalt-corrin portion of complete cob(III)amides (such as **2** and **3**) hardly changes structure.

Photolysis of methylcobalamin (MeCbl, **3**) in deoxygenated aqueous solution saturated with (pressurized) carbon monoxide, gave acetyl-cobalamin in good yield and in a radical reaction, which was considered to finally involve the (re)combination of cob(II)alamin (**11**) with an acetyl radical [177]. This experiment turned out not to be relevant for the biological

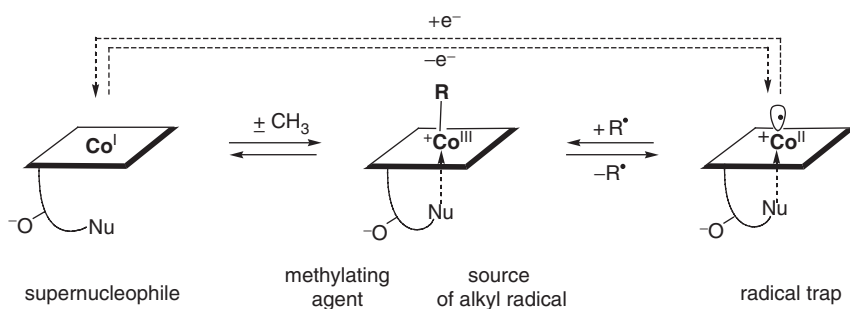


Figure 12. Elementary reaction steps in organometallic and redox transformations of “complete” corrinoids, and their patterns of reactivity relevant for their cofactor function in B_{12} -dependent enzymes.

assembly of acetyl-CoA by the now well known enzyme acetyl-CoA synthase [178]. However, this organometallic transformation with a B₁₂-derivative turned out to find considerable interest as a model for the “slaving-in” mechanism in radical reactions [179].

Another special mode of the formation of (Co–C) bonds in alkyl Co(III)-corrins involves nucleophilic alkylating agents and the electrophilic properties of aqua-Co(III)-corrins [156,157,180].

A further means of preparing methyl-corrinoids is opened by methyl group transfer reactions between corrinoids and methyl-corrinoids and some other methyl-organic compounds [22,126,181].

4.2. Cleavage of the (Co–C) Bond in Organocorrinoids

As coenzyme B₁₂ (AdoCbl, **2**) is considered to be a “reversible carrier of an alkyl radical” (or a reversibly functioning “radical source” [159]), the homolytic mode of the cleavage of the (Co–C) bond of **2** is of particular importance in its role as a cofactor. The strength of the (Co–C) bond of AdoCbl has been calculated to be about 30 kcal/mol by using detailed kinetic analyses of the thermal decomposition of **2** [159,160,182]. Considerable cage effects, and the presence of both “base-on” and “base-off” forms of **2**, caused complications in the quantitative treatment of the homolytic (Co–C) bond dissociation energy (BDE) [160]. In several organocobalamins, the nucleotide coordinated “base-on” forms decomposed faster than their corresponding nucleotide-deficient organocobinamides or their protonated (“base-off”) forms of the organocobalamins [183,184]. The intramolecular coordination of the nucleotide was therefore considered to cause a “mechanochemical” means of labilizing the (Co–C) bond of organometallic B₁₂ derivatives [159,183,184]. The extension of this idea to the enzymatic reactions with **2** as cofactor was disputed [58]. In the time since, crystallographic studies of coenzyme B₁₂-dependent enzymes also helped to dismiss much of the original idea concerning the direct “mechanochemical” mechanism. They rather suggested the specific stabilization of the homolysis fragments to be an important means of producing destabilization in the protein-bound AdoCbl (**2**) – and thus activating **2** towards homolysis [58]. From the more recent crystal structures of AdoCbl-dependent enzymes, a distant aden(os)ine-binding pocket is now recognized to provide the required structural means for this [58,86,88,185].

