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Research Letter

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Cobamide remodeling in the freshwater microalga *Chlamydomonas reinhardtii*

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One sentence summary: The ability of *Chlamydomonas reinhardtii* to remodel exogenous B_{12}-vitamers was investigated with bacterial norcobamides and the results pointed towards a novel mode of B_{12}-deconstruction that acts in the microalga.

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ABSTRACT

Microalgae are not able to produce cobamides (Cbas, B_{12} vitamers) *de novo*. Hence, the production of catalytically active Cba-containing methionine synthase (MetH), which is present in selected representatives, is dependent on the availability of exogenous B_{12} vitamers. Preferences in the utilization of exogenous Cbas equipped with either adenine or 5,6-dimethylbenzimidazole as lower base have been reported for some microalgae. Here, we investigated the utilization of norcobamides (NorCbas) for growth by the Cba-dependent *Chlamydomonas reinhardtii* mutant strain (Δ*metE*). The growth yields in the presence of NorCbas were lower in comparison to those achieved with Cbas. NorCbas lack a methyl group in the linker moiety of the nucleotide loop. *C. reinhardtii* was also tested for the remodeling of NorCbas (e.g. adeninyl-norcobamide) in the presence of different benzimidazoles. Extraction of the NorCbas from *C. reinhardtii*, their purification, and identification confirmed the exchange of the lower base of the vitamers. However, the linker moiety of the NorCbas nucleotide loop was not exchanged. This observation strongly indicates the presence of an alternative mode of Cba deconstruction in *C. reinhardtii* that differs from the amidohydrolase (CbiZ)-dependent pathway described in Cba-remodeling bacteria and archaea.

Keywords: microalgae; *Chlamydomonas*; methionine synthase; vitamin B_{12}; norcobamides; cobamide remodeling

INTRODUCTION

Either biosynthesis of cobamides (Cba) or salvaging of incomplete precursors thereof meets the requirement of Cba cofactors (B_{12} vitamers) for the function of Cba-dependent enzymes in microbes. Almost 90% of the known bacterial genomes contain a gene encoding a Cba-dependent enzyme, but the number of bacterial species equipped with such genes was found to be double the number of potential producers of the cobalt-containing macrocycles (Shelton *et al.* 2019). Hence, Cba cross

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feeding is essential for covering the demand of Cba in microbial communities (Seth and Taga 2014). Higher plants are free of Cba-dependent metabolic pathways, but freshwater and marine microalgae harbor the genetic information for Cba-dependent enzymes such as the methionine synthase (MetH; Croft *et al.* 2005; Helliwell *et al.* 2011). However, complete Cba biosynthetic pathways have not been reported for these organisms, which makes them strictly dependent on the exogenous supply of Cbas.

Cba cofactors differ in the structure of the nucleotide loop, which is bound to the corrin-ring, a contracted tetrapyrrole carrying a cobalt as central atom (Hodgkin *et al.* 1956; Weissbach *et al.* 1959). The incorporation of either purines, benzimidazoles (Bzas), azabenzimidazoles, imidazoles, or phenols as terminal component of the nucleotide loop generates a pool of structurally different biogenic Cbas that are selectively bound by Cba-dependent enzymes (Keller *et al.* 2018; Schubert *et al.* 2019; Sokolovskaya *et al.* 2019). The discovery of norcobamides (NorCbas; Kräutler *et al.* 2003) further extended the group of biologically produced B_{12} vitamers. In contrast to Cbas, which harbor an aminopropanol phosphate moiety as linker between the corrin ring and the nucleotide, NorCbas possess an ethanolamine phosphate moiety at this position (Fig. 1). The precursor of the linker moiety is produced by a recently identified serine phosphate decarboxylase (Keller *et al.* 2016, 2019).

