Acetyl Coenzyme A Synthesis from Unnatural Methylated Corrinoids: Requirement for "Base-Off" Coordination at Cobalt

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This communication reports that methylcobinamide[1] (MeCbi), which lacks a lower axial nitrogen donor ligand to cobalt, is a substrate for acetyl-CoA synthesis by the nickel iron−sulfur enzyme, acetyl-CoA synthase (ACS). In contrast, methylcobalamin (MeCbl), which contains a dimethylbenzimidazole ligand, is 2000-fold less reactive than MeCbi or a MeCbl analogue in which the coordinating nitrogen is methylated and cannot bind cobalt. Furthermore, CO dehydrogenase (CODH) catalyzes the CO-dependent reduction of Cbi at a rate approximately 10 000-fold faster than that of Cbl. These results support the hypothesis that lack of a lower axial nitrogen donor ligand to cobalt in the native corrinoid iron−sulfur protein (CFeSP) from methanogenic and acetogenic microbes enhances its propensity for reductive activation and demethylation reactions.

The bifunctional enzyme, CODH/ACS, is central to the Wood−Ljungdahl pathway of autotrophic CO_2 fixation.[2,3] CODH catalyzes the two-electron reduction of CO_2 to CO (eq 1), and ACS catalyzes acetyl-CoA synthesis from CO, the methyl-donor, and CoA (eq 2). An intermediate step in eq 2 is transfer of the cobalt-bound methyl group from the methylated CFeSP (Me-CFeSP) to ACS (eq 3). This appears to be an S_N2 attack of a nucleophilic center of ACS, presumably Ni,[4] on the methyl-Co(III) state of the CFeSP, generating Co(I)[5,6] and methylating ACS. The physiological methyl donor is the methylated CFeSP.[7,8]

$$CO_2 + 2 \text{ electrons} + 2\ H^+ \rightarrow CO + H_2O \quad (1)$$

$$CO + \text{methyl-X} + HSCoA \rightarrow CH_3\text{-}CO\text{-}SCoA + HX \quad (2)$$

$$CH_3\text{-Co(III)}\text{-CFeSP} + ACS \rightarrow CH_3\text{-ACS} + \text{Co(I)}\text{-CFeSP} \quad (3)$$

The CFeSP is an 88 kDa heterodimeric protein[8] that contains a corrinoid, 5-methoxybenzimidazolylcobamide, in its 33 kDa subunit and a $[Fe_4S_4]^{2+/1+}$ cluster in its 55 kDa subunit.[7,9] The cluster is involved in reductive activation of the CFeSP; it directs electrons from CODH/ACS to the cobalt site and does not directly participate in the transmethylation reaction.[5,6] Cobalt is the active site for the methyl-transfer reaction. Co(I) undergoes methylation, and methyl-Co(III) suffers demethylation at each catalytic cycle.

Table 1. Rates of Acetyl-CoA Synthesis and Co(II) Reduction[a]

substrates	products	rate constants, $M^{-1}\ s^{-1}$	ratio[b]
Me-CFeSP + CO + CoA	AcCoA	20000 ± 1000	100
Me-Cbi + CO + CoA	AcCoA	200 ± 10	1.0
Me-(Me_3Bmz)Cbl + CO + CoA	AcCoA	190 ± 10	1.0
Me-Cbl + CO + CoA	AcCoA	<0.12	<0.0006
Co(II)−C/FeSP	Co(I)−CFeSP	55000 ± 4000	0.433
Co(II)−Cbi(OH)	Co(I)−Cbi	127000 ± 11000	1.0
Co(III)−$Cbi(CN)_2$	Co(I)−Cbi	11800 ± 100	0.09
Co(II)−Cbl(OH)	Co(I)−Cbl	11 ± 1	0.00009

[a] Conditions: The methyl-transfer reactions were performed at 55 °C in a solution containing 1 mM CoASH, 1 atm CO, 0.1 M Tris, pH 7.6, 500 μM 3,4-dyhydroxybenzoic acid, and protocatechuate dioxygenase. The conditions were the same for the Co(II) reduction experiments, except the buffer was 0.05 M Tris, pH 7.6, 2 mM DTT. Demethylation was followed at 450 and 390 nm for the CFeSP, 464 and 388 nm for Me-Cbi, and 460 and 388 nm for Me-(Me_3-benzimidazolyl)cobamide. [b] The ratio of the rate constant obtained with the particular corrinoid divided by the rate constant using Cbi.