Indeed, the contribution of the nucleotide coordination to the ease of homolytic cleavage of AdoCbl (**2**) was found to be relatively small: On the basis of available thermodynamic data concerning the coordination of the nucleotide in **2** and of the homolysis product cob(II)alamin (**6**), the

coordination of the nucleotide was estimated to weaken the (Co–C) bond by only 0.7 kcal/mol [22,126]. In contrast, in MeCbl (**3**) the intramolecular coordination of the nucleotide was determined to increase the homolytic (Co–C)-BDE of **3**, by a bout 4 kcal/mol according to studies of the methyl-group transfer equilibrium between MeCbl (**3**)/cob(II)inamide (**41**) and methylcobinamide (**44**)/cob(II)alamin (**6**) [22], see Figure 13.

The nucleophile-induced dealkylations of alkyl-Co(III)-corrins is another well known means for cleavage of the (Co–C) bond, in particular for methyl-Co(III)-corrins. It has been less studied with MeCbl (**3**), due to the impediment of the nucleophile-induced demethylation by the intramolecular coordination of the nucleotide base [125,186]. Indeed, thiolates demethylate methylcobinamide (**44**) to cob(I)inamide (**42**) approximately 1000 times faster than MeCbl (**3**) to B_{12s} (**39**[−]) [186], reflecting the strong stabilizing effect of the coordinated nucleotide in **3** [22,126]. This effect is of relevance also for enzymatic methyl group transfer reactions involving protein-bound Co(I)- and methyl-Co(III)-corrins, where considerable axial base effects are seen [187].

The electrophile-induced dealkylation of the cobalt-bound methyl group by polarizable metal ions, such as Hg²⁺ ions, is a crucial path to methyl-metal complexes, such as the poisonous Hg-CH₃ ion [26]. Aqua-Co(III)-corrins can also demethylate methyl-Co(III)-corrins slowly at room temperature [181]. The coordination of the DMB nucleotide modifies the reactivity of the cobalt center by enhancing the ease of abstraction by electrophiles, in both a kinetic and thermodynamic sense [125]. The (Co–C) bond of alkyl-Co(III)-corrins is rather inert against proteolytic cleavage under physiological conditions. The acid-induced heterolytic cleavage of the (Co–C) bond of MeCbl (**3**) is not documented, the cleavage of coenzyme B₁₂

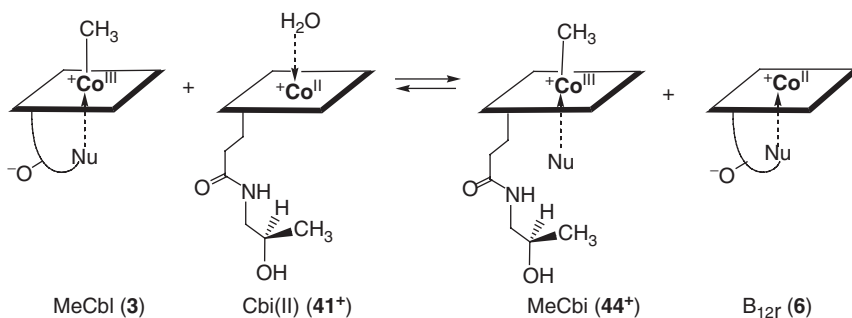


Figure 13. Methyl transfer reaction involving MeCbl (**3**) and methyl-cob(III)inamide (**44**⁺) as methyl group donors and B_{12r} (**6**) and cob(II)inamide (**41**⁺) as methyl group acceptors.

