B_{12}-Radical Chemistry

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More Radical Magic with B_{12}: B_{12}-Catalyzed, Light-Induced Cleavage of DNA

Bernhard Kräutler and Barbara Puffer*

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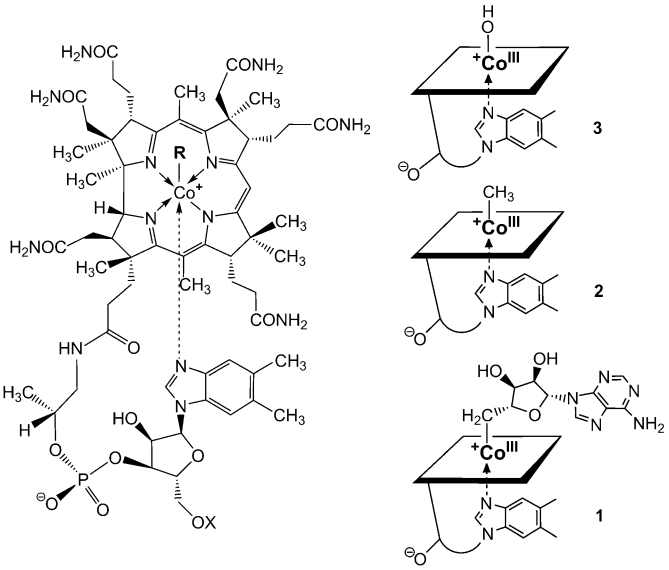
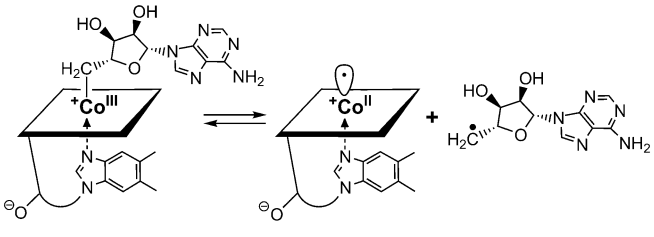
Coenzyme B_{12} (**1**) is a most fascinating cofactor.[1,2] Its inherent reactivity—to decompose reversibly into a reactive primary radical[3] and an efficient radical trap[4]—is the chemical basis for its known biological roles.[5] The radical-(oid) fragments of **1** are used, in enzyme-controlled processes, to catalyze a range of "chemically difficult" radical processes that are essential to most living organisms, including humans.[5–7] The simpler relative of **1**, methylcobalamin (**2**), carries a cobalt-bound methyl group and is the cofactor of enzymes that catalyze the transfer of methyl groups through mostly heterolytic processes.[5,8] The performance of B_{12} cofactors in organometallic reactions[8,9] is closely associated with the metabolic functions of the corrinoids.[5] The unique chemical reactivity of the cofactors **1** and **2** is the consequence of their specific structure consisting of a complex cobalt–corrin complex, a cobalt-coordinating dimethylbenzimidazole nucleotide function and an organometallic ligand[8,9] (Scheme 1). Vitamin B_{12} (cyanocobalamin) and hydroxocobalamin (**3**) are other forms of B_{12} that are commercially important, but appear to have no direct physiological role.

Thermolysis of **1** or excitation of **1** or **2** with visible light, induce the well-investigated homolysis of the Co–C bond and the formation of (primary) 5′-deoxyadenosyl radicals from **1** (Scheme 2) or of methyl radicals from **2**. In contrast, vitamin B_{12} and hydroxocobalamin (**3**) are thought to be rather stable (in aqueous solutions), even in the presence of light.[10] However, a recent investigation provided evidence for the formation of a hydroxy radical by photolysis of **3** (Scheme 3).[11] In this study, photolysis of **3** in the presence of oxygen was used to cleave plasmid DNA via photochemically generated hydroxy radicals. This process led to multiple breaks of the (double-stranded) circular plasmid DNA as the proposed result from attack by the hydroxy radicals. This finding indicated multiple turnover of the B_{12}-derivative **3** and supported the notion that **3** was functioning as a photocatalyst. The role of **3** as a real catalyst was rationalized as the consequence of a two-stage catalysis cycle that involved, first, the photo-induced formation of a hydroxy radical and cob(II)alamin (B_{12r}), and second, reoxidation of B_{12r} to the Co^{III}–corrin **3** by molecular oxygen[10,12] (Scheme 3).