Structural changes in the nucleotide of Cbas or NorCbas can interfere with the function of Cba-containing enzymes (Keller *et al.* 2018, Sokolovskaya *et al.* 2019). The hydrophobicity of the lower base is mainly defined by the substituents of the benzimidazole or purine moiety and influences the interaction of the Cba cofactor and the apoprotein. The presence of nitrogen atoms in the aromatic ring structure of the lower base also determines the efficiency of Cba binding to methylmalonyl-CoA mutase (Sokolovskaya *et al.* 2019). Selected bacteria and archaea possess the ability to exchange the nucleotide loop substructure (Cba remodeling; Woodson and Escalante-Semerena 2004; Gray and Escalante-Semerena 2009; Yi *et al.* 2012). In such organisms the amidohydrolase CbiZ disconnects the nucleotide loop from the corrin ring (Woodson and Escalante-Semerena 2004; Gray and Escalante-Semerena 2009) and via the action of the *cobD*, *cbiB*, *cobU*, *cobT*, *cobS* and *cobC* gene products the nucleotide loop is reconstructed with an alternative lower base (Escalante-Semerena 2007; Yi *et al.* 2012). The Cba remodeling in selected Cba auxotrophs might allow for the adaptation of the structure of the complex metal cofactor to specific structural prerequisites of the Cba-dependent enzyme(s) in the organism. In this study, we monitored the capacity of the model freshwater microalga *Chlamydomonas reinhardtii* to remodel B_{12} vitamers and produce Cbas that optimally function as cofactors in the organism's Cba-dependent methionine biosynthesis.

MATERIALS AND METHODS

Sources of (nor-)cobamides and benzimidazoles

Cyanocobalamin (vitamin B_{12}, DMB-Cba) and dicyanocobinamide (cobinamide, Cbi) were purchased from Merck (Darmstadt, Germany). The cobamides (Cbas) and norcobamide (NorCbas) used in this study were purified from bacterial sources according to a protocol described earlier (Keller *et al.* 2014). Adeninyl-Cba (Ade-Cba) was purified from *Propionibacterium acidipropionici* (Hoffmann *et al.* 2000). The 5,6-dimethylbenzimidazolyl-Cba (DMB-Cba), the 5-methylbenzimidazolyl-Cba (5-MeBza-Cba), the 5-methoxybenzimidazolyl-Cba (5-OMeBza-Cba) and the mixture of 5-hydroxybenzimidazolyl-Cba and 6-hydroxybenzimidazolyl-Cba (5/6-OHBza-Cba; ratio 1.7:1) was obtained by purification from *Desulfitobacterium hafniense* strain DCB-2 (Schubert *et al.* 2019). All the norcobamides (NorCbas) used in this study were extracted from *Sulfurospirillum multivorans* (Keller *et al.* 2018).

Cultivation of *C. reinhardtii* (Δ*metE*)

The cultivation of *C. reinhardtii* (Δ*metE*) was carried out in six-well plates for 5 days at 18°C, shaking at 100 rpm and under continuous blue light (18–23 μmol photon/m^2/s). A single well contained 5 mL Tris-Acetate-Phosphate medium (TAP; Sueoka 1960, Gorman and Levine 1965) including 10 pM of a B_{12}-vitamer. The blue light was generated through a filter (Lee Filter Roll 183 Moonlight Blue; Thomann, Burgebrach, Germany) placed in front of PowerStar HQI-T lamps (Osram, Munich, Germany). Prior to inoculation, cells from 10 mL culture were sedimented by centrifugation (4000 × *g*, 10 min, 10°C; Biofuge 15R, Heraeus, Hanau, Germany) and washed three times with 10 mL TAP medium. Cultures were inoculated with an initial OD_{730nm} of 0.005. For growth yield determination the OD_{730nm} of the cultures was measured after 5 days of incubation. Cultures for cobamide extraction were cultivated in 1 L TAP medium containing 10 nM Cba or NorCba. Benzimidazoles were added from aqueous stock solutions (4 mM). The large cultures were continuously stirred during incubation.