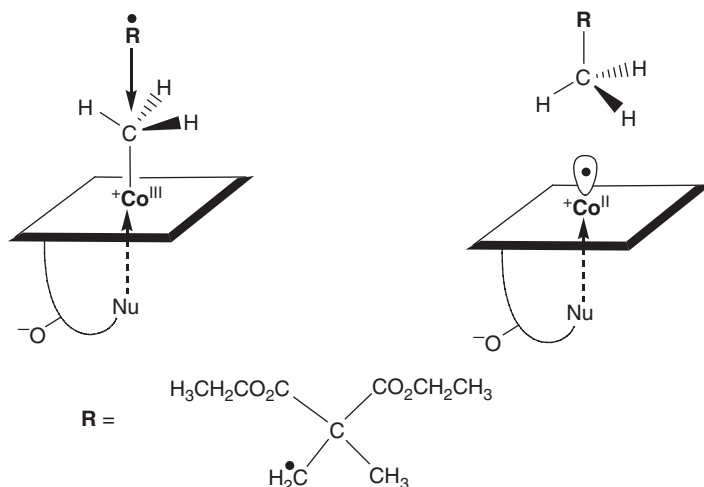


Figure 14. Methylcobalamin (3) as a methylating agent for organic radicals, a hypothetical new mechanism of biological methylation [189].

(2) occurs in acidic aqueous solution at low pH [188], but less readily when compared to 2'-deoxycoenzyme B₁₂ (48) and 2',3'-di-deoxycoenzyme B₁₂ (49) [163]. The reactivity difference can be traced back largely to the effect of the ease of protonation of the cobalt-bound organic group [163]. Interestingly, the replacement of the DMB base by an imidazole in Co_β-adenosyl-imidazolyl-cobamide also results in a more readily dealkylated analogue of AdoCbl [188].

A recently recognized further mode of cleavage of the (Co–C) bond of organometallic B₁₂ derivatives, is represented by the radical-induced substitution at the cobalt-bound carbon center [22,98,189] (Figure 14). This type of thermodynamically favorable reaction holds strong interest due to the observation of unusual biological (C–C) bond forming reactions and methylations at seemingly inactivated carbon centers [190,191].

In a formally related radical abstraction reaction, the cobalt-bound methyl group of methylcobalamin (3) and other methylcorrinoids is rapidly abstracted by Co(II)-corrinoids, such as cob(II)inamide (41⁺), (giving methylcob(III)inamide, 44⁺) and cob(II)alamin (B_{12r}, 6) (Figure 14) [22]. This type of reaction does not involve free methyl radicals and, under appropriate conditions (aprotic solvents), it is not (even) sensitive to the presence of molecular oxygen [192].

The (Co–C) bond of various organocorrinoids is cleaved homolytically by absorption of visible light and organometallic B₁₂ derivatives have long been known to be sensitive to visible light [193], which induces cleavage with a

quantum yield of about 0.3 [141,194,195]. Organo-cob(III)amides are also labile to strong one-electron reducing agents, as it has been found that after one electron reduction of organyl-Co(III)-corrins the (Co–C) bond is considerably weakened [139,155]. As noted above, this aspect may render it difficult to prepare organo-cob(III)amides with electron-withdrawing substituents, via alkylation of the strongly reducing cob(I)amides [173].

5. ORGANOMETALLIC B₁₂ DERIVATIVES AS COFACTORS AND INTERMEDIATES IN ENZYMES

“Complete” methyl- and adenosyl-corrinoids, such as MeCbl (**3**) and AdoCbl (**2**), typically are considered to be the relevant cofactor forms of corrinoids in enzymatic reactions. These organometallic corrinoids are frequently observable in the resting states (and may be found in isolation forms) of functioning enzymes, and may then be bound characteristically “base-on”, “base-off/His-on” or “base-off” to the protein part of the enzyme [18–23]. The catalytically equally important dealkylated cofactor forms, such as the Co(II)- and Co(I)-corrinoids cob(II)alamin (**6**) and cob(I)alamin (**39**[−]), are less well observable species, and transient in the enzyme reactions, for reasons of their thermodynamic instability under typical physiological conditions.

In most organisms, physiologically inactive forms of the (“complete”) corrinoids are taken up and converted into active organometallic cofactor forms enzymatically. In the human metabolism, (inactive) vitamin B₁₂ (**1**) is converted into the adenosyl-corrinoid coenzyme B₁₂ (**2**) by an ATP-using adenosyl-transferase, or into the methyl-corrinoid MeCbl (**3**) by methylation with *S*-adenosyl-methionine (SAM) in the methionine synthase complex [17]. However, in the course of the biosynthesis of “complete” corrinoids in microorganisms, such as of the cobalamins, “incomplete” organometallic B₁₂ derivatives already play an important role at an early stage as obligate biosynthesis intermediates [196]: Thus, the biosynthetic build-up of the “complete” corrinoids firsts leads to the “incomplete” (organometallic) Co_β-5'-deoxy-5'-adenosyl-cobinamide (**32**), to proceed further to the “complete” B₁₂ derivatives by assembly of the nucleotide moiety [196].

