Photoswitching

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Coenzyme B_{12} Repurposed for Photoregulation of Gene Expression

Karl Gruber and Bernhard Kräutler**

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The existence of evolved forms of life on Earth depends upon the ability to harvest and harness light.[1] Solar radiation drives photosynthesis,[2] by which plants fix carbon dioxide and generate atmospheric oxygen, thereby providing the basis for all other higher forms of life.[3] However, energy-rich sunlight is a mixed blessing; it is a source of photo-oxidative stress and its UV components cause damage to our genome.[4] Living organisms have therefore developed intricate ways to adapt to the multifaceted impact of solar irradiation, and cells have evolved sophisticated photoregulatory networks in order to sense light and adapt to it.[5]

Photoregulation of gene expression is based on photoreceptor proteins that respond to light via sensitive chromophores, such as flavins, bilins, and retinal.[5,6] In a striking recent twist from its known biological roles in radical enzymes,[7] coenzyme B_{12} (adenosylcobalamin, AdoCbl)[8] has joined the list of light-sensing cofactors for the task of photoregulation of gene expression (Figure 1). As recently shown by Perez-Marín, Murillo, Padmanabhan, Elias-Arnanz et al.,[9] the B_{12}-based photoreceptor CarH regulates the biosynthesis of photoprotecting carotenoids in the bacterium *Myxococcus xanthus*. This discovery and the associated bioinformatics-based evidence for a remarkable abundance of B_{12}-based photoregulation in bacteria (among them, *Thermus thermophilus*) raised the question of how the organometallic B_{12} cofactor could be repurposed to play a broadly relevant light-sensing gene-regulatory role. Astounding crystallographic, mutational, and mechanistic studies by Jost, Drennan et al.[10] have now provided precise structural insights into the remarkable light-dependent gene regulation carried out by the B_{12}-dependent CarH protein of *T. thermophilus*.

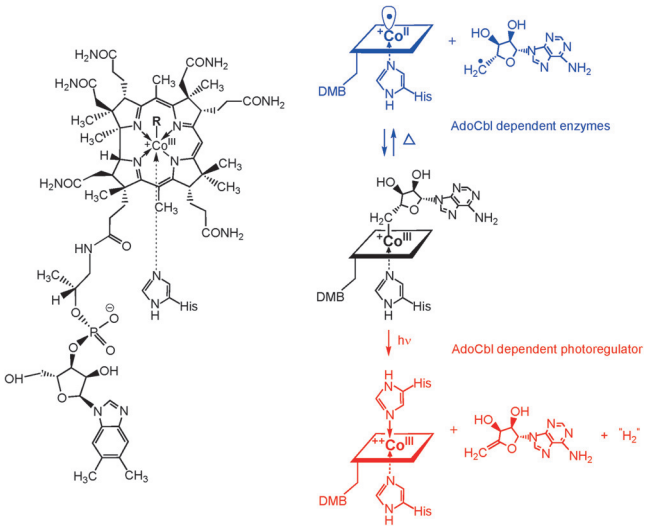

Figure 1. Left: Chemical formula of a cobalamin bound in a base-off/His-on fashion to a protein. Right: Two options for the cleavage of coenzyme B_{12} (adenosylcobalamin, AdoCbl,). In AdoCbl-dependent enzymes, the Co–C bond is cleaved homolytically to reversibly yield a reactive Ado radical (blue), whereas the photolytic cleavage of AdoCbl bound to CarH results in the irreversible formation of 4′,5′-anhydroadenosine (red).

The mechanism of gene regulation by CarH relies on modulation of the oligomeric state of the protein by the nature of the bound cofactor. Under low-light conditions, CarH binds intact AdoCbl and forms a dimer-of-dimers-type tetramer. In this state CarH binds with high affinity to the promoter region of genes coding for carotenoid biosynthesis, thereby inhibiting their transcription (Figure 2 A). Upon irradiation and photolytic cleavage of the Co–C bond of AdoCbl, a conformational change of the protomer leads to disintegration of the tetramer into monomers, which can no longer bind strongly to DNA.

