Homogeneous ferredoxin (flavodoxin):NADP⁺ reductase and flavodoxin A proteins served as electron donors for the reduction of co(III)rrinoids to co(II)rrinoids in vitro. The resulting co(II)rrinoids served as substrates for the ATP:co(II)rrinoid adenosyltransferase (CobA) enzyme of Salmonella enterica serovar Typhimurium LT2 and were converted to their respective adenosylated derivatives. The reaction products were isolated by reverse phase high performance liquid chromatography, and their identities were confirmed by UV-visible spectroscopy, mass spectrometry, and in vivo biological activity assays. Adenosylcobalamin generated by this system supported the activity of 1,2-propanediol dehydratase as effectively as authentic adenosylcobalamin. This is the first report of a protein system that can be coupled to the adenosyltransferase CobA enzyme for the conversion of co(III)rrinoids to their adenosylated derivatives.

Adenosylcobalamin (AdoCbl) is a structurally complex coenzyme (Fig. 1). Its biosynthesis has been studied in a number of procaryotes (1–5), where aerobic and anaerobic pathways for its generation have been identified (3, 5, 6). Conversion of biologically inactive forms of Cbl, such as CNCbl (vitamin B₁₂) to AdoCbl must occur before it can function as a coenzyme. This conversion requires reduction of the cobalt atom in the corrin ring to its Co(I) state before it can be covalently attached to the deoxyadenosyl moiety from ATP to yield AdoCbl. The process was thought to require three enzymatic interactions (7): a cob(II)alamin reductase (EC 1.6.99.8), a cob(II)alamin reductase (EC 1.6.99.9), and an ATP:co(II)rrinoid adenosyltransferase (EC 2.5.1.17) (CobA in Salmonella enterica). The adenosyltransferase enzyme has been studied in several organisms (7–13). Recently, the three-dimensional structure of the CobA enzyme of S. enterica was solved in its apoenzyme form, complexed with MgATP, and complexed with HOCbl and MgATP (14), revealing a new mode for corrinoid binding that is substantially different from that of Cbl-dependent enzymes (15–18).

The reducing system involved in the corrinoid adenosylation pathway is not well understood in any of the organisms in which Cbl biosynthesis has been studied. The corrinoid reducing activities described to date in procaryotes were reported to require pyridine nucleotides and free flavin nucleotides for activity (7, 8, 19), and thus activity has been attributed to easily dissociable flavoproteins. However, it was recently shown that co(III)rrinoid reduction can be chemically achieved by dihydroflavin nucleotides in the absence of any enzymes (20). The identity of the enzyme catalyzing the reduction of Co(II) to Co(I) remained unknown. Although a cob(II)pyrrolic acid a,c-diamide reductase was isolated from Pseudomonas denitrificans, neither the enzyme nor the gene responsible for this activity was identified (19).

At present, the only well documented cob(II)alamin reduction reaction is the one required for reductive activation of the E. coli methionine synthase enzyme (MetH). The MetH enzyme becomes inactive once every 2,000 turnovers when cob(II)alamin oxidizes to cob(II)alamin (21). The electrons for the reduction of cob(II)alamin to cob(I)alamin in MetH are provided by reduced flavodoxin (FldA) (22–24). It has been demonstrated that very specific interactions between FldA and MetH are required for cob(I)alamin reduction to occur (21, 24, 25). The data presented here show that the NADP⁺:ferredoxin (flavodoxin) reductase (Fpr) and FldA proteins can reduce co(III)rrinoids to co(II)rrinoids, the substrates of the CobA. The results from spectroscopic analyses and enzymic and biological activity assays are reported to show that the product of the in vitro adenosylation reaction was the adenosylated derivative of the corrinoid substrate used. It is proposed that the Fpr and FldA proteins are part of the corrinoid adenosylation pathway in S. enterica.

**EXPERIMENTAL PROCEDURES**

**Reagents**

Unless otherwise stated, all reagents including (CN)₂Cbi, HOCl, CNCl, and AdoCbl were obtained from Sigma.