Organometallic B₁₂ derivatives are also considered as intermediates in B₁₂-dependent reductive dehalogenases [97], which play an important role in the detoxification of chlorinated compounds [197,198]. Several B₁₂-dependent dehalogenases have been purified with nearly all containing one or more iron-sulfur clusters, in addition to the corrinoid cofactor [97,197]. In the anaerobic bacterium *Sulfurospirillum multivorans*, which catalyzes the reductive dehalogenation of tetrachloroethene and

trichloroethene to *cis*-1,2-dichloroethene [97], a novel corrinoid cofactor was found that had slightly different catalytic properties to other cobamides [66]. This cofactor was isolated as norpseudovitamin B₁₂ (**15**, Co_β-cyano-7''-adeninyl-176-norcobinamide or 176-norpseudovitamin B₁₂), a homologue of pseudovitamin B₁₂ (**16**) lacking the methyl group attached to carbon 176 [66].

In the B₁₂-catalyzed reductive dechlorination of tetrachloroethylene the first step is likely to involve an electron transfer from the fully reduced Co(I)corrin (such as **39**[−]) to tetrachloroethylene, leading to a Co(II)corrin (such as **6**) and the formation of a trichlorovinyl radical by loss of chloride [199]. Chlorovinylcobalamin (**29**) and vinylcobalamin (**28**) were thus synthesized as model compounds [200]. It was shown that chlorinated organometallic derivatives could be possible intermediates in reductive dehalogenation reactions, as **39**[−] promoted reactions can reduce such compounds back to the active form of the catalyst [95,96].

5.1. Methylcorrinoids in B₁₂-Dependent Methyltransferases

The B₁₂-dependent methyltransferases play an important role in amino acid metabolism in many organisms (including humans) as well as in one-carbon metabolism and CO₂ fixation in anaerobic microbes [21]. The reactivity of the “supernucleophilic” Co(I)-corrins and of methyl-Co(III)-corrins make B₁₂ derivatives ideal as cofactors in such enzymatic methyl group transfer reactions [11,12]. B₁₂-dependent methionine synthase has been particularly well studied (see e.g. [21,201]) as have methyltransferases in aerobic acetogenesis (see, e.g., [202]), methanogenesis (see, e.g., [203]), and in the anaerobic catabolism of acetic acid to methane and CO₂ (see, e.g., [204]). Various substrates act as sources of methyl groups, such as methanol, methyl amines, aromatic methyl esters, methylated heavy metals or N⁵-methyltetrahydro-pterins (such as N⁵-methyltetrahydromethanopterin or N⁵-methyltetrahydrofolate). For N⁵-methyltetrahydrofolate as a source of the methyl group it has been suggested that the methyl group donor is more likely to be the protonated form of N⁵-methyltetrahydrofolate [21]. Thiols are the methyl group acceptors in methionine synthesis (homocysteine) [21] and methanogenesis (coenzyme M) [23]. In the anaerobic biosynthesis of acetyl-coenzyme A from one-carbon precursors the methyl group acceptor is suggested to be the nickel center attached to the Fe/S cluster [205].

The methyl group transfers catalyzed by methionine synthase (MetH) from *E. coli* [21] and other B₁₂-dependent methyltransferases are all indicated to proceed with an overall retention of configuration (i.e., consistent with two nucleophilic displacement steps, each with inversion of

configuration) [206,207]. These stereochemical findings exclude free methyl cations or radicals as intermediates, even though, in a formal sense, the methyl transfer reactions catalyzed by B₁₂ enzymes involve (nucleophilic-bound) methyl “cations” and heterolytic cleavage/formation of the (CoCH₃) bond. The methyl group transfer thus, relies on the catalytic properties of enzyme-bound Co(I)corrins and methyl-Co(III)-corrins [22] and is amenable to considerable control from the protein environment [21], due to the great structural changes expected to accompany the transitions from (tetra-coordinate) Co(I)corrins to (hexacoordinate) methyl-Co(III)-corrins [11,12].