CarH exhibits a modular architecture, and each protomer consists of three distinct domains with specific functions. While the N-terminal winged-helix domain is responsible for DNA interaction, the C-terminal Rossman-type domain binds B_{12}. A four-helix-bundle domain separates these two domains. This region is mainly responsible for oligomer formation and "senses" the photochemical modifications of the bound cofactor. The DNA-binding domain shows struc-

[*] Prof. Dr. K. Gruber
Institute of Molecular Biosciences, University of Graz
Humboldtstraße 50/3, 8010 Graz (Austria)
E-mail: karl.gruber@uni-graz.at
Prof. Dr. B. Kräutler
Institute of Organic Chemistry and
Center for Molecular Biosciences (CMBI)
University of Innsbruck, Innrain 80/82, 6020 Innsbruck (Austria)
E-mail: bernhard.kraeutler@uibk.ac.at
The ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201601120.

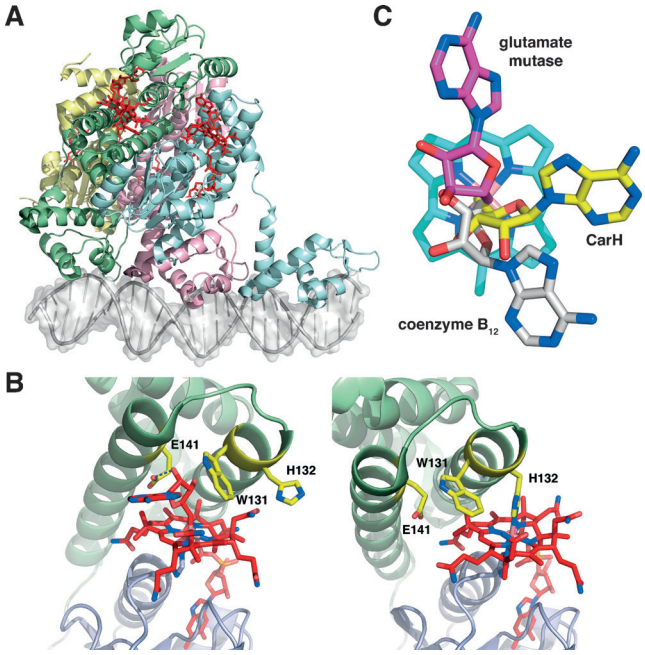

Figure 2. Crystal structures of CarH. A) Cartoon representation of the CarH tetramer (dark state) bound to DNA. The four protomers are colored green, cyan, magenta, and yellow. The bound AdoCbl is shown as a red stick representation and the DNA as a grey cartoon representation with a semitransparent surface. B) Comparison of the CarH protomer in the dark (left) and after photolysis (right) after superposition of the B_{12}-binding domains. The latter are shown in blue and the four-helix-bundle domains in green. The bound cobalamin is shown as a red stick representation. Residues assumed to be important for the functioning of the photoswitch are shown in yellow. C) Comparison of the relative orientation of the adenosyl group of AdoCbl in different environments (yellow: AdoCbl bound to CarH, magenta: AdoCbl bound to glutamate mutase, white: free AdoCbl = coenzyme B_{12}). The corrin ring is shown in cyan. The Figure was prepared using the program PyMOL (http://www.pymol.org).

tural similarity to the corresponding domains of the MerR-family transcription factors.[11] The B_{12}-binding domain is structurally very similar to the binding domain of MetH,[12] including the quite common base-off/His-on mode of cofactor binding (Figure 2B). The crystal structure of the CarH tetramer in complex with DNA shows that the flexible attachment of the DNA-binding domains to the remainder of the protein allows the three domains to interact simultaneously with the nucleic acid (Figure 2A), thereby enhancing the binding affinity. In the monomer that results from irradiation, this cooperativity is lost and thus the DNA binding affinity is considerably weakened.