Cobamide purification

The cells of a 1 L-culture were harvested by centrifugation (12 000 × *g*, 10 min, 10°C; Avanti JXN-26, Rotor JLA-10.500, Beckman Coulter, Krefeld, Germany). Wet cells (5 g) were resuspended in 15 mL 50 mM Tris/HCl (pH 7.5) and mixed with 5 g glass beads (0.25–0.5 mm, Roth, Karlsruhe, Germany) in a 15 mL conical tube. To disrupt the cells the tubes were clamped into a glass bead mill (Mixer Mill MM400, Retsch, Haan, Germany) and shaken for 4–6 h at 30 Hz. Afterwards the suspensions were transferred into 50 mL Schott bottles and the cobamide extraction and purification was performed according to the protocol described by Stupperich, Steiner and Rühlemann (1986). The pH of the crude extract was adjusted to ≤ 5 with acetic acid. Subsequently, 0.1 M KCN was added. The sample was boiled for 15 min in a water bath. After centrifugation of the sample (17 420 × *g*, 10 min, 10°C; Avanti JXN-26, Rotor JA-20, Beckman Coulter, Krefeld, Germany) the supernatant was collected and the pellet was resuspended in 10 mL ultrapure water (UPW). The extraction procedure was repeated twice. The combined supernatants from all three extractions were mixed with Amberlite XAD4 (Merck, Darmstadt Germany; 0.25 g XAD4 per mL extract) and incubated on a shaker overnight. The next day the supernatant was discarded and the XAD4 material was washed 10 times with an equal volume of UPW. Subsequently, the XAD4 material was incubated for 1 h with 15 mL 100% methanol. The supernatant was collected and the elution was repeated twice. The combined cobamide-containing eluates were dried completely using a rotation evaporator (Rotavaporator R-114, Büchi, Essen, Germany) and a vacuum concentrator (Savant SpeedVac SVC100). The dried sample was resuspended in 2 mL UPW and transferred to a column containing 3 g aluminum oxide (Merck, Darmstadt, Germany). The cobamides were recovered from the column by washing with ≥ 40 mL UPW. The eluate was dried as described above up to a volume of approximately 8 mL. Finally, the eluate was transferred on a CHROMABOND HR-X column (200 mg, 3 mL; Macherey-Nagel, Düren, Germany). After washing with 10 mL UPW, the cobamides were eluted with 10 mL 50% methanol. The eluate was dried, resuspended in 85 μL UPW and

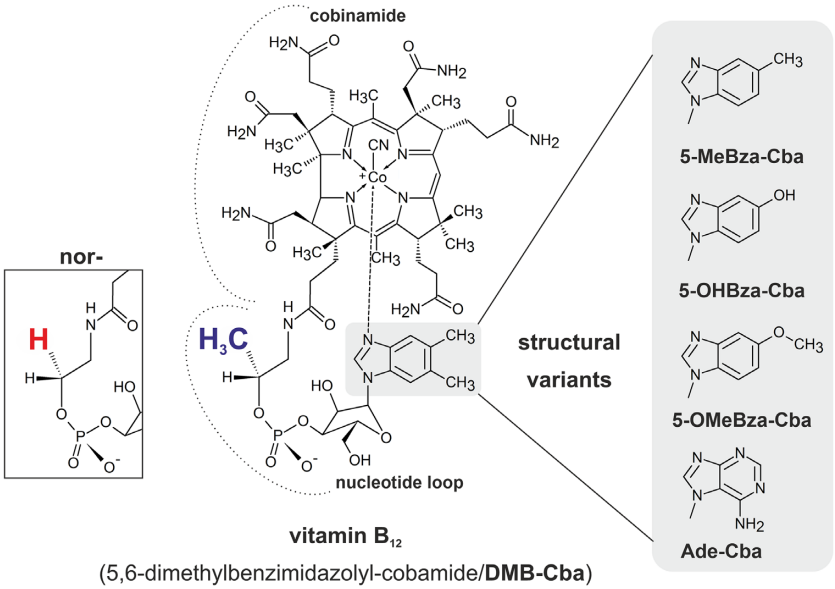

Figure 1. Structure of vitamin B_{12} (5,6-dimethylbenzimidazolyl cobamide, DMB-Cba). Structural variants used in this study harbor different lower bases such as methylbenzimidazole (MeBza), hydroxybenzimidazole (OHBza), methoxybenzimidazole (OMeBza), or adenine (Ade). Furthermore, norcobamides (NorCbas) have been used that lack the methyl group in the linker moiety (inset on the left). The cyano group (-CN) that serves as artificial upper ligand to the central cobalt ion is a result of the (nor-)cobamide purification in the presence of KCN.