The X-ray crystal analysis of the B₁₂-binding domain of MetH provided the first insight into the three-dimensional structure of a B₁₂-binding protein [36,116,208,209]. The astounding revelation of this work was the finding that the cobalt-coordinating DMB nucleotide tail of the protein-bound cofactor MeCbl (**3**) was displaced by a histidine imidazole and extended into the core of the “Rossmann fold” [36,209]. Consequently, in methionine synthase the corrinoid cofactor is bound by histidine ligation to the metal center and in a “base-off” constitution, i.e., bound in a “base-off/His-on” mode. Various other B₁₂-dependent methyltransferases are indicated to have either a “base-off/His-on” bound methyl-Co(III)-corrinoid, or even a corrinoid cofactor in “base-off” form (where His-coordination is absent) [128] (Figure 15).

In a catalytic cycle of B₁₂-dependent methyltransferases the corrinoid is indicated to cycle between a methyl-Co(III)-corrin and a Co(I)-corrin [21,23]. The changing between the hexacoordinate methyl-Co(III) form and (presumably) tetracoordinate Co(I) form is accompanied by constitutional/conformational changes which are highly likely to provide a means for controlling the organometallic reactivity of the bound cofactor [22], subject to H⁺ uptake or H⁺ release (see Figure 15). In response, a H⁺-mediated switch mechanism may result, mediated via the “regulatory” His-Asp-Ser triad, which provides the crucial conformational alterations associated with the enzyme [21,201,210]. The nucleophile-induced methyl group transfers, involving heterolytic cleavage and formation of the organometallic (Co-CH₃) bond at the corrin-bound cobalt center, are expected to be in-line attacks (incoming nucleophile/CH₃-group/leaving group) and to be subject to strict geometric control: a main role of the His-Asp-Ser-triad appears to be participating in maintaining conformational control of the mutual placement of the corrinoid cofactors and the enzyme-bound substrates [210,211].

A significant second role of the His-Asp-Ser triad in organometallic reactions is associated with the thermodynamic effect of the α -axial base coordination on the strength of the (Co _{β} -CH₃) bond. Solution studies showed a significant thermodynamic *trans* effect of the DMB coordination in methylcobalamin (**3**) [11,22,125] and of the imidazole coordination in Co _{β} -CH₃-imidazolyl-cobamide (**27**) [94] on heterolytic methyl group

