Published in final edited form as:

J Biol Inorg Chem. 2016 September ; 21(5-6): 669–681. doi:10.1007/s00775-016-1371-x.

Resonance Raman Spectroscopic Study of the Interaction Between Co(II)rrinoids and the ATP:Corrinoid Adenosyltransferase PduO from *Lactobacillus reuteri*

Kiyoung Park^{1,*}, Paola E. Mera², Jorge C. Escalante-Semerena³, and Thomas C. Brunold^{4,*}

¹Department of Chemistry, Korea Advanced Institute of Science and Technology, Yuseong-gu, Daejeon 305-701, Republic of Korea

²Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico 88003, USA

³Department of Microbiology, University of Georgia, Athens, Georgia 30602, USA

⁴Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA

Abstract

The human-type ATP:corrinoid adenosyltransferase PduO from *Lactobacillus reuteri* (*Lr*PduO) catalyzes the adenosylation of Co(II)rrinoids to generate adenosylcobalamin (AdoCbl) or adenosylcobinamide (AdoCbi⁺). This process requires the formation of “supernucleophilic” Co(I)rrinoid intermediates in the enzyme active site that are properly positioned to abstract the adeonsyl moiety from co-substrate ATP. Previous magnetic circular dichroism (MCD) spectroscopic and X-ray crystallographic analyses revealed that *Lr*PduO achieves the thermodynamically challenging reduction of Co(II)rrinoids by displacing the axial ligand with a non-coordinating phenylalanine residue to produce a four-coordinate species. However, relatively little is currently known about the interaction between the tetradentate equatorial ligand of Co(II)rrinoids (the corrin ring) and the enzyme active site. To address this issue, we have collected resonance Raman (rR) data of Co(II)rrinoids free in solution and bound to the *Lr*PduO active site. The relevant resonance-enhanced vibrational features of the free Co(II)rrinoids are assigned on the basis of rR intensity calculations using density functional theory to establish a suitable framework for interpreting rR spectral changes that occur upon Co(II)rrinoid binding to the *Lr*PduO/ATP complex in terms of structural perturbations of the corrin ring. To complement our rR data, we have also obtained MCD spectra of Co(II)rrinoids bound to *Lr*PduO complexed with the ATP analogue UTP. Collectively, our results provide compelling evidence that in the *Lr*PduO active site, the corrin ring of Co(II)rrinoids is firmly locked in place by several amino acid side chains so as to facilitate the dissociation of the axial ligand.

Keywords

resonance Raman spectroscopy; density functional theory; ATP:corrinoid adenosyltransferase; adenosylcobalamin

*To whom correspondence should be addressed. kiyoung.park@kaist.ac.kr; brunold@chem.wisc.edu.

Introduction

Adenosylcobalamin (AdoCbl), also known as coenzyme B₁₂, is one of the biologically active corrinoid species.¹ These species are characterized by the presence of a naturally occurring tetrapyrrole macrocycle termed the corrin ring that chelates a low-spin Co(III) ion in a tetradentate manner. On the “upper” and “lower” faces of the corrin ring of AdoCbl, the Co(III) ion is axially coordinated by, respectively, an adenosyl (Ado) moiety and a 5,6-dimethylbenzimidazole (DMB) group that is tethered to the macrocycle through a nucleotide loop.² AdoCbl serves as a cofactor in numerous enzymes, such as ribonucleotide triphosphate reductase,^{3–5} glutamate mutase,^{6,7} diol dehydratase,^{8–11} ethanolamine ammonia lyase,^{12–14} and methylmalonyl-CoA mutase,^{15,16} all of which dramatically (by up to 12 orders of magnitude) accelerate the rate of homolytic Co–C bond cleavage of AdoCbl.^{6,15,17,18} The Ado• radical that is generated in this process (along with cob(II)alamin [Co(II)Cbl], Fig. 1) initiates radical-mediated enzymatic catalysis by abstracting a hydrogen atom from substrate.^{19–23}

Lactobacillus reuteri is one of few prokaryotic species that can synthesize AdoCbl *de novo*, employing a PduO-type ATP:corrinoid adenosyltransferase (*L*PduO)²⁴ to catalyze the formation of the Co–C(Ado) bond. *L*PduO utilizes Co(II)rrinoids and ATP as substrates, transferring the Ado moiety from ATP to a transiently generated Co(I)rrinoid species.²⁵ The generation of this Co(I)rrinoid intermediate is a thermodynamically challenging task, given that the reduction potentials of free Co(II)rrinoids [e.g., E° (SHE) = –610 mV for Co(II)Cbl]²⁶ are considerably lower than those of putative *in vivo* reducing agents [e.g., E° (SHE) = –440 mV for the semiquinone/reduced flavin couple of flavodoxin A (FldA)].²⁷ Nevertheless, several *in vitro* studies of *L*PduO and other ATP:corrinoid adenosyltransferases (ACATs) complexed with co-substrate ATP have revealed that these enzymes can accomplish the Co(II) → Co(I)rrinoid reduction when supplied with FldA or even free dihydroflavins [e.g., E° (SHE) = –228 mV for FMN at pH 7.5].^{28–30}

Previously, the mechanisms by which the CobA-type ACAT from *Salmonella enterica*,^{31,32} the PduO-type human ACAT,³³ *L*PduO,³⁴ and the EutT-type ACAT from *Salmonella enterica*^{35,36} can overcome the thermodynamically challenging Co(II) → Co(I)rrinoid reduction were investigated using magnetic circular dichroism (MCD) and electron paramagnetic resonance (EPR) spectroscopies. These studies led to the proposal that the ACAT/ATP complexes convert Co(II)rrinoids to effectively four-coordinate, square-planar species, thereby stabilizing the redox-active Co 3d_{z²}-based molecular orbital (MO) and raising the reduction potential into the physiologically accessible range.³¹ In support of this proposal, the X-ray crystal structure of *L*PduO complexed with Co(II)Cbl and MgATP²⁵ revealed that the side chain of a phenylalanine residue (F112) displaces the DMB moiety from the Co(II) ion upon Co(II)Cbl binding to the active site. By providing this hydrophobic environment below the corrin ring, the active site of the *L*PduO/ATP complex is capable of converting both Co(II)Cbl and cob(II)inamide [Co(II)Cbi⁺, a natural cobalamin precursor that lacks the nucleotide loop with its DMB tail and instead features an axially bound water molecule] to four-coordinate species.³⁴

These enzyme-bound, four-coordinate Co(II)rrinoid species exhibit distinct spectral signatures, most notably an intense, positively signed band in the near-IR spectral region of the MCD spectrum and a series of widely spread resonances in the low-field region of the EPR spectrum, which reflect the presence of a significantly stabilized Co 3d_z²-based MO. While the MCD features associated with the corrin $\pi \rightarrow \pi^*$ transitions are also notably perturbed relative to those displayed by five-coordinate free Co(II)rrinoids, relatively little is currently known about the effects of corrin ring conformational changes on the electronic structure of Co(II)rrinoids. Yet, it is obvious that non-bonding (electrostatic/hydrophobic) interactions between the corrin ring and the enzyme active site must play an important role in the mechanism of axial ligand removal, as amino acid substitutions in *L*PduO that disrupt the interactions between the peripheral amide groups of the corrin ring and the enzyme active site result in a significantly decreased yield for the five- to four-coordinate Co(II)rrinoid conversion and thus slow down the formation of AdoCbl.^{37,38}