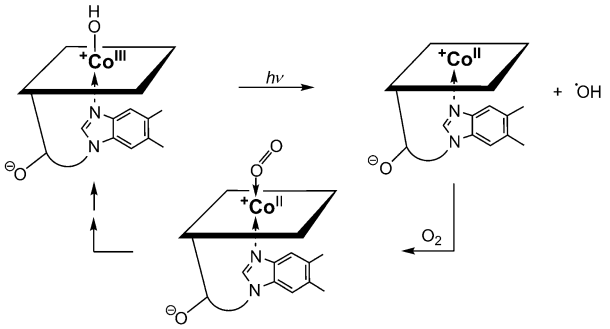


Scheme 1. Structural formula and symbols of the cob(III)alamins coenzyme B_{12} (R = 5′-deoxy-5′-adenosyl, X = H, **1**), methylcobalamin (R = CH_3, X = H, **2**), hydroxocobalamin (R = OH, X = H, **3**), and a conjugate of **3** with spermine (R = OH, X = C(O)-NH-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-NH_2, **4**).



Scheme 2. Coenzyme B_{12} (**1**) is a reversibly functioning source of the primary 5′-deoxyadenosyl radical and of cob(II)alamin (B_{12r}), which is an efficient radical trap.

[*] Prof. Dr. B. Kräutler
Institute of Organic Chemistry & Centre of Molecular Biosciences (CMBI), University of Innsbruck
Innrain 52a, 6020 Innsbruck (Austria)
E-mail: bernhard.kraeutler@uibk.ac.at



Scheme 3. Photohomolysis of hydroxocobalamin (**3**) gives hydroxy radicals and cob(II)alamin, which is reoxidized into **3** by molecular oxygen.

Hydroxy radicals have frequently been used to analyze the structure of nucleic acid oligomers, and they have been helpful in the characterization of DNA with unusual shape, as well as for the study of DNA–protein interactions and RNA folds.[13] The major advantage of hydroxy radicals over other foot-printing reagents is their outstanding reactivity, resulting in a largely sequence-independent strand scission, which is determined by the solvent accessibility of the corresponding nucleotides. However, the standard method to generate hydroxy radicals—the Fenton reaction using Fe^{II} EDTA and H_2O_2[13]—reaches its limits in intracellular applications because the initiation and termination of the radical reaction can not be precisely controlled. The approach developed by Shell and Lawrence,[11] using hydroxocobalamin (**3**) as the radical source, may allow intracellular temporal control as a result of the light dependence of the photoinitiated reaction. Furthermore, conjugation of **3** with the DNA-binding moiety spermine gave the photocatalyst **4**, which exhibited strand-cleavage efficiency that was larger by about two orders of magnitude than that of **3**.

The fascinating effective inertness of the B_{12}-derivative **3** under the free radical reaction conditions outlined by Shell and Lawrence,[11] relates to the behaviour of coenzyme B_{12} (**1**) and methylcobalamin (**2**). These photolabile organometallic cofactors are also known to effectively remain unaltered, over all, when their (oxygen-free) aqueous solutions are irradiated with light[8]. These observations can be rationalized by the remarkably efficient and selective recombination of the photochemically generated radical(oid)s to the starting cobalamins, with insignificant net conversion into other products. The surprising resistance of the complex and multifunctional cobalamins against degradation by radicals is likely a result of, in large part, the highly efficient combination of radicals with the homolysis fragment cob(II)-alamin. This radicaloid Co^{II}–corrins is an ideal trap for radicals, as its structure corresponds to the cobalt–corrin part of Co^{III}–corrins (such as **1** to **3**)[8]. Indeed, the cobalt-coordinating nucleotide function directs any of the recombination reactions to the ("upper") β face and thus helps to retain the structural integrity of the resulting cob(III)alamins. In view of the proposed pre-enzymatic origin of the (basic elements of the) B_{12} structure, and the deduced prebiotic availability of the basic reactivity of cobalt–corrinoids (such as the cobalamins **1** to **3**)[1], the noted unique resistance of these highly substituted cobalt–corrins against decomposition by aggressive radicals is truly remarkable.

The outstanding property of coenzyme B_{12} (**1**) to act as a reversibly functioning source of organic radicals is the basis of its role as a cofactor in enzymes that catalyze "chemically difficult" transformations of important metabolites. Shell and Lawrence have now extended the scope of the remarkable basic versatility of cobalamins in radical reactions. They have done this by using hydroxocobalamin as a photocatalytic source for hydroxy radicals in investigations of complex biological samples, such as DNA.[11] Their new strategy is particularly promising for intracellular applications, since both, initiation and termination of the radical reaction, can be controlled with visible light. The "old vitamin" has thus become a new tool that expands the available chemical techniques for investigating the structures of complex cellular components.

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