AdoCbl is not directly involved in DNA binding. Rather, destabilization of the tetramer is caused by conformational changes to the protein, which are induced by photolytic Co–C bond cleavage and loss of the adenosyl group. In response, the four-helix bundle is reorganized, thereby destroying the binding interactions between the protomer interfaces. In the tetramer, the sidechain of Trp131 in the helix-bundle domain is in intimate contact with the ribose moiety of the adenosyl group of AdoCbl (Figure 2B), thereby furnishing the dark-state conformation of the protein. The loss of this tight interaction upon light-triggered release of the adenosyl group enables the conformational change, which is eventually "locked-in" by His132 through fixing of the remaining cobalamin moiety in a two-fold axial His coordination (Figure 2B). The Ado group of AdoCbl bound to one domain of CarH, and Trp131 and His132 at the other domain, thus constitute the crucial elements of a one-way light-sensing "mechanical" switch. This arrangement is somewhat reminiscent of a compressed spring, which relaxes upon cleavage of the Co–C bond, with the indole ring of Trp131 scooping the Ado group out of the way. Mutagenesis experiments indeed showed that Trp131 is crucial for the functioning of the photoswitch, whereas removal of His132 does not interfere with the photoregulation of CarH itself but increases the rate of B_{12} loss after illumination.[10]

Remarkably, *Myxococcus xanthus* has been shown to lack the capacity for biosynthesis of corrinoids,[9b] and it relies on external sources for Cbls. Indeed, AdoCbl and related Cbls are only biosynthesized by some prokaryotes.[13] The observed trapping of the cofactor in a photo-inactive, bis-His-ligated form of the photoreceptor[10] may thus help the organism to economize on the expensive AdoCbl, which is consistent with the need for recuperation and recycling of nature's most beautiful[14] and, probably, most complex cofactor.[15]

The crystal structure of the dark state of CarH reveals a solvent-exposed adenosyl group, oriented towards the molecular "east" of the bound B_{12} cofactor, which is quite different to what has been observed in the free coenzyme or in AdoCbl-dependent enzymes (Figure 2C). Light-induced (formal) heterolysis of the Co–C bond produced unreactive 4′,5′-anhydroadenosine[16] and an uncharacterized, unstable B_{12} derivative, likely to be the rather elusive "hydrido-cobalamin",[17] in an irreversible process without evidence for direct formation of a radical species.[18] In contrast, 4′,5′-anhydroadenosine is only a minor side product of the corresponding light-induced reaction of AdoCbl in solution, which primarily generates the Ado radicals.[19] Similarly, in AdoCbl-dependent radical enzymes, such as glutamate mutase, reversible thermal homolysis of the Co–C bond of AdoCbl generates the Ado radical (Figure 1).[20] This highly reactive primary radical is tightly fixed by a net of H-bonding interactions that precisely direct its activity towards H-atom abstraction from the substrate,[20,21] or alternatively towards recombination to AdoCbl, a form of "negative catalysis".[7a] By contrast, in the CarH structure, only one H-bond between the ribose and Glu141 was observed (Figure 2B). This H-bond and the intimate contact with the indole of Trp131 are elements that appear to be crucial for the novel Ado conformation observed in CarH (Figure 2C). It is also tempting to speculate that these two binding partners of the repurposed photoregulating AdoCbl play a still elusive, but decisive role in "reprogramming" the path of the light-triggered cleavage of the Co–C bond.

There is increasing evidence for the involvement of AdoCbl in bacterial gene-regulation. For example, AdoCbl is a ligand of the wide-spread B_{12} riboswitches,[22] which help regulate B_{12} biosynthesis and the expression of proteins for B_{12} uptake and transport.[22b,23] B_{12} riboswitches undergo precise restructuring upon binding of the intact B_{12} cofactor.[24]

By contrast, in the present case, photoregulation by AdoCbl and CarH is based on the light-induced, irreversible destruction of bound AdoCbl,[9b,10,16] and it concerns genes involved in the biosynthesis of non-corrinoid pigments. Indeed, a related situation has been reported for a Cbl-dependent photoregulator in the photosynthetic bacterium *Rhodobacter capsulatus*, which is, however, capable of biosynthesizing Cbls.[25] Here, the B_{12}-binding protein AerR binds CrtJ (a regulator of genes coding for tetrapyrrole biosynthesis) when carrying the aerobic photoproduct of AdoCbl, aquocobalamin. B_{12}, commonly known as a vitamin, may thus have the potential for more intriguing 'non-canonical" biological roles, such as the one now revealed with impressive structural detail in the AdoCbl-dependent photoregulator CarH.

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