Because corrinoids exhibit intense electronic absorption (Abs) features associated with corrin $\pi \rightarrow \pi^*$ transitions in the UV/visible region, resonance Raman (rR) spectroscopy provides a particularly sensitive probe of geometric perturbations of the corrin ring and has been used to investigate interactions between AdoCbl and AdoCbl-dependent enzymes.^{39–41} The strong coupling between the corrin $\pi \rightarrow \pi^*$ transitions and the stretching motion of the conjugated C–C and C–N bonds in the corrin ring leads to the predominant enhancement of corrin-based vibrational modes upon laser excitation in the UV/visible region. However, assigning these corrin-based vibrational modes is far from straightforward due to the structural complexity and lack of symmetry of the corrin ring. Originally, the two most strongly enhanced features in the rR spectra of Co(III)rrinoids were assigned on the basis of their rR enhancement patterns.^{42–46} The lower-energy feature at $\sim 1500\text{ cm}^{-1}$ was found to be predominantly enhanced upon laser excitation into the so-called α -band, which arises from the lowest-energy corrin $\pi \rightarrow \pi^*$ transition that is polarized along the C5 $\cdots$ C15 axis (the long axis, LA in Fig. 1), while the higher-energy feature at $\sim 1550\text{ cm}^{-1}$ was preferentially enhanced upon laser excitation into the so-called γ -band, which arises from a corrin $\pi \rightarrow \pi^*$ transition polarized along the Co $\cdots$ C10 axis (the short axis, SA in Fig. 1). On the basis of these findings, the ~ 1500 and $\sim 1550\text{ cm}^{-1}$ features were assigned as vibrational modes that primarily entail stretching motion of the conjugated C–C and C–N bonds oriented along the LA (the LA-stretching mode) and the SA (the SA-stretching mode), respectively.^{45,46} In support of these assignments, the $\sim 1550\text{ cm}^{-1}$ feature was shown to undergo a significant down-shift upon H/D exchange at C10, while the $\sim 1500\text{ cm}^{-1}$ feature was largely unaffected by this isotopic substitution.⁴⁵ However, on the basis of a density functional theory (DFT)-assisted normal mode analysis for methylcobalamin (MeCbl),⁴⁷ Kozlowski and coworkers suggested that the frequency of the SA-stretching mode is in fact lower than that of the LA-stretching mode. This finding raised questions regarding the validity of the normal mode assignments that were based on the rR enhancement patterns.

An equally puzzling finding in previous studies of corrinoids was that the rR enhancement patterns of the corrin-based vibrational modes vary significantly as a function of the cobalt oxidation state.⁴⁸ In the rR spectra of Co(II)rrinoids, two features associated with corrin-based vibrational modes are similarly enhanced upon laser excitation in resonance with the LA-polarized “ α -band-type” corrin $\pi \rightarrow \pi^*$ transition.⁴⁹ Notably, one of these enhanced

features is considerably down-shifted upon H/D exchange at C10, indicating that it arises from a vibrational mode predominantly involving SA-stretching motion. Consistent with these observations, a recent DFT-based analysis of the relative rR intensities of the relevant corrin-based vibrational modes revealed that the relative coupling strengths of these modes to a given corrin $\pi \rightarrow \pi^*$ transition strongly depend on the exact compositions of the donor and acceptor orbitals.⁴⁸ As a result, vibrations mainly involving stretching of conjugated C–C and C–N bonds oriented along one axis of the corrin ring may, in fact, couple to a perpendicularly electronic transition.

In the present study, we have used rR spectroscopy to investigate how the conformation of the corrin ring is perturbed upon Co(II)rrinoid binding to the active site of the *Lp*PduO/ATP complex. To aid in the interpretation of our experimental data, we computed the frequencies and relative rR intensities for models of Co(II)Cbl, Co(II)Cbi⁺, and four-coordinate Co(II)rrinoids. Additionally, we obtained MCD spectra of Co(II)Cbl bound to *Lp*PduO in the presence of the ATP analogue UTP, which allowed us to discriminate between different possible interpretations of our computational results. Collectively, the spectroscopic and computational data obtained in this study provide significant new insight into the nature of the interaction between four-coordinate Co(II)rrinoids and the *Lp*PduO/ATP complex.

Materials and Methods

Sample preparation and spectroscopy

All chemicals including aquacobalamin (H_2OCbl^+), dicyanocobinamide $[(\text{CN})_2\text{Cbi}]$, potassium formate (HCOOK), sodium borohydride (NaBH_4), deuterium chloride (DCl), and deuterium oxide (D_2O) were purchased from Sigma and used as obtained. Dihydroxocobinamide $[(\text{OH})_2\text{Cbi}]$ was prepared as described previously.⁴⁹ Briefly, an aqueous solution of $(\text{CN})_2\text{Cbi}$ was reduced to Co(I)Cbi with NaBH_4 , while purging the reaction mixture with $\text{N}_2(\text{g})$ to remove the nascent $\text{HCN}(\text{g})$. The resulting Co(I)Cbi solution was then re-oxidized in air and loaded onto a C18 SepPack cartridge to quench the remaining NaBH_4 with distilled water, and the $(\text{OH})_2\text{Cbi}$ product was subsequently eluted with methanol. Solutions of free Co(II)Cbl and Co(II)Cbi⁺ were obtained anaerobically by first degassing aqueous solutions of H_2OCbl^+ and $(\text{OH})_2\text{Cbi}$, respectively, under vacuum and subsequently reducing with HCOOK. H/D isotopic labeling at C10 was accomplished by incubating H_2OCbl^+ or $(\text{OH})_2\text{Cbi}$ in a DCl/ D_2O solvent mixture.⁵⁰ The excess deuterium ions in the solutions of $\text{H}_2\text{OCbl}^+(d-10)$ and $(\text{OH})_2\text{Cbi}(d-10)$ were quenched with NaOH dissolved in D_2O before the reduction to Co(II)Cbl(*d-10*) and Co(II)Cbi⁺(*d-10*), respectively, was initiated with HCOOK.

The protocol for producing and purifying *Lp*PduO is described in references (9) and (18). In short, a pTEV3 plasmid encoding the *Lp*PduO protein with an N-terminal His₆ tag was overexpressed in *Escherichia coli*, and the tag-free enzyme was obtained using rTEV protease and a HisTrap FF column (Amersham Biosciences). Samples of enzyme-bound, four-coordinate Co(II)rrinoids were prepared by mixing solutions of free (i.e., five-coordinate) Co(II)Cbl or Co(II)Cbi⁺ and *Lp*PduO, which was contained in pH 8 Tris-HCl buffer with at least an 8-fold molar excess of MgATP, in a mole ratio of 0.9:1.0. The

Co(II)Cbl + *Lt*PduO/UTP sample was prepared in the same way, except that excess MgUTP instead of MgATP was added to the buffer containing *Lt*PduO.