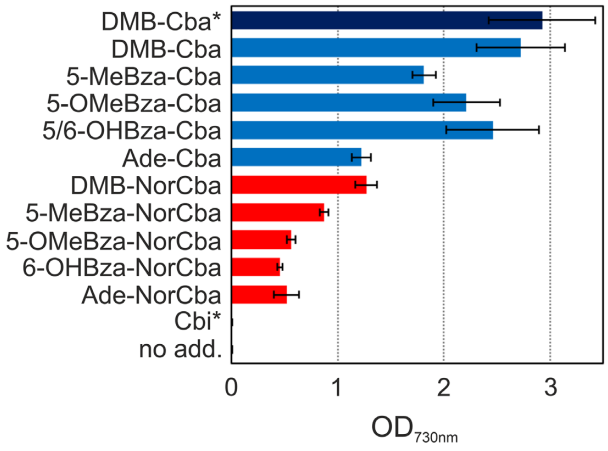

Figure 2. Growth yield of *C. reinhardtii* (Δ*metE*) cultures amended with various B_{12} vitamers (10 pM, all in the cyano-form) isolated from bacterial sources. Cobamides (Cbas) = blue, norcobamides (NorCbas) = red, no add. = no addition, ∗commercially available cyanocobalamin (DMB-Cba) and dicyanocobinamide (Cbi). The results were obtained from at least four biological replicates with at least two technical replicates. The mean values and standard deviations are depicted.

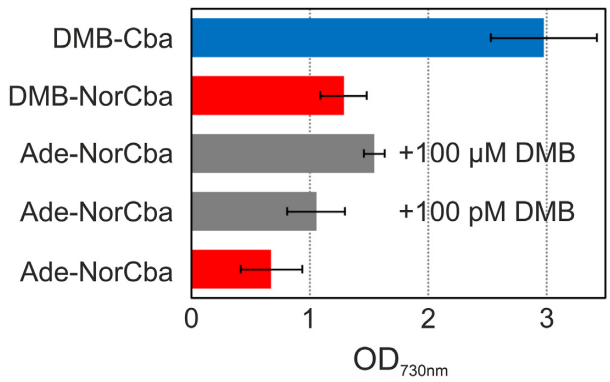

Figure 3. Growth yield of *C. reinhardtii* (Δ*metE*) cultures amended with Ade-NorCba (10 pM) and various concentrations of DMB. The results were obtained from at least four biological replicates with at least two technical replicates. The mean values and standard deviations are depicted.

stored at −20°C. The concentration of the purified cobamides or norcobamides (all in the cyano-form) was calculated from the absorbance maximum of the Co^{3+}-oxidation state at 360 nm. For the calculation the extinction coefficient of DMB-Cba was used (28.06 $mM^{-1}cm^{-1}$; Friedrich 1975). The absorbance spectra were recorded with a Cary 100 UV–visible spectrophotometer (Agilent Technologies, Waldbronn, Germany).

Cobamide analysis

Cbas and NorCbas were initially characterized via high-performance liquid chromatography (HPLC) as reported earlier (Schubert *et al.* 2019).