In many methyltransferases, methylcobalamin can replace the physiological methyl donor, which is a methylated corrinoid protein.[10−16] When ^{14}C-labeled MeCbl is reacted with CoA and CO in the presence of CODH/ACS,[17] ^{14}C-acetyl-CoA is formed at a meager steady-state rate of $<0.31\ \mu M\ h^{-1}$. MeCbi[18] is a much more active methyl donor; under the same conditions, a rate of $26.2\ \mu M\ h^{-1}$ is observed. The rates of MeCbi decay, which is monitored at 464 nm by UV−visible spectroscopy, and of Co(I) formation at 388 nm (Table 1) are equal (see Supporting Information Figure 1). Cbi lacks benzimidazole and the nucleotide loop that is appended to the corrin ring. The increased activity of MeCbi relative to MeCbl could derive from an effect of the axial Co−N bond or some inhibitory effect of benzimidazole. To distinguish between these alternatives, we used CH_3-(Me_3-benzimidazolyl) cobamide[18] as methyl donor (Figure 1). This compound is identical to MeCbl except that its coordinating

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(1) Abbreviations used: Cbl, cobalamin; Cbi, cobinamide; CODH, CO dehydrogenase; ACS, acetyl-CoA synthase; CFeSP, corrinoid iron−sulfur protein.

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(17) 0.1 mM $^{14}CH_3$−Cbl (9200 dpm/nmol), 0.44 mM CoA and 7.3 μM CODH/ACS and 1 atm of CO were reacted in the dark at 55 °C in 0.1 M Tris-HCl, pH 7.60. The reaction was followed for 20 h, and monitored by analyzing 50 μL aliquots by C_{18} analytical HPLC. The reaction with $^{14}CH_3$−Cbi contained 28 μM methyl donor, 1 atm CO, 164 μM CoA, and 1.5 μM CODH/ACS and analyzed by analytical HPLC. Fractions were collected, and the amount of acetyl-CoA formed in each case was determined from the radioactivity of the corresponding peak. The relative MeCbi:MeCbl reactivity ratio after normalizing for CODH/ACS and methyl donor concentrations is 1470:1.
(18) Me-(Me_3-benzimidazolyl)cobamide was prepared as previously described.[34] Cbi was prepared from (CN)Cbl (vitamin B_{12}) as described.[35] Initial rates were measured at 55 °C in a Cary UV−visible spectrophotometer modified by OLIS Instruments (Bogart, GA) using anaerobic 1 and 0.2 cm path length quartz cuvettes. All experiments were carried out under 1 atm of 100% CO (g). Initial rates were determined either by the initial slopes or by fits of the entire time courses to a double-exponential function followed by extrapolation to $t = 0$, with software provided by OLIS Instruments.

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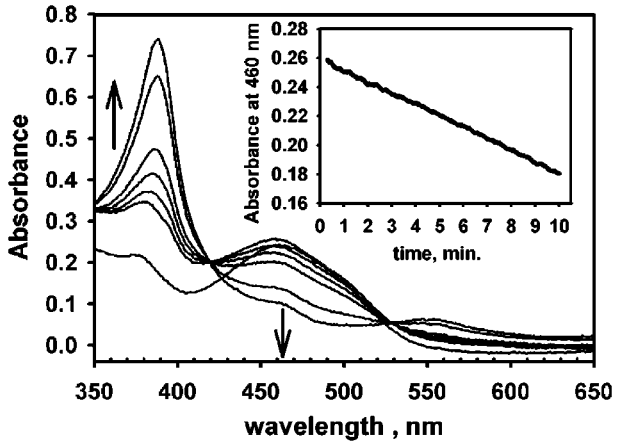

Figure 1. Acetyl-CoA synthesis from Me-(Me_3-benzimidazolyl)cobamide. The reaction mixture contained 28 μM methyl donor, 1 atm CO, 164 μM CoA, and 1.5 μM CODH/ACS. The lowest trace (at 350 nm) of the methyl donor in the absence of CODH/ACS is nonisosbestic because it lacks the CODH/ACS absorbance. The succeeding traces were collected 1, 2.5, 6, 9, 16, and 27 min after CODH/ACS addition.