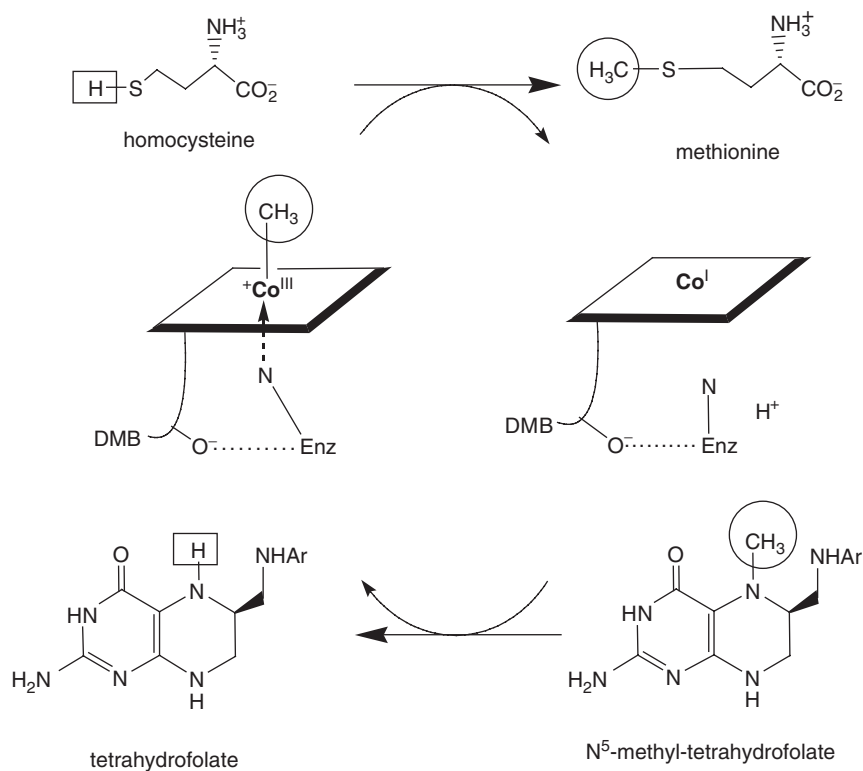


Figure 15. Illustration of methionine formation catalyzed by MetH (Enz signifies the MetH-apoenzyme) [21], where the bound corrinoid shuttles between MeCbl (3), in a “base-off/His-on” form, and cob(I)alamin (B₁₂s, 39⁻).

transfer reactions. The result showed that the stronger coordinating (nitrogen) ligand stabilizes the methyl-Co(III) form against nucleophilic abstraction of the methyl group by about 4 kcal/mol in **3** [126]. This may be seen mainly as an “electronic” effect [11,22,125], consistent with the observation of anomalous structural *trans* effects in other methyl-Co(III) complexes [100]. More recent studies with **27** suggested the imidazole base exerts similar “electronic effects” as the DMB base in **3** but **27** is more basic and, therefore, imidazolyl-cobamides (or the “base-off/His-on” form of **3**) are more readily protonated near neutral pH [94]. The His-Asp-Ser triad may then represent a “relay” for H⁺ uptake/release, assumed to function in the enzymatic methylation/demethylation cycles [212,213].

In conclusion, the axial (Co–N_{ax}) bond in the methyl-Co(III) form of the protein-bound cofactor of MetH (and other B₁₂-dependent

methyltransferases) appears to have three important consequences. The weakening of this bond activates both (i) the methyl group for heterolytic abstraction by a nucleophile and (ii) the Co(II) form for reduction to the Co(I) form and (iii) helps to position the methyl-cob(III)amide cofactor for methyl group transfer [22,201,211].

5.2. Adenosylcorrinoids in Enzymes Dependent on Coenzyme B₁₂

About ten coenzyme B₁₂-dependent enzymes are now known. These enzymes are four carbon skeleton mutases, two diol dehydratases, ethanolamine ammonia lyase, two amino mutases and B₁₂-dependent ribonucleotide reductase (see [18–20,214–216]). The coenzyme B₁₂-dependent enzymes are disproportionately distributed in the living world. Only methylmalonyl-CoA mutase is indispensable in human metabolism.

The coenzyme B₁₂-dependent enzymes perform chemical transformations that are difficult to achieve by typical “organic reactions” [18]. With the exception of the enzymatic ribonucleotide reduction [215], the results of coenzyme B₁₂-catalyzed enzymatic reactions correspond to isomerizations with vicinal exchange of a hydrogen atom and of a group with heavy atom centers. Homolytic cleavage of the (Co–C) bond of the protein-bound AdoCbl (**2**) to a 5'-deoxy-5'-adenosyl radical and cob(II)alamin (**6**) was indicated early to be the entry to H abstraction reactions induced by the 5'-deoxy-5'-adenosyl radical [217]. Therefore, homolysis of the (Co–C) bond of **2**, which is the thermally most easily achieved reaction of **2** in solution (homolytic Co–C BDE of about 30 kcal/mol [159,176]) appears to be its biologically most significant reactivity: coenzyme B₁₂ (**2**) is characterized as a “reversible free radical carrier” [159] (see Figures 11, 12, and 16).