The separation was performed isocratically on a Kinetex 5 µm EVO C18 100 Å LC Column 250 × 4.6 mm (Phenomenex, Aschaffenburg, Germany) connected to a Smartline HPLC System (Knauer, Berlin, Germany) with 14% methanol/0.2% acetic acid at 30°C and with a flow rate of 0.5 mL/min. The (nor-)cobamides were tentatively identified based on their characteristic absorbance spectrum monitored with a UV–Vis Smartline PDA Detector 2850 (Knauer, Berlin, Germany). For mass determination the different (nor-)cobamide containing fractions were collected using a Smartline Fraction Collector 3050 (Knauer, Berlin, Germany). The samples were dried and resuspended in 10 µL UPW.

For mass determination, UHPLC was coupled with an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). UHPLC was performed on an Ultimate 3000 system (RSLC, Dionex, Sunnyvale, CA) with an Acclaim 120 C18 column having a length of 150 mm, a diameter of 2.1 mm, a particle size of 2.2 μm and a pore size of 120 Å (Dionex, Sunnyvale,

Table 1. Mass determination of the Cbas and NorCbas purified from *C. reinhardtii* (Δ*metE*).

Amendment	Retention time (min)	Charge	Accurate mass (M/z)	Calculated mass (M/z)	(ppm)	Elemental composition	Identified compound
DMB-Cba	112	$[M + H]^+$	1355.57771	1355.57531	2.234	$C_{63}H_{88}O_{14}N_{14}CoP$	DMB-Cba **(1)**
DMB-NorCba	75.5	$[M + H]^+$	1341.56342	1341.55966	3.271	$C_{62}H_{86}O_{14}N_{14}CoP$	DMB-NorCba **(2)**
		$[M + 2H]^{2+}$	671.28547	671.27983	3.45		
Ade-NorCba	55.3	$[M + H]^+$	1330.53132	1330.52975	1.647	$C_{58}H_{81}O_{14}N_{17}CoP$	Ade-NorCba **(3)**
Ade-NorCba + DMB	57.1	$[M + H]^+$	-	-	-	$C_{58}H_{81}O_{14}N_{17}CoP$	Ade-NorCba **(3)**
		$[M + 2H]^{2+}$	666.77036	665.76487	3.241		
	75.5	$[M + H]^+$	1341.56414	1341.55966	3.808	$C_{62}H_{86}O_{14}N_{14}CoP$	DMB-NorCba **(2)**
		$[M + 2H]^{2+}$	671.28567	671.27983	3.748		
Ade-NorCba + OHBza	47.9	$[M + H]^+$	1329.52567	1329.52328	2.275	$C_{60}H_{82}O_{15}N_{14}CoP$	OHBza-NorCba **(4)**
	55.3	$[M + H]^+$	1330.53215	1330.52975	2.271	$C_{58}H_{81}O_{14}N_{17}CoP$	Ade-NorCba **(3)**
Ade-NorCba + OMeBza	57.4	$[M + H]^+$	1330.53481	1330.52975	4.27	$C_{60}H_{82}O_{15}N_{14}CoP$	Ade-NorCba **(3)**
		$[M + 2H]^{2+}$	665.77056	665.76487	3.541		
	58.9	$[M + H]^+$	1343.54288	1343.53893	3.412	$C_{61}H_{84}O_{15}N_{14}CoP$	OMeBza-NorCba **(5)**
		$[M + 2H]^{2+}$	672.27513	672.27280	3.486		
DMB-NorCba + OHBza	48.4	$[M + H]^+$	1329.52717	1329.52328	3.403	$C_{60}H_{82}O_{15}N_{14}CoP$	OHBza-NorCba **(4)**
		$[M + 2H]^{2+}$	665.26699	665.26164	3.05		
	74.8	$[M + H]^+$	1341.56291	1341.55966	2.891	$C_{62}H_{86}O_{14}N_{14}CoP$	DMB-NorCba **(2)**
		$[M + 2H]^{2+}$	671.28514	671.27983	2.959		
DMB-NorCba + OMeBza	56.8	$[M + H]^+$	1343.54276	1343.53893	3.323	$C_{61}H_{84}O_{15}N_{14}CoP$	OMeBza-NorCba **(5)**
		$[M + 2H]^{2+}$	672.27491	672.27280	3.159		
	73.4	$[M + H]^+$	1341.56336	1341.55966	3.227	$C_{62}H_{86}O_{14}N_{14}CoP$	DMB-NorCba **(2)**
		$[M + 2H]^{2+}$	671.28521	671.2798	3.063		