All rR spectra were collected on samples contained in NMR or EPR tubes that were submersed in either an ice bath or liquid N₂ and upon excitation with a Coherent I-305 Ar⁺ ion laser with 10–50 mW laser power at the sample. The ~135° backscattered light was dispersed by an Acton Research triple monochromator equipped with 1200 and 2400 grooves/mm gratings and analyzed with a Princeton Instruments Spec X:100BR deep depletion, back-thinned CCD camera. The HCOO[−] peak observed at 1348 cm^{−1} (with the ice peak aligned at 228 cm^{−1}) was used as an internal standard.^{51,52} Low-temperature Abs and MCD spectra were collected on a Jasco J-715 spectropolarimeter in conjunction with an Oxford Instruments SM-4000 8T magnetocryostat. All MCD spectra presented in this work were obtained by taking the difference between spectra collected with the magnetic field oriented parallel and antiparallel to the light propagation axis to remove contributions from the natural CD and glass strain.

Computational models and frequency calculations

The initial geometries of all Co(II)rrinoid models were derived from the X-ray crystal structure of Co(II)Cbl⁵³ by replacing all peripheral side chains with H atoms at a distance of 1.1 Å from adjacent C atoms, except for the methyl groups on the C5 and C15 methine bridges of the corrin ring (see Fig. 1 for the atom numbering scheme used in this study). These latter methyl groups were preserved because some corrin-based vibrational modes of interest involve significant methine-bridge movement.⁵⁴ Also, to prevent excessive flattening of the corrin ring during the geometry optimization of the Co(II)Cbl model,⁴⁹ the methyl group on the B5 position of the DMB group was maintained, while that on B6 was replaced by a H atom. In the Co(II)Cbi⁺ model, the oxygen atom of the coordinated water molecule was placed at the position originally occupied by the ligating nitrogen atom of the DMB group in the Co(II)Cbl model, while for the four-coordinate Co(II)rrinoid model the axial ligand was removed altogether. The geometries of these truncated models were optimized by performing DFT energy minimizations using the ADF 2006.01 program. The optimized structures were then subjected to analytical frequency calculations to ensure that the geometry optimizations converged to true minima of the potential energy hypersurfaces.^{55–60} In each case, the Vosko-Wilk-Nusair (VWN)⁶¹ local density approximation and the Perdew-Burke-Ernzerhof (PBE)⁶² gradient-corrected exchange and correlation functionals were employed, and the integration constant was set to 5.0. TZP (core double- ξ and valence triple- ξ with polarization) Slater-type orbital basis sets were used for all atoms and core orbitals were frozen through 1s (O, N, C) and 2p (Co). The ADF calculated eigenvector representations of the normal modes of vibration were visualized using the Jmol program.⁶³

Time-dependent DFT (TDDFT) and DFT excited-state calculations

TDDFT calculations were performed using the ORCA 2.6 program developed by Dr. Frank Neese.⁶⁴ The PBE exchange correlation functional⁶² was employed along with the RI approximation^{65–71} for accelerating the calculations. The valence double- ξ polarized Gaussian-type orbital basis set in conjunction with DeMon-J Coulomb fitting basis set were

used for all atoms except Co, for which Ahlrichs' valence triple- ξ with polarization function basis set was used.^{72,73} At least 60 excited states were calculated within an orbital energy window of ± 3 hartrees using the Tamm-Dancoff approximation.⁷⁴ On the basis of these TDDFT calculations, the corrin π_7 - and corrin π_8^* -based MOs were identified as the donor and acceptor MOs, respectively, for the “ α -band-type” transition. MOs were plotted with the gOpenMol program developed by Laaksonen using an isodensity value of 0.03 au.^{75–77}

The excited electronic configuration associated with the “ α -band-type” transition was specified using the block keyword “Occupations” in subsequent ADF calculations aimed at determining the excited-state potential-energy curves along the relevant corrin-based normal modes for the three Co(II)rrinoid models described above. In these calculations, the models were systematically distorted along the modes of interest, and for each nuclear configuration a single-point DFT excited-state calculation was performed using the same functionals and basis sets as those employed for the geometry optimizations and frequency calculations.

Results and Analysis

Abs spectra of Co(II)rrinoids

Fig. 2 shows the low-temperature Abs spectra of Co(II)Cbl and Co(II)Cbi⁺ free in solution and bound to the *L*PduO/ATP complex. In each case the dominant Abs band in the visible region is centered at ~ 470 nm (21000 cm^{-1}). This band was previously assigned to a transition with predominant corrin $\pi_7 \rightarrow$ corrin π_8^* character on the basis of spectroscopic and computational results.^{48,49} Because this same transition is responsible for the so-called α -band in the Abs spectra of Co(III)rrinoids, it is generally referred to as the “ α -band-type” transition. Previous TDDFT studies of Co(II)rrinoids revealed that the corrin π_7 -based donor MO contains a sizable contribution from the Co $3d_{z^2}$ orbital, making this orbital weakly σ -antibonding with respect to the axial ligand–Co(II) bond.⁴⁹ Because the DMB moiety is more basic than a water molecule, this σ -antibonding interaction is weaker in Co(II)Cbi⁺ than in Co(II)Cbl, and the “ α -band” of Co(II)Cbi⁺ is thus slightly blue-shifted from that of Co(II)Cbl (cf. dashed gray traces in the top and bottom panels of Fig. 2). A more pronounced blue-shift of the “ α -band” is observed upon binding of Co(II)Cbl or Co(II)Cbi⁺ to the *L*PduO/ATP complex (cf. dashed gray and solid black traces in Fig. 2), reflecting the large stabilization of the Co $3d_{z^2}$ orbital and, thus, greatly diminished contribution from this orbital to the corrin π_7 -based MO in response to the axial ligand dissociation from the Co(II) ion.

rR spectra of Co(II)rrinoids

A series of rR spectra were collected for each Co(II)rrinoid species described above using numerous Ar⁺ ion laser lines between 514.5 and 457.9 nm. Consistent with a previous report,⁴⁹ the rR excitation profiles of the corrin-based vibrational modes were found to trace the “ α -band” envelope in the corresponding Abs spectra (Supporting Information, Fig. S1). The rR spectra obtained with laser excitation at 465.8 nm, which is close in energy to the “ α -band” peak position of four-coordinate Co(II)rrinoids, are presented in Fig. 3. The rR spectrum of free Co(II)Cbl is dominated by two similarly intense features at 1482 and 1587 cm^{-1} (Fig. 3a, dashed gray trace). Our recent DFT-based analysis of the corrin-based

vibrational modes of this species revealed that the normal mode associated with the 1482 cm^{-1} feature (referred to as the symmetric $\nu_s(\#1)$ mode) primarily involves SA-stretching motion along the N22–C9 and N23–C11 bonds coupled in-phase with methine-stretching motion along the C5–C6 and C15–C14 bonds. Alternatively, the mode responsible for the 1587 cm^{-1} feature (the symmetric $\nu_s(\#3)$ mode) mainly involves LA-stretching motion along the N21–C4 and N24–C16 bonds coupled out-of-phase with the same methine-stretching motion (*vide infra*).⁴⁸