**Protein Purification**

**Purification of E. coli Fpr**

**Step 1: Mass Culturing of the Overexpressing Strain and Generation of Cell-free Extracts**—Fpr protein was overproduced from strain JE4937 (E. coli C-1a/pEE1010, a gift from Elisabeth Haggård-Ljungquist, Stockholm University, Stockholm, Sweden) grown overnight at 37 °C on Luria Bertani broth containing 100 µg/ml of ampicillin and 30 mM glucose. The cells were harvested by centrifugation at 10,000 × g for 10
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**Fig. 1. Chemical structure of AdoCbl.**

min using a Sorvall GSA rotor (DuPont Instruments, Wilmington, DE). Approximately 20 g of cells were resuspended in 50 mM Tris-Cl buffer pH 7.5 at 4 °C (buffer A) containing 16 µg/ml of protein inhibitor phenylmethanesulfonyl fluoride. The cell suspension was broken by sonication using a Sonics Dismembrator (model 550; Fisher) for 13 min (large tip, setting of 6, 50% duty). Cell-free extracts were generated by centrifugation at 40,000 × g for 1.5 h using a KOMP Spin rotor.

**Step 2: Anion Exchange Chromatography on DEAE-650M—Cell-free extracts (~1.5 g of protein) were applied to a column containing DEAE 650M resin (2.5 × 20 cm, 7.7 ml bed volume) equilibrated with buffer A. Approximately 22 mg of protein was applied per ml of resin. The column was washed with 70 ml of loading buffer followed by 140 ml of 100 mM sodium acetate buffer, pH 5.0. Bound proteins were eluted with a linear gradient 100–500 mM NaCl in 100 mM sodium acetate buffer, pH 5.0. The pH of the fractions containing the FldA protein was brought up to 7.0 with 2 M Tris-Cl buffer, pH 8.0, at 4 °C. Fractions containing the FldA protein were pooled and dialyzed against buffer A.

**Step 3: Anion Exchange FPLC—Fractions containing dialyzed FldA protein (~315 mg of total protein) were applied to a POROS HQ (PerSeptive Biosystems, Framingham, MA) anion exchange FPLC column (1.0 × 10 cm, 7-ml bed volume) equilibrated with buffer A. Forty-five mg of protein was applied per ml of resin. The column was washed with 14 ml of buffer A followed by a linear gradient of 0–1.5 M NaCl in buffer A. Fractions containing FldA were pooled, dialyzed against buffer A, and stored at ~90 °C in buffer A + 20% (v/v) glycerol. After this purification step, the FldA protein was assessed to be >95% homogeneous by SDS-PAGE. A total of 85 mg of FldA was obtained from 25 g of starting material.

**Purification of the CobA Enzyme of S. enterica**

Purification of the CobA enzyme was performed as described in Bauer et al. (14) without modifications.

**Purification of the NAD(P)Flavin oxidoreductase (Fre) Enzyme**

Hexahistidine-tagged Fre enzyme (H₆Fre) was purified to homogeneity as described (20).

**Protein Techniques**

Total protein concentration was determined by the Bradford method using the Bio-Rad protein reagent (26). Protein analysis was performed by SDS-PAGE (27) stained with Coomassie Blue (28). The concentration of the Fpr and FldA proteins was determined using the reported extinction coefficients for the protein-bound flavins (29).

**In Vitro Activity Assays**

**Cobrinoid Adenosylation Assays**

Potassium borohydride-dependent CobA assays were performed as described (13). The assay that demanded enzymic reduction of cob(I)alamin to cob(I)alamin was a modification of the corrinoid adenosylation assay previously reported (13). Conversion of (HOCbl) to AdoCbl was monitored spectrophotometrically using a PerkinElmer Lambda 6 Spectrophotometer equipped with a temperature-regulated cuvette holder. The assay mixtures (final volume, 1 ml) contained HOCbl (50 mM), NADPH (500 mM), ATP (400 mM), MnCl₂ or MgCl₂ (800 mM), FMN (50 mM), Tris-Cl buffer, pH 8.0, at 37 °C (200 µmol) homogenous NAD/P(NADP)FMN oxidoreductase (Fre) enzyme (50 units, where 1 unit of flavin reductase activity was defined as the amount of enzyme required to reduce 1 nmol of FMN) and Fre were omitted from these assays.