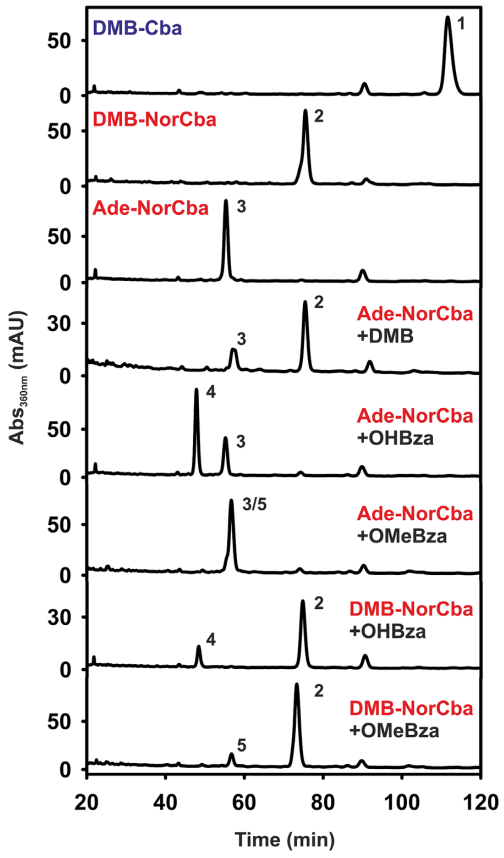

Figure 4. HPLC-elution profile of the Cbas or NorCbas purified from *C. reinhardtii* (Δ*metE*) cultures amended with different (nor-)cobamides and benzimidazoles. The numbers assigned to the signals represent the compounds identified by mass determination (Table 1, column 'Identified Compound')

CA). The (nor-)cobamides were eluted by linear gradient at a flow rate of 300 μL/min. The solvents used were water (solution A) and acetonitrile (solution B; suitable for LC-MS; Merck, Darmstadt, Germany). Both solutions contained 0.1% (v/v) of formic acid (suitable for LC-MS, Sigma–Aldrich, Munich, Germany). For the elution, the volume fraction of solution B was increased linearly from 0 to 100% over 15 min and kept constant at 100% for 5 min. The equilibration of the column was carried out for 5 min with 0% solution B. The ionization of the sample was performed by electrospray ionization (ESI) with a spraying voltage of 4 kV, a capillary voltage of 35 V and a temperature of 275°C. The samples were measured by the Orbitrap mass analyzer in the positive ion mode in a range of 100–2000 m/z with a resolution of 30 000 m/Δm. The data were evaluated via Xcalibur 3.0.63 (Thermo Fisher Scientific, Waltham, MA).

RESULTS AND DISCUSSION

Growth of *C. reinhardtii* (Δ*metE*) in the presence of norcobamides

The biosynthesis of norcobamides (NorCbas) has been reported exclusively for two organohalide-respiring representatives of the bacterial genus *Sulfurospirillum* (Kräutler *et al.* 2003; Goris *et al.* 2017; Keller *et al.* 2018). To our knowledge, NorCbas have not been identified in natural samples yet, which impedes the evaluation of their role in nature. However, the structural divergence of NorCbas in comparison to Cbas make NorCbas a valuable object of study for the investigation of cobamide cofactor function, selectivity and remodeling. For instance, NorCbas display a higher tendency to adopt the base-off conformation in aqueous solution, i.e. the lower ligand base does not coordinate the central cobalt ion (Butler *et al.* 2006; Sonnay and Zelder 2018). The latter might influence the affinity of the macrocycle to transporters involved in Cba uptake or Cba-dependent enzymes.