While all of the Co(II)rrinoid rR spectra presented in Fig. 3a exhibit two resonance-enhanced features at ~ 1490 and $\sim 1590 \text{ cm}^{-1}$, the relative intensities of the $\sim 1590 \text{ cm}^{-1}$ band of the four-coordinate Co(II)Cbl and Co(II)Cbi⁺ species bound to the *LtPduO*/ATP complex are greatly diminished (cf. solid black and dashed gray traces). Additionally, the frequency of the $\nu_s(\#1)$ mode varies noticeably as a function of axial ligation. From Co(II)Cbl to Co(II)Cbi⁺ and the four-coordinate Co(II)Cbl/Co(II)Cbi⁺ species, the $\nu_s(\#1)$ mode up-shifts by as much as 11 cm^{-1} (Fig. 3a). This trend reflects the decrease in charge donation from the cobalt center to the empty corrin π^* frontier orbitals upon replacement of the axial DMB ligand by a more weakly donating water molecule and the removal of the axial ligand, respectively. No such simple correlation exists between the frequency of the $\nu_s(\#3)$ mode and the electron density on the cobalt center. Specifically, removal of the axial ligand by the *LtPduO*/ATP complex results in an up-shift of the $\nu_s(\#3)$ mode of Co(II)Cbl by 5 cm^{-1} , but in a down-shift of this mode by 3 cm^{-1} in the case of Co(II)Cbi⁺. As a result, the rR spectra of four-coordinate Co(II)Cbl and Co(II)Cbi⁺ are nearly superimposable. This observation is consistent with the fact that in the X-ray crystal structure of Co(II)Cbl bound to the *LtPduO*/ATP complex,⁹ the DMB group is excluded from the enzyme active site and no longer interacting with the cobalt center.

H/D exchange at C10 causes the $\nu_s(\#1)$ mode to down-shift by $\sim 4\text{--}6 \text{ cm}^{-1}$, while the $\nu_s(\#3)$ mode is barely affected (Fig. 3b). The fact that a vibrational mode showing strong enhancement upon laser excitation into the “ α -band” is sensitive to isotopic labeling at C10 implies that significant coupling occurs between corrin SA-stretching motion and an LA-polarized electronic transition. Although it had generally been assumed that the corrin-centered vibrational modes mainly involving SA-stretching motion should only couple to SA-polarized electronic transitions,^{45,46} our recent DFT-based rR intensity calculations revealed that these modes can in fact also couple to LA-polarized electronic transitions.⁴⁸

Flanked by the rR features associated with the $\nu_s(\#1)$ and $\nu_s(\#3)$ modes, two additional features can be discerned whose intensities vary marginally as a function of laser excitation wavelength. The feature at $\sim 1530 \text{ cm}^{-1}$ was previously attributed to a symmetric mode [the $\nu_s(\#2)$ mode] involving LA-stretching motion coupled in-phase with methine-stretching motion and out-of-phase with SA-stretching motion. Despite its totally symmetric character, the $\nu_s(\#2)$ mode is only weakly enhanced upon laser excitation across the entire UV/vis region, but down-shifts upon H/D exchange at C10 due to the involvement of SA-stretching motion (Table 2). The feature at $\sim 1560 \text{ cm}^{-1}$ was assigned to an anti-symmetric vibrational mode (the ν_{as} mode) primarily involving LA-stretching motion, with out-of-phase stretching of the bonds related by the pseudo-mirror plane perpendicular to the corrin ring. Due to the

significant involvement of C10 motion, the ν_{as} mode shows the largest shift upon H/D isotopic exchange at C10 (Table 2).

DFT geometry optimizations and frequency calculations

To aid in the interpretation of the experimental data presented above, computational models of Co(II)Cbl, Co(II)Cbi⁺, and the four-coordinate Co(II)rrinoid species were generated via DFT energy minimization (Fig. 4) and subsequently subjected to analytical frequency calculations. As expected, the corrin ring in the optimized Co(II)Cbl model is considerably folded (see LA fold angle, θ , in Table 1), whereas that of the four-coordinate Co(II)rrinoid model is nearly planar due to the lack of steric interactions with an axial ligand. This prediction is consistent with the X-ray crystal structural changes observed from free Co(II)Cbl to Co(II)Cbl bound to the *LPduO*/ATP complex, which revealed that θ decreases by more than a factor of 2 upon removal of the axial DMB ligand from five-coordinate Co(II)Cbl.^{25,53}

Despite the variation in axial coordination, the DFT-computed eigenvector descriptions of the corrin-based normal modes are virtually identical for all three Co(II)rrinoid models (Fig. 5). Four corrin-based vibrational modes, including three totally symmetric vibrational modes in the parent C_{2v} point group, corresponding to ν_s (#1~3), and an anti-symmetric vibrational mode, ν_{as} , are predicted in the 1480–1600 cm^{-1} range (Fig. 3a and Table 2). Experimentally, the frequency of the ν_s (#1) mode increases with increasing positive charge on the cobalt center due to the consequent decrease in charge donation from the cobalt center to the empty corrin π^* frontier orbitals (*vide supra*). Consistent with this experimental trend, the computed frequency of the ν_s (#1) mode is larger for the four-coordinate Co(II)rrinoid model than for the five-coordinate models, though DFT predicts a higher frequency for the Co(II)Cbl model than for the Co(II)Cbi⁺ model. The DFT-predicted isotopic shifts for the ν_s (#1) mode upon H/D exchange at C10 are in excellent agreement with the experimentally observed shifts (Table 2).

The computed frequencies for the ν_s (#2) and ν_{as} modes also agree well with our experimental values (Table 2). The ν_{as} mode mainly involves LA-stretching motion whereby atoms belonging to one half of the corrin ring move out-of-phase relative to their counterparts belonging to the other half. Consistent with the anti-symmetric nature of this mode, it carries substantial “off-resonance” intensity in the experimental rR spectra (Supporting Information, Fig. S1). The large contribution from C10 motion to the ν_{as} mode leads to a sizable H/D isotope shift computed for this mode, which agrees well with the experimental observation that the ν_{as} mode displays the largest shift upon H/D exchange at C10 (Table 2). Lastly, the computations predict nearly identical frequencies and negligible H/D isotope shifts for the ν_s (#3) mode of all three Co(II)rrinoid models, consistent with our experimental data. Overall, the good agreement between the experimentally observed and computationally predicted frequencies and isotope shifts for the relevant corrin-centered normal modes lends support to our DFT-based normal-mode analysis for the different Co(II)rrinoids.

TDDFT and DFT excited-state calculations

While the computed eigenvector representations of the three ν_s modes vary only slightly as a function of axial ligation, the experimental rR spectra of four-coordinate Co(II)rrinoids bound to the *L*PduO/ATP complex show a significantly diminished intensity of the ν_s (#3) mode relative to that of the ν_s (#1) mode. To explore the origin of this puzzling result, TDDFT and single-point DFT excited-state calculations were carried out for all three Co(II)rrinoid models.

Because the methyl groups at C5, C15, and B5 are expected to affect the vibrational frequencies of the corrin-centered normal modes, they were included in our Co(II)rrinoid models.⁴⁸ However, because these groups contribute minimally to the corrin-based frontier MOs, the TDDFT-computed Abs spectra for our Co(II)Cbl and Co(II)Cbi⁺ models (Supporting Information, Figure S2) are nearly identical to those reported previously for models lacking the peripheral methyl groups.⁴⁹ The most intense band in the visible region of the computed Abs spectra for all three Co(II)rrinoid models considered in this study arises from the “ α -band-type” transition. The donor MO involved in this transition, the corrin π_7 -based MO, is symmetric with respect to reflection about the pseudo-mirror plane oriented along the SA, while the acceptor MO, the corrin π_8^* -based MO, is anti-symmetric (Fig. 6, left). Therefore, the “ α -band-type” transition is LA-polarized.