However, the homolysis of the (Co–C) bond of the protein-bound coenzyme needs to be accelerated by a factor of about 10¹² to agree with the observed rates of reaction of catalysis by the coenzyme B₁₂-dependent enzymes [159,160]. The deduced dramatic labilization of the bound organometallic cofactor towards homolysis of the (Co–C) bond is an intriguing feature of the coenzyme B₁₂-dependent enzymes [159,160,215]. The mechanism of the enzyme- (and substrate-) induced labilization of the (Co–C) bond still is a much discussed problem. Covalent restructuring of the bound cofactor (except for the formation of the “base-off/His-on”-form in the carbon skeleton mutases) is not indicated [86,216]. In addition, protein and solvent molecules can only weakly stabilize a radical center [218]. Steric distortions of the protein-bound cofactor are thus considered as a likely means for the enhanced rate of (Co–C) bond homolysis [85,86,88,216]. In

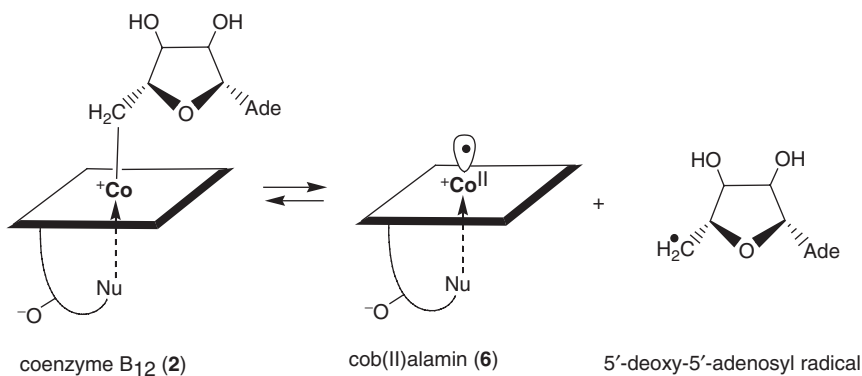


Figure 16. Coenzyme B₁₂ (AdoCbl, **2**), a reversible source of the 5'-deoxy-5'-adenosyl radical and of cob(II)alamin (B₁₂, **6**).

view of the available crystal structures of cob(II)alamin (**6**) [58] and of coenzyme B₁₂-dependent enzymes [36–44], Halpern's earlier suggestion of an “upwards conformational distortion” of the cobalt-corrin part of **2** [159] is not likely to be of relevance. However, labilization may come about largely from a protein- and substrate-induced strain on the organometallic group, separation of the largely nonstrained homolysis fragments and strong binding by the protein of the separated pair, 5'-deoxy-5'-adenosyl radical and **6** (in either a “base-off/His-on” or “base-on” form) [85,86,88,216]. One explanation is the existence in some of these enzymes of a binding interface (e.g., of an “adenosine-binding pocket”) which does not allow for unstrained binding of the organometallic moiety [85,86,88,216]. Fixed placement of the corrin moiety at the interfaces of the B₁₂-binding and substrate-binding/activating domains appears to be of high significance and movements of the corrin moiety are not required. The “regulatory triads” logically appears not to be involved in proton-transfer steps and may conserve its structure largely during enzymatic turnover. “Electronic effects” of the axial *trans* ligand on the (Co–C) bond homolysis in **2** and MeCbl (**3**) are now seen to be of less importance [22].

To conclude, all coenzyme B₁₂-dependent enzymes appear to rely on the reactivity of bound organic radicals, which are formed (directly or indirectly) by a H atom abstraction by the 5'-deoxy-5'-adenosyl radical, that originates from the homolysis of the (Co–C) bond of AdoCbl (**2**). In these enzymatic reactions, the 5'-deoxy-5'-adenosyl radical is the established reactive partner in the actual enzymatic reaction, so that **2** should be looked at as a “pre-catalyst” (or catalyst precursor) [22]. Coenzyme B₁₂ (**2**) might then be considered to be a structurally highly sophisticated, reversible source for an

alkyl radical, whose (Co–C) bond is labilized in the protein bound state [159], and the main role of the bound cofactor AdoCbl (**2**) is indeed, the production and controlled presentation of the 5'-deoxy-5'-adenosyl radical from homolysis of its (Co–C) bond [159]. The function of the remaining Co(II)-corrin fragment **6** of the coenzyme (as a “spectator” or a “conductor”) has recently again become a matter of discussion [129] and has been re-addressed by calculations [219].

