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Cobalamin's (Vitamin B₁₂) Surprising Function as a Photoreceptor

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Summary

Cobalamin (Vitamin B₁₂) is an adenosyl- or methyl-donating cofactor for many enzymes, yet many proteins with unknown or non-enzymatic function also contain B₁₂ binding domains. Recent studies show that light excitation energy can promote covalent linkage of B₁₂ to transcription factors with this linkage affecting gene expression. Thus B₁₂ now has a newly described regulatory function. Here, our bioinformatics analysis reveals other transcription factors, photoreceptors, kinases and oxygen sensors that harbor a B₁₂-binding domain that could potentially also regulate activity in response to light absorption.

Keywords

Photobiology; light regulated gene regulation

The known roles of cobalamin in enzymology

Cobalamin (Vitamin B₁₂) is one of the most complex nonpolymeric biomolecules that archaeal and prokaryotic cells synthesize [1]. This tetrapyrrole contains a bound cobalt atom (Co) with two axial ligands (Fig. 1A). The lower ligand in unbound B₁₂ typically involves a linked 5, 6-dimethylbenzimidazole. This lower ligand is displaced in enzymes that bind B₁₂ with a His (called “base off/His on” binding). In regards to the upper Co axial ligand there are two biologically active forms of B₁₂ that are commonly used as enzyme cofactors; adenosylcobalamin (AdoB₁₂), which has 5'-deoxyadenosyl as an upper axial ligand, and methylcobalamin (MeB₁₂), where a methyl group functions as an upper axial ligand (Fig. 1A). The upper carbon-cobalt bond is relatively weak with a disassociating energy of ~30 kcal mol⁻¹, thereby generating a reactive leaving group that enables B₁₂ to function as a cofactor for methyltransferases, reductases and isomerases, the mechanisms of which are well studied [1]. All of these B₁₂-dependent enzymes contain a well-conserved B₁₂ binding domain that is characterized by 5 parallel β -sheets surrounded by 4–5 α helices that together

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Appendix A. Supplementary Materials

Supplementary data associated with this article can be found in the online version, at <http://>

form a Rossmann fold [2]. In some B₁₂ binding proteins there is an adjacent N terminus domain that is also involved in B₁₂ binding, which forms a 4-helical bundle cap. Also indicative of B₁₂ binding is a conserved B₁₂ binding sequence Asp/Glu-X-His-X-X-Gly-(41)-Ser/Thr-X-Leu-(26–28)-Gly-Gly, with the His forming a lower axial ligand to the corrin-bound cobalt [3]. The binding of this His also facilitates reactivity of the upper cobalt ligand with 5'-deoxyadenosyl or methyl groups [1].

B₁₂ has a newly defined role of controlling of gene expression in response to light absorption

CarH

The first evidence that B₁₂ can biologically function as a photosensor to control gene expression was a study by Ortiz-Guerrero, *et al.* [4] on light regulation of carotenoid biosynthesis in *Myxobacteria*. Their study demonstrated that the DNA binding activity of CarH, a light-responding repressor of carotenoid gene expression, was regulated by light excitation of a bound AdoB₁₂. CarH is a MerR-type DNA binding transcription factor that has an N-terminal DNA binding domain followed by a canonical B₁₂ binding domain. A recent structural study of a CarH-DNA co-complex shows that CarH binds AdoB₁₂ in the absence of light, which stimulates the formation of a stable CarH-AdenoB₁₂ tetramer. This dark state CarH tetramer stably binds to carotenoid promoters allowing it to inhibit transcription in the dark [4–5] (Fig. 2A). These structural studies reveal that AdoB₁₂ is bound to CarH in the dark state in a “base-off/His-on” form, with the 5, 6-dimethylbenzimidazole displaced by H177. The binding of AdoB₁₂ mediates CarH tetramer formation, positioning the DNA-binding domain on the surface to engage DNA. Surprisingly, the B₁₂ binding domain of CarH that binds AdoB₁₂ is more structurally homologous to the B₁₂-binding domain of methionine synthase (MetH), which uses MeB₁₂ as a cofactor [2, 5]. The only difference is a small shift of the four-helix bundle that occurs in the space occupied by the Co upper face in CarH to accommodate binding of the much bulkier AdoB₁₂. When the corrin ring of the bound AdoB₁₂ undergoes exposure to light there is photolysis of the bulky upper 5'-deoxyadenosyl Co ligand to generate a bis-His ligated cobalamin and a nonreactive 4', 5'-anhydroadenosine photolysis product [5, 6]. This leaves a large cavity on the Co upper face, promoting re-orientation of a helix bundle allowing H132 to move into this cavity to form a covalent linkage with the Co [5]. This reorientation of the 4-helix bundle relative to the Rossmann-fold B₁₂ binding domain leads to tetramer disassembly, dissociation of CarH from DNA and subsequent transcription activation of carotenoid gene expression.

AerR

The involvement of B₁₂ in the control of gene expression may not be that surprising given that CarH has a B₁₂ domain linked with a DNA binding output domain. However, there is also a recent example of a stand-alone B₁₂ binding domain, without any obvious output domain, that controls gene expression in response to light absorption. In this case, Cheng *et al.* [7] showed that *Rhodobacter capsulatus* regulates synthesis of their photosystem in a light dependent manner *via* AerR, which is a small stand-alone B₁₂-binding domain protein.