The experimental rR excitation profiles for both the ν_s (#1) and ν_s (#3) modes of Co(II)rrinoids roughly follow the Abs envelope in the region of the LA-polarized “ α -band-type” transition (Supporting Information, Figure S1). To compute the relative rR intensities of the three ν_s modes for excitation in resonance with this transition, the displacement () of the corresponding excited state from the ground state along these modes was estimated by systematically distorting the computational models along each ν_s mode and performing DFT calculations for the distorted models in the excited-state associated with the corrin $\pi_7 \rightarrow \pi_8^*$ transition. Since in ADF the eigenvectors of all normal modes are expressed in terms of normalized displacements of the atoms in non-mass-weighted Cartesian coordinates, the distorted models were generated by adding a variable fraction of the ν_s normal mode displacements to the optimized ground-state geometry in Cartesian coordinates. The excited-state potential-energy curves obtained using this approach are shown in Fig. 6.⁷⁸ Note that the values (Table 3) are not dimensionless; rather, they correspond to the fraction of the normal mode eigenvectors added to the ground-state geometry in Cartesian coordinates.

In agreement with our previous vibrational analysis of the normal modes of free Co(II)Cbl,⁴⁸ the excited state associated with the “ α -band-type” (corrin $\pi_7 \rightarrow \pi_8^*$) transition is displaced more substantially along the ν_s (#1) mode than along the other ν_s modes, regardless of the identity of the axial ligand (Table 3). This computationally predicted coupling between the ν_s (#1) mode, which primarily involves SA-stretching motion, and an LA-polarized electronic transition explains the puzzling observation that the rR band associated with the ν_s (#1) mode is enhanced upon laser excitation into “the α -band” while at the same time it down-shifts upon H/D exchange at C10.

The relative rR intensities of the other ν_s modes were estimated using the semi-classical theory of Shorygin^{79,80} for the A-term intensity, $I \propto \Delta^2 \bar{\nu}^{-4}$ (thus, we assumed that the ratio of values is unaffected on going from dimensionless units to fractions of normal mode displacements in Cartesian coordinates, which is reasonable because the same atoms are involved in the vibrations of interest). While the intensity of the $\nu_s(\#2)$ mode relative to that of the $\nu_s(\#1)$ mode, I_2/I_1 , is correctly predicted for the Co(II)Cbi⁺ and four-coordinate Co(II)rrinoid models, it is overestimated for Co(II)Cbl, primarily because of the small $|z_1|$ value predicted for this model (Table 3). The I_3/I_1 intensity ratio is underestimated for all three Co(II)rrinoid models, but most significantly for Co(II)Cbi⁺, as for this model the ground and excited states are predicted to be minimally displaced along $\nu_s(\#3)$ (i.e., $|z_3| \approx 0$).

In the experimental rR spectra (Fig. 3), the intensities of the corrin-based modes of the four-coordinate Co(II)rrinoid species bound to the *LrPduO*/ATP complex are considerably different from those observed for their unbound, five-coordinate counterparts. In particular, the I_3/I_1 ratio decreases by a factor of ~ 2 upon binding of Co(II)Cbl or Co(II)Cbi⁺ to the enzyme active site. Yet, the DFT-predicted I_3/I_1 intensity ratio is substantially larger for the four-coordinate Co(II)rrinoid model than for both of the five-coordinate models. This discrepancy may be rationalized in two ways. First, it is conceivable that the computational approach employed in this study to calculate the relative rR intensities of the corrin-based normal modes is not sufficiently accurate to replicate the experimentally observed variations among the different Co(II)rrinoid species. This seems unlikely, though, considering that we successfully used the same approach to reproduce the key experimental trends regarding the frequencies and relative rR intensities of the corrin-based modes from CNCbl to Co(II)Cbl and Co(I)Cbl⁻.⁴⁸ Second, it is plausible that our four-coordinate Co(II)rrinoid model does not accurately mimic the actual conformation of the enzyme-bound Co(II)rrinoid species in the *LrPduO*/ATP complex. In this scenario, the diminished I_3/I_1 intensity ratio and, thus, $|z_3|/|z_1|$ ratio reflect a conformational change of the coring ring imposed by hydrogen-bonding, electrostatic, and/or hydrophobic interactions with amino acid residues in the enzyme active site. To discriminate between these two possibilities, we used MCD spectroscopy as a complementary probe of the corrin ring conformation of Co(II)rrinoids bound to the active site of *LrPduO*.

Corrin ring perturbations in the active site of *LrPduO*

The MCD spectrum of Co(II)Cbl in the presence of *LrPduO* incubated with the ATP analogue uridine-5'-triphosphate (UTP) lacks the features characteristic of four-coordinate Co(II)Cbl species (Fig. 7a). However, in the region of the corrin $\pi \rightarrow \pi^*$ transitions ($\sim 20,000 - 30,000 \text{ cm}^{-1}$), this spectrum is much more similar to that of the four-coordinate Co(II)Cbl species bound to the *LrPduO*/ATP complex than the MCD spectra of Co(II)Cbl free in solution and in the presence of *LrPduO* devoid of nucleotide [which is unable to bind Co(II)Cbl].¹⁸ These similarities suggest that the MCD spectral changes in the region of the corrin $\pi \rightarrow \pi^*$ transitions observed upon Co(II)Cbl binding to the *LrPduO*/ATP complex primarily reflect perturbations to the corrin ring conformation induced by non-bonded interactions with active site residues, rather than the dissociation of the axial DMB ligand.

To test this hypothesis, we also collected rR spectra for the sample of Co(II)Cbl in the presence of the *LPduO*/UTP complex. As shown in Fig. 7b, the intensity of the $\nu_s(\#3)$ mode relative to that of the $\nu_s(\#1)$ mode is substantially lower in the rR spectrum of Co(II)Cbl + *LPduO*/UTP than in the free Co(II)Cbl spectrum, indicating that a significant decrease in the $| \nu_3 / \nu_1 |$ ratio occurs upon Co(II)Cbl binding to the *LPduO*/UTP complex (though not quite as large as in the case of Co(II)Cbl + *LPduO*/ATP). Yet, the frequency of the $\nu_s(\#3)$ mode is virtually unaffected when Co(II)Cbl binds to *LPduO*/UTP while the $\nu_s(\#1)$ mode is up-shifted by a mere 2 cm^{-1} , providing further evidence that Co(II)Cbl retains the axial DMB ligand in the active site of the *LPduO*/UTP complex. Thus, we conclude that the unusually small $| \nu_3 / \nu_1 |$ ratio observed for the four-coordinate Co(II)Cbl species generated in the active site of the *LPduO*/ATP complex stems primarily from perturbations imposed on the corrin ring, rather than the dissociation of the axial ligand.