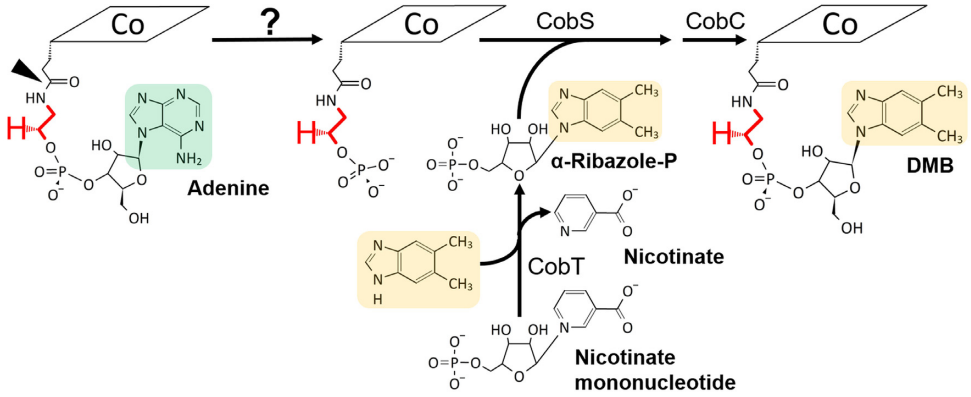

Figure 5. Tentative scheme of NorCba remodeling in *C. reinhardtii*. The cleavage site of the bacterial amidohydrolase CbiZ is indicated by a black arrowhead.

The utilization of NorCbas by *C. reinhardtii* (Δ*metE*) was analyzed by growth yield determination. Besides the NorCbas, several Cbas and cobinamide (an incomplete Cba lacking the ribosylated lower base) have been included in the analysis (Fig. 1). All NorCbas and Cbas, which have been purified beforehand from bacterial sources (see Materials and Methods), were applied in the cyano-form with a CN-group as upper ligand to the cobalt. As depicted in Fig. 2, *C. reinhardtii* (Δ*metE*) grew in the presence of Cbas as well as NorCbas. However, in comparison to the Cbas, the yield monitored for the cultures amended with the respective NorCbas was substantially reduced (≤50%). From these results it was concluded, that besides the identity of the lower base, structural changes in the linker moiety of the nucleotide loop can also interfere with Cba utilization. The linker moiety might be involved in the recognition and binding of the cofactors by the Cba uptake system of *C. reinhardtii*. A Cba transport system in *C. reinhardtii* still awaits identification. No growth was observed with Cbi, which underpins the role of an intact nucleotide loop for Cba utilization. A general preference for benzimidazolyl-Cbas over adeninyl-Cba (Ade-Cba) was detected. This observation was in good agreement with previous findings by Helliwell *et al.* (2016), who reported the preferential use of 5,6-dimethylbenzimidazolyl-Cba (DMB-Cba) over Ade-Cba by the organism. Although, the presence of 5,6-dimethylbenzimidazolyl-norcobamide (DMB-NorCba) resulted in a higher growth yield compared to adeninyl-NorCba (Ade-NorCba), the presence of other benzimidazolyl-NorCbas such as 5-methoxybenzimidazolyl-NorCba (5-OMeBza-NorCba) or 6-hydroxybenzimidazolyl-NorCba (6-OHBza-NorCba) did not (Fig. 2). Differences in hydrophobicity of the various Bzas tested were due to the different substituents and appeared to have no influence on the utilization of the various cofactors. The Cba-dependent methionine synthase (MetH) of *C. reinhardtii* might not strictly select among the Cba or NorCba cofactors with different lower ligand bases. Earlier studies showed that the type of substituent at the Bza moiety interferes with Cba utilization by Cba-dependent enzymes such as the reductive dehalogenase or the methylmalonyl-CoA mutase (Keller *et al.* 2018; Sokolovskaya *et al.* 2019). All three Cba-dependent enzymes bind the cofactor in the base-off conformation with the lower base displaced from the central cobalt ion and involved in anchoring the cofactor in the proteins structure.