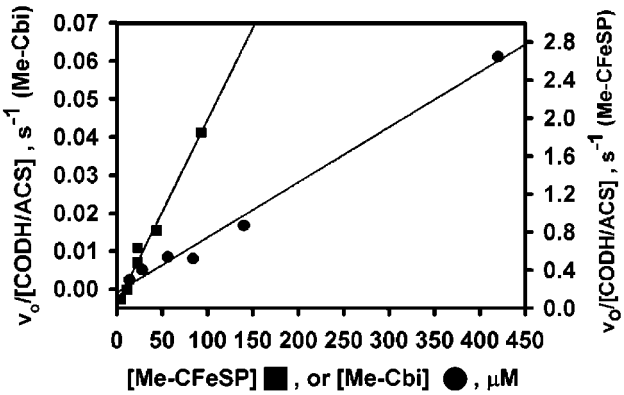

Figure 2. Acetyl-CoA synthesis from the methylated CFeSP and methylcobinamide.

nitrogen is methylated and, thus, is unable to bind cobalt. With Me-(Me_3-benzimidazolyl)cobamide as the methyl donor, the same spectral changes are observed and its reactivity equals that of MeCbi (Table 1). For all three methyl donors, the observed rate constants are linear with concentration allowing comparison of the second-order rate constants for acetyl-CoA synthesis (Figure 2).

The above results indicate that ligation of the benzimidazole nitrogen to cobalt causes a >2000-fold decrease in the transmethylation reaction rate. The role of the lower axial ligand in cobalamin-dependent reactions has been much discussed (for review, see ref 20). MeCbi transfers its methyl group to alkylselenides or alkylthiols approximately 1000-fold faster than MeCbl,[21] which is strikingly similar to our enzymatic results. In an enzymatic methyl transfer to a thiol group, MeCbi is more effective than MeCbl in methylating coenzyme M by the methanol:CoM methyltransferase from *Methanosarcina barkeri* and imidazole inhibits the reaction.[12]

We have speculated that the lack of a lower axial nitrogen donor ligand in the CFeSPs from methanogenic and acetogenic microbes enhances reductive activation, stabilizes the methyl−Co bond against homolytic cleavage, and enhances demethylation reactions.[11,22] The Me-CFeSP transfers its methyl group 10^5-fold faster than MeCbl, which apparently includes a 10^3-fold rate enhancement offered by removal of the N-donor ligand plus an additional 100-fold rate increase derived from interactions between ACS and the CFeSP. These CFeSPs are distinct from most cobalamin-containing proteins, which contain a histidine ligand replacing the benzimidazole group when the cofactor binds the protein.[23,24] Proteins that have the histidine ligand usually contain a conserved motif, DxHxG-(41-42)-gxSxL-(21/22)-GG.[23] In such proteins, the cobalt is "base-off" and "his-on". In contrast, the CFeSPs lack the ligating histidine residue and the conserved motif;[9,25] therefore, they remain in a "base-off" "his-off" form in all redox and coordination states.[7,26−28] This is mechanistically important because the imidazole of histidine confers similar donor ligand properties as benzimidazole.[29]

In the presence of CODH, CO acts as a natural electron donor to activate Co(II)−CFeSP to the Co(I) state. We compared the initial rates of Co(II) reduction for Cbi, Cbl, and the CFeSP by CODH (Table 1), which were linear with corrinoid concentration. The second-order rate constants for Co(II) reduction were strikingly similar for the CFeSP and Cbi(OH),[19] but 5000-fold slower with Cbl(OH). This likely reflects in part the ∼120 mV lower redox potential of the Co(II)/(I) couple for cobalamin relative to cobinamide.[30]

The rate enhancement of Co(II) reduction and Me-Co(III) demethylation upon removal of the nitrogen donor ligand is consistent with the view that lower electron density at the metal facilitates electron transfer to Co(II), renders Co(I) a better leaving group for S_N2 reactions, and decreases Co affinity for the CH_3^+ group. Over 20 corrinoids (0.5 μmol g^{-1} cell wet weight) have been isolated from *Moorella thermoacetica*; in the methyl-Co state some are converted to the methyl group of acetate.[31−33] Possibly, some corrinoids, like cobyric acid or Cbi, supplement the activity of the CFeSP in acetyl-CoA synthesis under physiological conditions.

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Supporting Information Available: Figure showing spectral changes during acetyl-CoA synthesis from MeCbi (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The second-order rate constant for the reduction of the CFeSP was found to be 270 000 M^{-1} s^{-1} with CFeSP concentrations below 8 μM and 55 000 with higher concentrations. The explanation for this variation is under investigation.

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