Mass spectrophotometric analysis shows that light excitation of AdoB₁₂ also leads to photohydrolysis of the upper 5'-deoxyadenosyl Co ligand to generate OHB₁₂, which subsequently forms a strong covalent linkage between His10 and Co, not unlike that observed with CarH [7] (Fig. 1B). Thus, photohydrolysis of the upper 5'-deoxyadenosyl Co ligand, followed by formation of a stable His adduct, may be a common mechanism of this class of B₁₂-dependent photoreceptors. In the case of AerR, subsequent regulation of gene expression occurs through the formation of a stable interaction between B₁₂-bound AerR and a well-characterized repressor of photosystem gene expression termed CrtJ [7, 8]. This interaction affects the oligomerization state of CrtJ to alter its DNA binding affinity (Fig. 2B) [5]. In addition, AerR homologs have now been isolated from six species and have been shown to tightly bind OHB₁₂, the photohydrolysis product of light excitation of AdoB₁₂ and MeB₁₂ [9]. These AerR homologs are also tightly chromosomally linked with CrtJ homologs indicating that the use of B₁₂ as a light sensor to control photosynthesis gene expression may be a common mechanism among purple bacteria species [7, 9].

There are many proteins of uncharacterized function that exhibit a B₁₂ binding domain

Analysis of proteins that have well defined B₁₂ binding domains indicates that there are currently 51,093 protein sequences present in 11,568 different bacterial, archaeal and eukaryotic species. These B₁₂ binding proteins can be categorized into 179 different groups based on different output domain architectures [10]. The three most common groupings are enzymes that use B₁₂ as a cofactor for methylation reactions, such as methionine synthase (9,123 sequences); methylmalonyl-CoA mutases (1,697 sequences); and methyltransferases (189 sequences). However, analysis of potential regulatory proteins indicates that there are 6,749 examples of stand-alone B₁₂ binding domains, similar to that of AerR (Supplemental Table S1), and 1,123 cases containing tandem B₁₂ binding domains without output domains, which have not been biochemically characterized (Fig. 2C; Supplemental Table S2). In regards to transcription factors, there are 93 examples of amino terminal helix-turn-helix (HTH) domains followed by a B₁₂ binding domain, similar to CarH (Supplemental Table S3), 1,199 examples of proteins with a HTH domain followed by tandem B₁₂ binding domains (Supplemental Table S4) and 10 examples of a B₁₂ binding domain followed by a HTH domain (Supplemental Table S5). With the exception of CarH, none of these putative transcription factors have been characterized although it seems likely that they may also function as B₁₂-dependent light sensors in a broad spectrum of species. Beyond transcription factors, there are 23 examples of hybrid proteins that contain combinations of heme/oxygen sensing globin domains linked to B₁₂ domains, none of which have been characterized (Supplemental Tables S6–S9). Intriguingly, there are also 17 examples of photoreceptors containing well-characterized flavin BLUF domains or biliverdin-binding GAF domains linked to B₁₂ domains (Supplemental Tables S10–S12). These photoreceptors could be utilizing the B₁₂ domain to sense wavelengths of light that differ from those absorbed by flavin or biliverdin. Finally, there are 7 examples of histidine kinases and serine/threonine kinases that also contain well defined B₁₂ domains suggesting that their kinase activity may be regulated in response to light absorption by B₁₂ (Fig. 2C; Supplemental Tables S13–S14).

Concluding remarks

It has long been known that B₁₂ functions as a cofactor for many enzymes that catalyze a range of important biochemical reactions. However, two recent studies provide evidence that B₁₂ can also function as a chromophore for light absorption to facilitate an output response that affects gene expression. Thus, B₁₂ can be added to a list of light absorbing chromophores, such as flavin mononucleotide, flavin adenine dinucleotide, retinal, p-coumaric acid, and linear tetrapyrroles (bilins and biliverdins), which are used by cells to regulate protein activity [11]. What is generally not appreciated is that there are many examples of proteins of unknown or nonenzymatic functions harbored by species in the Bacteria, Archaea and Eukarya kingdoms that contain well-defined B₁₂ binding domains. This suggests that the use of B₁₂ as a light sensor may be a widespread feature with a deep evolutionary history.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Structural features of cobalamin (B₁₂) and its newly defined photoreceptor role
 (A) Structure of B₁₂ with the upper Co linkage as either 5'-deoxyadenosyl (AdoB₁₂) or a methyl group (MeB₁₂). (B) Upper structure represents free unbound B₁₂, the middle structure represents the "base off/His on" dark bound form and the lower structure represents the light excited version of B₁₂ that is tightly bound to CarH and AerR *via* upper and lower His axial ligands.

Fig. 2. Models for the light-responsive B₁₂-based regulatory switches in *M. xanthus* and *R. capsulatus*

(A) Light mediated photolysis of the AdoB₁₂::CarH tetramer to the OHB₁₂::CarH monomer allows *M. xanthus* to synthesize carotenoids in the light. (B) Light converts AdoB₁₂ to OHB₁₂, which enables OHB₁₂ binding to AerR and thus binding to CrtJ, derepressing photosynthesis gene expression in *R. capsulatus*. (C) Representative B₁₂ binding protein architectures that have potential light driven regulatory functions. The number of proteins found in each category is indicated. Details of these derivatives and the search method used for their identification can be found in Supplementary Materials.