Discussion

Corrin-centered normal modes of Co(II)rrinoids

Two symmetric corrin-centered vibrations, the $\nu_s(\#1)$ and $\nu_s(\#3)$ modes, are simultaneously enhanced in the rR spectra of Co(II)rrinoids obtained with laser excitation into the “ α -band”. The $\nu_s(\#1)$ mode down-shifts upon H/D exchange at C10, indicating that this mode primarily entails C–C/N stretching motions along the corrin SA (Fig. 5). This coupling between a vibrational mode mainly involving SA-stretching motion and an LA-polarized electronic transition is reproduced quite well by our DFT-assisted rR intensity calculations (Table 3). The rR enhancement patterns of the corrin-based modes of Co(II)rrinoids in the region of the “ α -band” are strikingly different from those observed for Co(III)rrinoids and Co(I)rrinoids, even though the eigenvector representations of the ν_s modes and the compositions of the corrin π - and π^* -based MOs involved in the “ α -band-type” transition differ marginally among these species.⁴⁸ This variation in the degree of coupling between corrin ring vibrations and corrin $\pi \rightarrow \pi^*$ transitions as a function of the Co oxidation state reflects the changes in the extent of mixing between the Co 3d valence orbitals and corrin frontier orbitals that occur from Co(III)- to Co(II)- and Co(I)rrinoids. With decreasing cobalt oxidation state, the filled Co 3d orbitals shift closer in energy to the empty corrin π^* frontier orbitals, which leads to an increased mixing between these orbitals. Because in the lowest-energy corrin π^* -based MOs the Co ion and N atoms of the corrin ring engage in π -antibonding interactions, the way by which the corrin ring distorts upon population of these MOs in electronic excited states will depend on the extent of Co 3d $\rightarrow$ corrin π^* “backbonding”, which increases from Co(III)- to Co(II)- and Co(I)rrinoids. It is due to this delicate interplay between bonding and antibonding interactions between the Co 3d and corrin π/π^* frontier orbitals that the direction of the corrin ring distortion in the excited state corresponding to the “ α -band-type” transition varies as a function of the Co oxidation state.⁴⁸

Spectral probes of corrin ring distortions and axial ligation changes in Co(II)rrinoids

The frequency of the $\nu_s(\#1)$ mode of corrinoids decreases with decreasing formal oxidation state of the cobalt center; thus, this mode is observed at lower frequencies for Co(II)rrinoids than for Co(III)rrinoids. In the case of Co(II)rrinoids, even relatively subtle changes in the

electron density on the Co(II) center caused by axial ligand substitutions have a noticeable effect on the frequency of the $\nu_s(\#1)$ mode [i.e., $\text{Co(II)Cbl} < \text{Co(II)Cbi}^+ < \text{four-coordinate Co(II)Cbl}$ and Co(II)Cbi^+ , Table 2]. Therefore, rR spectroscopy can be used as a probe of axial ligand–Co bonding interactions in Co(II)rrinoids to complement the information obtained from EPR and MCD studies.

No obvious correlation exists between the charge on the Co(II) ion and the frequency of the $\nu_s(\#3)$ mode. The DFT computed frequencies of the $\nu_s(\#3)$ mode for the three different Co(II)rrinoid models are nearly identical (Table 2), suggesting that the small differences observed experimentally from Co(II)Cbl to Co(II)Cbi⁺ and the four-coordinate Co(II)rrinoid species are not a direct consequence of the changes in axial ligation. Indeed, our rR spectra obtained for Co(II)Cbl bound to the *LrPduO*/ATP and *LrPduO*/UTP complexes reveal that the frequency of the $\nu_s(\#3)$ mode is sensitive to changes in the corrin ring conformation; i.e., in both spectra the $\nu_s(\#3)$ mode is observed at $\sim 1590\text{ cm}^{-1}$ despite the differences in the Co(II) coordination number (4 and 5, respectively). These spectra also show that the intensity of the $\nu_s(\#3)$ mode relative to that of the $\nu_s(\#1)$ mode decreases substantially upon Co(II)Cbl binding to the *LrPduO* active site, even when the axial DMB ligand is retained. The diminished relative rR intensity of the $\nu_s(\#3)$ mode is accompanied by significant changes of the MCD features associated with the corrin $\pi \rightarrow \pi^*$ transitions, in support of our hypothesis that the frequency and relative intensity of the $\nu_s(\#3)$ mode are modulated by changes in the corrin ring conformation.

As expected, the frequency of the $\nu_s(\#1)$ mode remains essentially unchanged when Co(II)Cbl binds to the *LrPduO*/UTP complex, because the axial DMB ligand is retained in this process (Fig. 7a). Likewise, minimal differences in the region dominated by LF transitions are observed between the MCD spectra of Co(II)Cbl free in solution and bound to the *LrPduO*/UTP complex (Fig. 7b), and the EPR spectra obtained for these two species are almost identical (Supplementary material, Fig. S3 and Table S1). These findings indicate that the relative energies of the Co 3d-based MOs in five-coordinate Co(II)Cbl are insignificantly affected by changes in the corrin conformation and confirm that the $\nu_s(\#1)$ and $\nu_s(\#3)$ modes provide selective probes of perturbations to the axial ligand–Co(II) bonding interaction and the corrin ring conformation, respectively.

Corrin ring conformation of Co(II)rrinoids in the active site of *LrPduO*

Our rR and MCD data provide evidence for a distorted corrin ring conformation in the four-coordinate and five-coordinate Co(II)Cbl species bound to the *LrPduO*/ATP and *LrPduO*/UTP complexes, respectively (Fig. 7). Because of the largely diminished $| \nu_3 / \nu_1 |$ ratios observed for these species, we conclude that the corrin ring is primarily distorted along the $\nu_s(\#3)$ mode, which will reduce the displacement of the ground-state potential relative to the excited-state potential associated with the “ α -band-type” transition along this mode. In support of this conclusion, inspection of the X-ray crystal structure of Co(II)Cbl bound to the active site of the *LrPduO*/MgATP complex²⁵ (Fig. 8) reveals that three of the four edges of the corrin ring are in close contact with active site residues, while the remaining edge that connects the A and D pyrrole rings (Fig. 1) faces the substrate access channel. Thus, the active site can modulate the conformation of the corrin ring along the LA,

but not along the SA. Because the $\nu_s(\#1)$ and $\nu_s(\#3)$ modes primarily involve C–C/N stretching motions along the SA and LA of the corrin ring, respectively, only the latter is significantly affected by Co(II)rrinoid binding to the *LrPduO* active site.

Although the unique conformation of the corrin ring imposed by the enzyme active site may aid in the conversion of five- to four-coordination Co(II)rrinoids, probably the most important finding from this study with regards to the mechanism of substrate activation by *LrPduO* is that certain residues in the enzyme active site serve to lock the corrin ring in place, as evidenced by the unusually small $| \nu_3 / \nu_1 |$ ratios and perturbed corrin $\pi \rightarrow \pi^*$ transitions observed for Co(II)Cbl bound to the *LrPduO*/ATP and *LrPduO*/UTP complexes (Fig. 7). This tight binding of the corrin ring is expected to be critical for preventing the Co(II)rrinoid substrate from repositioning itself while retaining the axial ligand when the side chain of F112 moves into the position originally occupied by the DMB and H₂O ligands of Co(II)Cbl and Co(II)Cbi⁺, respectively. In support of this proposal, we have recently shown that alanine substitutions of active site residues located in the plane of the corrin ring drastically lower the relative yield of four-coordinate Co(II)rrinoid formation achieved by the *LrPduO*/ATP complex.³⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Science Foundation grant MCB-0238530 (to T.C.B.), the National Institutes of Health grant R37-GM40313 (to J.C.E.-S.), the National Research Foundation of Korea NRF-2015R1C1A1A02036917 and Korea Institute of Science and Technology Information KSC-2015-C2-044 (to K.P.). P.E.M was supported in part by Chemical Biology Interface Training Grant T32-GM008505 (L. L. Kiessling, P. I.) from the National Institute of General Medical Sciences (NIGMS).