Norcobamide remodeling

From the complementation of the growth limitations observed in the presence of Ade-Cba by the addition of exogenous DMB, Helliwell and coworkers (2016) proposed that *C. reinhardtii* is capable of Cba remodeling, i.e. the enzymatic exchange of the lower base. However, the final product of the remodeling process was not identified. In order to test whether this positive effect of DMB holds also true for NorCbas, *C. reinhardtii* (Δ*metE*) was cultivated in the presence of Ade-NorCba and DMB (Fig. 3). Indeed, the growth yield measured in the presence of Ade-NorCba was increased when DMB was added, which strongly indicated NorCba remodeling. However, the yield measured in the presence of DMB-Cba was not reached even in the presence of a large excess of DMB in the medium. A similar cell yield would have been expected if the mechanism of Cba remodeling in *C. reinhardtii* was similar to the mode of enzymatic deconstruction and reconstruction of the Cba's nucleotide loop in bacteria and archaea. In the latter, the complete nucleotide loop substructure including the linker moiety is removed and rebuilt (Woodson and Escalante-Semerena 2004; Gray and Escalante-Semerena 2009). The Cba-remodeling bacteria and archaea analyzed so far produce Cbas rather than NorCbas by incorporating a linker moiety derived from aminopropanol phosphate rather than from ethanolamine phosphate (Woodson and Escalante-Semerena 2004; Gray and Escalante-Semerena 2009; Yi *et al.* 2012; Ma, Tyrell and Beld 2020).

In order to analyze the capability of *C. reinhardtii* (Δ*metE*) to remodel NorCbas, cobamide extractions were performed with cells cultivated with either DMB-Cba, DMB-NorCba, or Ade-NorCba in the absence or presence of different Bzas. To our knowledge, Cba purification from *C. reinhardtii* has not been reported before, hence, we adapted an extraction protocol previously used for bacteria (Keller *et al.* 2014). When analyzed via HPLC, the extracts displayed clear signals representing (nor-)cobamides that were identified at first glance by observing the distinct Cba absorbance spectrum (Keller *et al.* 2018). Supplementation of lower base precursors such as DMB, OHBza, or OMeBza to culture media containing Ade-NorCba resulted in the production of alternative NorCbas such as DMB-NorCba, OHBza-NorCba, or OMeBza-NorCba (Fig. 4). These findings were verified by LC-MS (Table 1). The production of Cbas has never been observed under these conditions. At the end of the cultivation period, more than half of the NorCbas in the cells contained the alternative lower base. The same result was not obtained when DMB-NorCba was provided in lieu of Ade-NorCba. Addition of OHBza or OMeBza to cultures growing in the presence of DMB-NorCba correlated with an exchange of the lower base, but this remodeling was less efficient than the conversion of Ade-NorCba to DMB-NorCba. The latter observation suggested a specificity of the remodeling machinery in *C. reinhardtii* that

probably used Ade-NorCba as a substrate more efficiently than DMB-NorCba. The reduced efficiency in the deconstruction of DMB-NorCba agreed with its preferential use by the organism (Fig. 2). No Ade-NCba deconstruction or remodeling was observed in cell free extract of *C. reinhardtii* (Δ*metE*), which indicated the necessity of intact cells for the conversion.

Chlamydomonas reinhardtii possesses the gene products that are required for the activation and incorporation of lower base precursors such as benzimidazoles (Fig. 5; Helliwell *et al.* 2016). However, a gene encoding a homologue of the aminohydrolase (CbiZ) that is responsible for Cba deconstruction in bacteria and archaea is not encoded in the *C. reinhardtii* genome. Thus, we surmise that *C. reinhardtii* uses an alternative enzyme to remove the ribosylated base. From the data obtained in this study, it is highly feasible that this alternative enzyme act on another part of the nucleotide loop and does not remove the linker moiety. Therefore, we hypothesize that an alternative mechanism of Cba deconstruction is used by *C. reinhardtii* and most probably also in other B_{12}-auxotrophic microalgae.

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Conflicts of interest. None declared.

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