The rearrangement steps of B₁₂-dependent enzymatic rearrangements are now assumed to be accomplished by tightly protein-bound radicals that are controlled in their reaction space [18]. Consequently, the major functions of the enzyme concern not only the catalysis of its proper reactions but also the reversible generation of the radical intermediates and the protection of its proteinic environment from non-specific radical chemistry, called “negative catalysis” [217].

6. CONCLUDING REMARKS AND FUTURE DIRECTIONS

The discovery of B₁₂ coenzymes by Barker et al. [220] and of their organometallic nature by Lenhert and Hodgkin [1], as well as subsequent studies of the organometallic chemistry and biological function of AdoCbl and MeCbl have helped to open the field of “bioorgano-metallic” chemistry. Clearly, Nature makes use of the capacities of organometallic catalysis in a remarkable way, as is particularly apparent, e.g., in alternative pathways of carbon fixation in anaerobes [205,221].

In the B₁₂-dependent metabolism, the B₁₂ cofactors are bound to proteins and are subjected to the mutual interaction with the proteins. Recently, natural B₁₂-binding nucleotides have also been discovered and suggested to function as “riboswitches”, relevant in a new form of controlling gene expression [222]. This finding has begun to open a new area of research in the B₁₂ field [223,224] and to induce further studies and complementary work with B₁₂ nucleotide conjugates [49,225]. Indeed, the capacity for mutual interaction between the evolved B₁₂ cofactors and functional nucleotides is hardly explored. This subject may intensify the search for evidence for a role of corrinoids in an early form of life, such as is represented by the (hypothesis of an) RNA world [226]. Corrinoids clearly are unique compounds extending the capacity of (biological) organometallic catalysis.

In another direction, recent studies on the proteins involved in the uptake of B₁₂ derivatives in microorganisms [227], humans [133], and other

mammals are giving a good foundation for investigations on other complex questions, such as:

- (i) how corrinoids are selectively acquired from the environment, and what forms of mutual dependencies and symbiosis may result from the metabolic need of B₁₂-catalyzed organometallic processes (see, e.g., [228]).
- (ii) how vitamin B₁₂ derivatives can be used as carriers to shuttle small ligands or larger molecules into cells, simple and higher organisms [74], to diagnose and to influence their metabolism [73].

The growing understanding of the unique reactivity of corrinoids in organometallic processes may also lead to an increasing use of these natural cobalt complexes in the (*in vitro* and *in vivo*) analysis of normal and aberrant life processes, such as chemical modifications and damage to DNA [229]. Along the same lines, organometallic processes with B₁₂ derivatives also provide a remarkable potential as the general basis of novel synthetic and analytical developments [137]. Clearly, the “most beautiful” cofactor [230] and its unique organometallic reactivity [22] will continue to fascinate not only the “B₁₂ fraternity”, but it will keep a special place in a range of current and future scientific developments.

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ABBREVIATIONS

Ado	adenosyl
AdoCbl	5'-deoxy-5'-adenosylcobalamin, adenosylcobalamin, coenzyme B ₁₂
BDE	bond dissociation energy
Cbi	cobinamide
Cbl	cobalamin (DMB-cobamide)
CE	calomel electrode
CNCbl	cyanocobalamin
CoA	coenzyme A
DMB	5,6-dimethylbenzimidazole

DMF	dimethylformamide
ESR	electron spin resonance
H ₂ OCbl	aquacobalamin, B _{12a}
HOCbl	hydroxocobalamin
HPLC	high performance liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
MeCbl	methylcobalamin
MetH	methionine synthase
NHE	normal hydrogen electrode
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
Nu	nucleotide function
SAM	<i>S</i> -adenosylmethionine
SCE	saturated calomel electrode
UV-Vis	ultraviolet visible absorbance spectrum

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