Fpr protein was used to reduce cob(III)alamin to cob(I)alamin, and thus FMN and Fre were omitted from these assays.

**AdoCbl formation was monitored by the increase in the absorbance of the solution at 525 nm as a function of time. The amount of product generated was calculated using the difference in molar extinction coefficient between the substrate, cob(I)alamin, and the product, AdoCbl, at 525 nm (ε₅₂₅ = 4.8 × 10⁻³ M⁻¹ cm⁻¹). One unit of CobA activity was defined as the amount of enzyme required to generate 1 nmol of AdoCbl/min (13).**

**Cytochrome c Reductase Assays**

Activity of the Fpr protein was monitored using the cytochrome c reductase assay as reported by Fujii et al. (22, 23), except the assays were performed under anoxic conditions to mimic the conditions used in the corrinoid adenosylation assays.

**1,2-Propanediol Dehydratase Assays**

In vitro assays for AdoCbl-dependent 1,2-propanediol dehydratase (a gift from Perry A. Frey) were performed as described (30). The conver-
sion of 1,2-propanediol to propionaldehyde was measured by derivatization of the product with 3-methyl-2-benzothiazoline hydrazone yielding an azine product that can be measured spectrophotometrically at 305 nm ($\varepsilon_{305} = 13,300 \text{ M}^{-1} \text{ cm}^{-1}$). This assay was used as a measure of coenzymic activity of enzymically generated AdoCbl. One unit of 1,2-propanediol dehydratase is defined as the amount of enzyme required to generate 1 μmol of propionaldehyde/min under the conditions of the assay.

**Synthesis of Aquocobinamide (H₂OCbi)**

H₂OCbi was synthesized in vitro from (CN)₂Cbi. First, AdoCbi was synthesized from (CN)₂Cbi using the potassium borohydride-dependent corrinoid adenosylation assays. The assays were performed as described (13), except (CN)₂Cbi (50 μM) was substituted for HOCbl. After substrate conversion was complete, the reaction mixture was exposed to air and filtered through a 45-μm (25-mm diameter) syringe filter (Nalgene, Rochester, NY). The corrinoid product was isolated using a C₁₈ SepPak cartridge (Waters, Milford, MA) and dried using a SpeedVac concentrator (Savant Instruments, Farmingdale, NY). Concentrated AdoCbi was resuspended in 400 μl of water and photolyzed aerobically using a tungsten/halogen lamp placed 20 cm away from the tube containing the sample. Using this procedure, AdoCbi was converted by heterolysis to H₂OCbi. The sample was passed through a C₁₈ cartridge as described above. H₂OCbi was converted to (CN)₂Cbi by photolysis in 0.1 M KCN, pH 10.0, using a tungsten/halogen lamp and quantitated using the molar extinction coefficient for (CN)₂Cbi at 367 nm ($\varepsilon_{367} = 30,800 \text{ M}^{-1} \text{ cm}^{-1}$) (31).

**Reverse Phase HPLC (RP-HPLC) Analysis of Reaction Products**

The products of the corrinoid adenosylation assays were isolated and analyzed by RP-HPLC on a Prodigy ODS (8) column (Phenomenex, Torrance, CA) attached to an HPLC system as described (32). A photodiode array detector (Waters) was used to identify corrinoids by their UV-visible spectra. Authentic HOCbl and AdoCbl were used as standards for UV-visible spectra. The isolated corrinoids were desalted using a C₁₈ SepPak cartridge and dried in a SpeedVac concentrator.