References

- (1). Banerjee R, Ragsdale SW. Annual Review of Biochemistry. 2003; 72:209.
- (2). Bouquiere JP, Finney JL, Lehmann MS, Lindley PF, Savage HF. J. Acta. Crystallogr. Sect. B-Struct. Commun. 1993; 49:79.
- (3). Gerfen GJ, Licht S, Willems JP, Hoffman BM, Stubbe J. J. Am. Chem. Soc. 1996; 118:8192.
- (4). Sintchak MD, Arjara G, Kellogg BA, Stubbe J, Drennan CL. Nat Struct Mol Biol. 2002; 9:293.
- (5). Crona M, Hofer A, Astorga-Wells J, Sjöberg B-M, Tholander F. PloS one. 2015; 10:e0134293. [PubMed: 26225432]
- (6). Marsh ENG, Ballou DP. Biochemistry. 1998; 37:11864. [PubMed: 9718309]
- (7). Yoon M, Kalli A, Lee H-Y, Håkansson K, Marsh ENG. Angew. Chem. Int. Ed. 2007; 46:8455.
- (8). Johnson CLV, Pechonick E, Park SD, Havemann GD, Leal NA, Bobik TA. Journal of Bacteriology. 2001; 183:1577. [PubMed: 11160088]
- (9). Toraya T. Cell. Mol. Life Sci. 2000; 57:106. [PubMed: 10949584]
- (10). Wei XQ, Meng XL, Chen YL, Wei YT, Du LQ, Huang RB. Biotechnol. Lett. 2014; 36:159. [PubMed: 24078133]
- (11). Toraya T. Arch. Biochem. Biophys. 2014; 544:40. [PubMed: 24269950]
- (12). Buan NR, Suh S-J, Escalante-Semerena JC. Journal of Bacteriology. 2004; 186:5708. [PubMed: 15317775]
- (13). Robertson WD, Wang M, Warncke K. J. Am. Chem. Soc. 2011; 133:6968. [PubMed: 21491908]

- (14). Jones AR, Rentergent J, Scrutton NS, Hay S. Chem.-Eur. J. 2015; 21:8826. [PubMed: 25950663]
- (15). Chowdhury S, Banerjee R. Biochemistry. 2000; 39:7998. [PubMed: 10891081]
- (16). Yabuta Y, Kamei Y, Bito T, Arima J, Yoneda K, Sakuraba H, Ohshima T, Nakano Y, Watanabe F. Biosci. Biotechnol. Biochem. 2015; 79:710. [PubMed: 25516375]
- (17). Conrad KS, Jordan CD, Brown KL, Brunold TC. Inorg. Chem. 2015; 54:3736. [PubMed: 25839944]
- (18). Larsson KM, Logan DT, Nordlund P. ACS Chem. Biol. 2010; 5:933. [PubMed: 20672854]
- (19). Banerjee R. Biochemistry. 2001; 40:123.
- (20). Marsh ENG, Patterson DP, Li L. ChemBioChem. 2010; 11:604. [PubMed: 20191656]
- (21). Frey PA. Acc. Chem. Res. 2014; 47:540. [PubMed: 24308628]
- (22). Dowling DP, Croft AK, Drennan CL. Annu. Rev. Biophys. 2012; 41:403. [PubMed: 22577824]
- (23). Toraya T. Chem. Rev. 2003; 103:2095. [PubMed: 12797825]
- (24). Santos F, Vera JL, van der Heijden R, Valdez G, de Vos WM, Sesma F, Hugenholtz J. Microbiology. 2008; 154:81. [PubMed: 18174128]
- (25). St. Maurice M, Mera P, Park K, Brunold TC, Escalante-Semerena JC, Rayment I. Biochemistry. 2008; 47:5755. [PubMed: 18452306]
- (26). Lexa D, Saveant JM. Acc. Chem. Res. 1983; 16:235.
- (27). Sykes GA, Rogers LJ. Biochem. J. 1984; 217:845. [PubMed: 6424653]
- (28). Mera PE, Escalante-Semerena JC. Journal of Biological Chemistry. 2010; 285:2911. [PubMed: 19933577]
- (29). Fonseca MV, Escalante-Semerena JC. Journal of Biological Chemistry. 2001; 276:32101. [PubMed: 11408479]
- (30). Moore TC, Mera PE, Escalante-Semerena JC. Journal of bacteriology. 2014; 196:903. [PubMed: 24336938]
- (31). Stich TA, Buan NR, Escalante-Semerena JC, Brunold TC. J. Am. Chem. Soc. 2005; 127:8710. [PubMed: 15954777]
- (32). Pallares IG, Moore TC, Escalante-Semerena JC, Brunold TC. Biochemistry. 2014; 53:7969. [PubMed: 25423616]
- (33). Stich TA, Yamanishi M, Banerjee R, Brunold TC. J. Am. Chem. Soc. 2005; 127:7660. [PubMed: 15913339]
- (34). Park K, Mera PE, Escalante-Semerena JC, Brunold TC. Biochemistry. 2008; 47:9007. [PubMed: 18672897]
- (35). Park K, Mera PE, Moore TC, Escalante-Semerena JC, Brunold TC. Angew. Chem. Int. Ed. 2015; 54:7158.
- (36). Pallares IG, Moore TC, Escalante-Semerena JC, Brunold TC. J. Am. Chem. Soc. 2016; 138:3694. [PubMed: 26886077]
- (37). Mera PE, St. Maurice M, Rayment I, Escalante-Semerena JC. Biochemistry. 2007; 46:13829. [PubMed: 17988155]
- (38). Park K, Mera PE, Escalante-Semerena JC, Brunold TC. Inorg. Chem. 2012; 51:4482. [PubMed: 22480351]
- (39). Dong SL, Padmakumar R, Maiti N, Banerjee R, Spiro TG. J. Am. Chem. Soc. 1998; 120:9947.
- (40). Dong SL, Padmakumar R, Banerjee R, Spiro TG. J. Am. Chem. Soc. 1999; 121:7063.
- (41). Huhta MS, Chen HP, Hemann C, Hille CR, Marsh ENG. Biochem. J. 2001; 355:131. [PubMed: 11256957]
- (42). George WO. Applied Spectroscopy. 1973; 27:390.
- (43). Mayer E, Gardiner DJ, Hester RE. Journal of the Chemical Society, Faraday Transactions. 1973; 69:1350.
- (44). Mayer E, Gardiner DJ, Hester RE. Molecular Physics. 1973; 26:783.
- (45). Puckett JM, Mitchell MB, Hirota S, Marzilli LG. Inorg. Chem. 1996; 35:4656.
- (46). Salama S, Spiro TG. Journal of Raman Spectroscopy. 1977; 6:57.
- (47). Andruniow T, Zgierski MZ, Kozlowski PM. Journal of Physical Chemistry A. 2002; 106:1365.