**Mass Spectrometry**

Mass spectrometry of purified reaction products was performed at the Mass Spectrometry Facility at the University of Wisconsin-Madison Biotechnology Center.

**In Vivo Assessment of Adenosylcorrinoid Synthesis**

**In vitro synthesis of adenosylcorrinoids was assessed in vivo by using strain JE1096 (metE205 ara-9 cobA343::MudJ) as an indicator strain in a biological activity assays.** The cells of the indicator strain grown overnight in 2 ml of nutrient broth were concentrated by centrifugation and washed with 14.5 ml sterile saline; 0.1 ml of the cell suspension (∼10⁸ cells) was added to 3 ml of molten soft agar (0.7% w/v) and used

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**Fig. 2.** Denaturing polyacrylamide gel electrophoretic analysis of purified of proteins required for in vitro corrinoid adenosylation. Lane A, homogeneous Fpr enzyme. Lane B, homogeneous FldA protein. Lane C, homogeneous CobA enzyme. Molecular mass standards are shown on the right (in kDa).

**Fig. 3. A.** In vitro conversion of cob(III)alamin to AdoCbl by homogeneous Fpr, FldA, and CobA proteins. Coupled corrinoid adenosylation assays contained: 100 μmol of NADPH, 50 nmol of HOCbl, 400 nmol of ATP, 800 nmol of MnCl₂, 50 nmol of FMN, 50 units of purified H₆Fre enzyme, 4.7 nmol of purified Fpr enzyme, 3.2 nmol of purified FldA protein, and 1.4 nmol of purified CobA enzyme in Tris-Cl buffer, pH 8.0, at 37°C (200 μmol). Spectrum 1, AdoCbl product of the complete reaction mixture; spectrum 2, cob(I)alamin product of a reaction in which the FldA protein was omitted; spectrum 3, cob(II)alamin product of a reaction in which the Fpr enzyme was omitted; spectrum 4, cob(I)-alamin product of a reaction in which the CobA enzyme was omitted. B. Spectral changes associated with the conversion of cob(III)alamin to AdoCbl. Protein concentrations were as follows: 0.7 nmol of Fpr; 2.1 nmol of FldA, and 0.9 nmol of CobA. Spectrum 1, UV-visible spectrum of the solution at the start of the reaction; spectrum 2, UV-visible spectrum of the solution 10 min after the reaction was started; spectrum 3, UV-visible spectrum of the solution 20 min after the reaction was started; spectrum 4, UV-visible spectrum of the solution 30 min after the reaction was started; spectrum 5, UV-visible spectrum of the solution one hr after the reaction was started. C, time course for the enzymatic conversion of cob(III)alamin to AdoCbl. Protein concentrations were as follows: 0.7 nmol of Fpr; 2.1 nmol of FldA, and 0.9 nmol of CobA. The reaction conditions were as described under “Experimental Procedures.”
to overlay a plate of Vogel-Bonner minimal medium (33) containing glucose (11 mM) as carbon and energy source. The corrinoid standards AdoCbi, (CN)2Cbi, and Cbl (50 pmol each) and reaction products were spotted onto the overlay and incubated overnight at 37 °C.

**RESULTS**

Homogeneous Fpr, FldA, and CobA Proteins Are Sufficient for In Vitro Conversion of Cob(III)alamin to AdoCbl—NADPH-dependent synthesis of AdoCbl was conducted when cell-free extracts of strains overexpressing Fpr (ferredoxin/flavodoxin): NADP⁺ oxidoreductase) and FldA (flavodoxin A) proteins were added to corrinoid adenosylation assay mixtures in place of potassium borohydride (data not shown). Control experiments in which either cell-free extract was omitted failed to yield a product, suggesting a requirement for both Fpr and FldA proteins. These proteins were isolated to homogeneity to further investigate their involvement in the corrinoid adenosylation reaction.

**FIG. 4.** Isolation of the reaction product by RP-HPLC. A, elution profile of the reaction product of the coupled corrinoid adenosylation assays as isolated by RP-HPLC. The product of the reaction had an elution time of 21.9 min. Commercially available AdoCbl subjected to the same procedure eluted at 22.0 min (data not shown). B, UV-visible spectrum of the isolated corrinoid. Inset, UV-visible spectrum of authentic AdoCbl.

**FIG. 5.** Mass spectrometry analysis of the product of the coupled corrinoid adenosylation assay. A, positive and negative ion ESI mass spectra of the reaction product isolated by RP-HPLC. B, positive and negative ion ESI mass spectra of authentic AdoCbl isolated by RP-HPLC.
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Identification of the Product of the Reaction—The product of the corrinoid adenosylation reaction was isolated by RP-HPLC. When HOCbl was used as substrate, the elution time (Fig. 4A, 21.9 min) and UV-visible spectrum of the isolated product was identical to an AdoCbl standard isolated by the same procedure (Fig. 4B). When the reducing system (Fpr, FldA) was omitted from the reaction mixture, a product with an elution time (18.7 min) and UV-visible spectrum identical to that of a HOCbl standard was obtained (data not shown).

The isolated product was analyzed by electrospray ionization (ESI) mass spectrometry (Fig. 5). The positive-ion ESI mass spectrum showed signals with m/z values of 829.2 and 1582.2, which corresponded to masses of KAdoCbl (z = +2) and AdoCbl (z = +1), respectively. The negative-ion ESI mass spectrum of the product showed a parent peak with an m/z value 1367.8 (z = +1), in agreement with the molecular mass for NaHOCbl and suggesting that the Ado group was removed during analysis. This was confirmed by the negative-ion ESI mass spectrum of authentic AdoCbl isolated by HPLC (Fig. 5B). The positive ion ESI mass spectrum of authentic AdoCbl showed a

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Homogeneous Fpr protein (Fig. 2, lane A) used in this work had a cytochrome c reductase specific activity of 0.36 μmol cyt c min⁻¹ mg⁻¹ of protein, and a specific content of 42 nmol of Fpr/mg of protein. Initially, the cob(II)alamin substrate was generated using NAD(P)H:flavin oxidoreductase H6Fre enzyme, NADH, and FMN (20). Once the Fpr protein was isolated, it was used in lieu of H6Fre to reduce HOCbl to cob(III)alamin. Elimination of the H6Fre protein simplified the reaction conditions.

The FldA protein was purified to >95% homogeneity (Fig. 2, lane B). Approximately 85 mg of protein was obtained from 22 nmol of FldA/mg of protein. FldA protein was reduced by Fpr, and reduced FldA was coupled to CobA enzyme in the corrinoid adenosylation assays. This system converted cob(III)alamin or cob(III)iminamide to their corresponding adenosylated derivatives. NADPH was required, but unlike other systems, no further addition of FAD or FMN to the reaction mixture was necessary (7, 8, 19, 20).

When Fpr and FldA proteins were coupled to the CobA enzyme, AdoCbl synthesis was observed (Fig. 3A). The characteristic changes associated with conversion of cob(II)alamin to the final product, AdoCbl (Fig. 3B), were not observed when either one of the enzymes was omitted from the reaction mixture. These spectral changes were in good agreement with those observed for this conversion in other systems (7). The identity of the products of these reactions was further confirmed by the analyses shown below.

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**A.**

**B.**

**FIG. 8.** Amino acid sequence alignments of the *E. coli* and *S. enterica* Fpr and FldA proteins. Sequence alignments were performed using the CLUSTAL W program (43). Sequences were also analyzed using the National Center for Biotechnology Information BLAST 2.0 program (44). Preliminary sequence information for *S. enterica* serovar Typhimurium LT2 was obtained from the Genome Sequencing Center (Washington University, St. Louis, MO) data base (Genome Sequencing Center, http://genome.wustl.edu/geo/Blast/client.pl). Identical amino acid residues are marked by *asterisks*. Differences between sequences are highlighted in gray.

Enzymic Reduction and Adenosylation of Corrinoids—Corrinoid adenosylation assays were performed using either (CN)$_2$Cbi or H$_2$OCbi as substrate. When (CN)$_2$Cbi was used, the rate conversion of this substrate to AdoCbi was slow, as judged by the changes in the UV-visible spectrum associated with this conversion (Fig. 6A). High concentrations of the flavoprotein system and of the CobA enzyme were required to drive the reaction to near completion (Fig. 6A, *spectrum 5*). The UV-visible spectrum of the product obtained under these conditions was in good agreement with that reported for AdoCbi (13, 34). The absorbance peak at 579 nm (Fig. 6A, *spectrum 5*) is indicative of unreacted substrate. These results indicated that the cyanide ligands were removed upon reduction of the cobalt ion to generate the four-coordinate cob(II)alamin substrate for CobA. When H$_2$OCbi was used as substrate (Fig. 6B), the apparent rate for adenosylation was approximately twice as fast, indicating that removal of the cyanide ligands was the rate-limiting step when (CN)$_2$Cbi was used as substrate.

To confirm that the product of the in vitro reaction was AdoCbi, strain JE1096 was used to test for biological activity (Fig. 7). Strain JE1096 is a methionine auxotroph correctable with AdoCbi or Cbl (11). A strong growth response was observed when the product of the reaction was provided to the indicator strain. Growth of the cobA mutant strain was also observed with AdoCbi generated in KBH$_4$-dependent adenosylation assays (Fig. 7). As expected, a growth response was not observed when (CN)$_2$Cbi was provided in the assay. These results confirmed that the product was AdoCbi.

**DISCUSSION**

The data reported herein show that the reducing system comprised of the Fpr and FldA proteins is sufficient for the generation of the corrinoid substrate of the ATP:corrinoid adenosyltransferase CobA enzyme of *S. enterica*. To the best of our knowledge, this is the first report of a reducing system that can be coupled to the CobA enzyme for the generation of adenosylated corrinoids.

The involvement of the FldA protein in the corrinoid adenosylation reaction is consistent with the ability of this protein to work at low redox potentials as a one-electron carrier (21, 22, 35, 36) and with its involvement in the reduction of cob(II)alamin to cob(I)alamin on the Cbl-dependent methionine synthase MetH enzyme (21–24, 37). The validity of the conclusions drawn from this work is not affected by the use of *E. coli* FldA and Fpr proteins given the high degree of identity shared by the proteins in these bacteria (Fig. 8), strongly suggesting that these proteins perform equivalent functions in these procaryotes. Thus it is reasonable to think that reduced FldA might represent a natural reducing system for corrinoid adenosylation in enterobacteria (*e.g. E. coli* and *S. enterica*).

**The Site of Corrinoid Reduction**—The very low redox potentials of the free cob(II)alamin/cob(I)alamin couple (E$^\circ$ = −610 mV) (38) and the extreme reactivity of the cob(I)alamin nucleophile (39) strongly suggest that reduction of cob(II)alamin occurs on the CobA enzyme. It is unlikely that cob(II)alamin reduction occurs in solution because the midpoint redox potentials for the semiquinone and hydroquinone forms of free FldA (−260 and −440 mV, respectively) (21) are too high to account for the generation of the cob(II)alamin nucleophile via a second order reaction. The redox potential for this reaction is expected to be close to the redox potential for free cob(II)alamin and thus considerably lower than that of the cob(II)alamin form of free methionine synthase (21, 38). In addition, the lower axial ligand of HOCbl has been shown to be coordinated to the cobalt in the CobA crystal structure (Fig. 8) (14). Generation of the cob(II)alamin substrate for CobA ligand would require lower

signal at mlz = 829.4, consistent with the molecular masses of KAdoCbl (z = +2).

AdoCbl Generated by the Enzymic System Has Coenzymic Activity—Approximately 0.1 nmol of enzymically generated AdoCbl was added to 1,2-propanediol dehydratase reaction mixtures. The amount of propionaldehyde generated from the dehydration of 1,2-propanediol during the reaction was identical to that of a reaction performed using 0.1 nmol of commercially available AdoCbl (specific activity, 0.02 unit/mg), indicating that enzymically generated AdoCbl was biologically active.
ligand dissociation from the cobalt ion. This reaction is known to raise the redox potential of the cob(II)alamin/cob(I)alamin couple (21, 40). Interactions between FldA and CobA coupled to product formation are therefore expected to raise the redox potential to facilitate the reaction. The documented mechanisms for MetH reactivation as well as the FldA-dependent anaerobic ribonucleotide reductase activation reaction support this hypothesis (24, 41). Interactions between the FldA and CobA proteins are an attractive possibility, when one also considers the location of the corrinoid-binding site in CobA (14). The ternary complex structure between HOCbl, ATP, and CobA show that the corrinoid binding site is located near the surface of the enzyme (Fig. 9). The interactions between FldA and CobA and/or the reduction of the corrinoid may be responsible for the hypothesized conformational changes CobA must undergo to bring the target C5/H11032 of the ribose moiety closer to the cobalt atom for the nucleophilic attack to occur (14). Work addressing these important aspects of the reaction is currently being performed.

Is FldA Involved in Corrinoid Adenosylation in Vivo?—The answer to this question is complicated by the fact that mutations in the βlA gene have been found to be lethal under aerobic and anaerobic growth conditions, suggesting that FldA is an essential function (42). Thus a genetic approach to this question is not possible. Because of this, we are not able to determine whether FldA is one redundant function in co(II)rrinoid reduction for adenosylation or whether it is solely responsible for this process. If FldA were an in vivo corrinoid reductase involved in corrinoid adenosylation, it would explain why cob(II)alamin reductase mutants have not been isolated. The chemistry of the reaction together with the data presented herein suggest that a true in vivo reductant for this process must be a low redox one-electron carrier and that, if the proposed interactions with the CobA enzyme are required, at least some specificity would be expected.

The Corrinoid Adenosylation Pathway in S. enterica—The schematic of the corrinoid adenosylation pathway shown in Fig. 10 incorporates the findings reported in this paper. In this model, reduction of co(III)rrinoids to co(I)rrinoids are donated by NADPH through flavins, Fpr, or FldA. The Fpr enzyme reduces FldA to the semiquinone form for generation of the co(I)rrinoid substrate of CobA. ε−, electron; Fpr-FAD, oxidized form of Fpr; Fpr-FADH2, hydroquinone form of Fpr; FldA-FMN, oxidized form of FldA; FldA-FMNH2, semiquinone form of flavodoxin; PPI, tripolyphosphate.

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FIG. 9. Stereo view of the ternary complex between CobA, HOCbl and ATP (14). Shown is a ribbon representation of the complex between CobA and its substrates. Only one HOCbl molecule is observed in this representation, bound at the surface of subunit A. The N-terminal α-helix from subunit B is shown interacting with the nucleotide loop of the HOCbl molecule bound to subunit A. The lower ligand, 5,6-dimethylbenzimidazole, is shown coordinated to the cobalt ion of the corrin ring.

FIG. 10. The corrinoid adenosylation pathway in S. enterica. Electrons for reduction of co(III)rrinoids to co(I)rrinoids are donated by NADPH through flavins, Fpr, or FldA. The Fpr enzyme reduces FldA to the semiquinone form for generation of the co(I)rrinoid substrate of CobA. ε−, electron; Fpr-FAD, oxidized form of Fpr; Fpr-FADH2, hydroquinone form of Fpr; FldA-FMN, oxidized form of FldA; FldA-FMNH2, semiquinone form of flavodoxin; PPI, tripolyphosphate.

2 M. V. Fonseca and J.C. Escalante-Semerena, unpublished results.
We thank the Genome Sequencing Center, Washington University, St. Louis, MO (http://genome.wustl.edu/gsc/Search/policy.shtml) for allowing access to 
S. enterica DNA sequence data prior to publication.

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