- (48). Park K, Brunold TC. The Journal of Physical Chemistry B. 2013; 117:5397. [PubMed: 23477417]
- (49). Stich TA, Buan NR, Brunold TC. J. Am. Chem. Soc. 2004; 126:9735. [PubMed: 15291577]
- (50). Hill H, Pratt J, Williams R. Journal of the Chemical Society (Resumed). 1965:2859.
- (51). Chou IM, Blank JG, Goncharov AF, Mao HK, Hemley RJ. Science. 1998; 281:809. [PubMed: 9694649]
- (52). Heyns AM, Range KJ; Muller K. Canadian Journal of Chemistry-Revue Canadienne De Chimie. 1991; 69:1774.
- (53). Krautler B, Keller W, Kratky C. J. Am. Chem. Soc. 1989; 111:8936.
- (54). Liptak MD, Brunold TC. J. Am. Chem. Soc. 2006; 128:9144. [PubMed: 16834388]
- (55). SCM, Theoretical Chemistry. Vrije Universiteit; Amsterdam, The Netherlands: ADF2006.01; <http://www.scm.com>
- (56). Berces A, Dickson RM, Fan LY; Jacobsen H, Swerhone D, Ziegler T. Computer Physics Communications. 1997; 100:247.
- (57). Guerra CF, Snijders JG, te Velde G, Baerends EJ. Theoretical Chemistry Accounts. 1998; 99:391.
- (58). Jacobsen H, Berces A, Swerhone DP, Ziegler T. Computer Physics Communications. 1997; 100:263.
- (59). Velde GT, Bickelhaupt FM, Baerends EJ, Guerra CF, Van Gisbergen SJA, Snijders JG, Ziegler T. Journal of Computational Chemistry. 2001; 22:931.
- (60). Wolff SK. International Journal of Quantum Chemistry. 2005; 104:645.
- (61). Vosko SH, Wilk L, Nusair M. Canadian Journal of Physics. 1980; 58:1200.
- (62). Perdew JP, Burke K, Ernzerhof M. Physical Review Letters. 1996; 77:3865. [PubMed: 10062328]
- (63). Jmol: an open-source Java viewer for chemical structures in 3D. <http://www.jmol.org/>
- (64). Neese, F. 2.6 revision 4 ed. Institut fuer Physikalische und Theoretische Chemie, Universitaet Bonn; Germany: Jul. 2007
- (65). Baerends E, Ellis D, Ros P. Chemical Physics. 1973; 2:41.
- (66). Dunlap BI, Connolly J, Sabin J. The Journal of Chemical Physics. 1979; 71:3396.
- (67). Eichkorn K, Treutler O, Öhm H, Häser M, Ahlrichs R. Chemical physics letters. 1995; 240:283.
- (68). Eichkorn K, Weigend F, Treutler O, Ahlrichs R. Theoretical Chemistry Accounts. 1997; 97:119.
- (69). Kendall RA, Früchtl HA. Theoretical Chemistry Accounts. 1997; 97:158.
- (70). Van Alsenoy C. Journal of computational chemistry. 1988; 9:620.
- (71). Whitten JL. Journal of Chemical Physics. 1973; 58:4496.
- (72). Godbout N, Salahub DR, Andzelm J, Wimmer E. Canadian Journal of Chemistry-Revue Canadienne De Chimie. 1992; 70:560.
- (73). Schafer A, Horn H, Ahlrichs R. Journal of Chemical Physics. 1992; 97:2571.
- (74). Hirata S, Head-Gordon M. Chemical Physics Letters. 1999; 314:291.
- (75). Bergman DL, Laaksonen L, Laaksonen A. Journal of Molecular Graphics & Modelling. 1997; 15:301. [PubMed: 9640561]
- (76). Laaksonen L. Journal of Molecular Graphics. 1992; 10:33. [PubMed: 1504051]
- (77). Laaksonen, L. 3.00 ed. Center for Scientific Computing, Espoo; Finland: 2005.
- (78). Neese F, Petrenko T, Ganyushin D, Olbrich G. Coordination Chemistry Reviews. 2007; 251:288.
- (79). Shorygin PP. Dokl. Akad. Nauk. SSSR. 1952; 87:201.
- (80). Shorygin PP. Dokl. Akad. Nauk. SSSR Ser. Fiz. 1953; 17:58.

Figure 1.

Chemical structure of cob(II)alamin [Co(II)Cbl]. In cob(II)inamide [Co(II)Cbi⁺], the nucleotide loop with its DMB tail is absent and the Co(II) center is axially ligated by a water molecule. In AdoCbl, an Ado moiety is bound axially to the central Co(III) ion trans to the DMB group.

Figure 2.

Electronic absorption spectra at 4.5 K of free Co(II)Cbl (top, dashed gray), Co(II)Cbl bound to the *LtPduO/ATP* complex (top, solid black), free Co(II)Cbi⁺ (bottom, dashed gray), and Co(II)Cbi⁺ bound to the *LtPduO/ATP* complex (bottom, solid black). The laser excitation wavelength (465.8 nm) used for collecting the rR spectra presented in Figure 3 is indicated by an arrow.

Figure 3.

(a) rR spectra of free Co(II)Cbl (dashed gray, top), Co(II)Cbl + *Lt*PduO/ATP (solid black, top), free Co(II)Cbi⁺ (dashed gray, bottom), and Co(II)Cbi⁺ + *Lt*PduO/ATP (solid black, bottom). (b) rR spectra of Co(II)Cbl (solid black, top), Co(II)Cbl(*d-10*) (dashed gray, top), Co(II)Cbi⁺ (solid black, top) and Co(II)Cbi⁺(*d-10*) (dashed gray, bottom). All spectra were obtained with 465.8 nm laser excitation.

Figure 4. Geometry-optimized computational models of **(a)** Co(II)Cbl, **(b)** Co(II)Cbi⁺, and **(c)** the four-coordinate Co(II)rrinoid species.

Figure 5. DFT-computed vector representations of the relevant corrin-centered normal modes of Co(II)Cbl (top), Co(II)Cbi⁺ (middle), and four-coordinate Co(II)rrinoid (bottom).

Figure 6.

Results from TDDFT and single-point DFT excited-state calculations used to estimate the values for four-coordinate Co(II)rrinoids. **(a)** Experimental (red) and computational (solid black) Abs spectra of four-coordinate Co(II)rrinoids. The calculated LA- and SA-polarized contributions to the Abs envelope are shown by dashed black and gray lines, respectively. **(b)** Molecular orbitals involved in the LA-polarized “ α -bandtype” (corrin $\pi_7 \rightarrow \pi_8^*$) transition. **(c)** Computed potential-energy curves of the ground state and excited state associated with the corrin $\pi_7 \rightarrow \pi_8^*$ transition for the four-coordinate Co(II)rrinoid model along the ν_s (#1) mode (yellow triangles), the ν_s (#2) mode (green circles), and the ν_s (#3) mode (blue squares).

Figure 7.

(a) MCD spectra collected at 7 T and 4.5 K and (b) rR spectra obtained at 77 K upon 465.8 nm laser excitation of free Co(II)Cbl (solid gray), five-coordinate Co(II)Cbl in the presence of *L*PduO/UTP (solid black), and four-coordinate Co(II)Cbl in the presence of *L*PduO/ATP (dashed gray). The major spectral changes in the region of the corrin $\pi \rightarrow \pi^*$ transitions that occur upon binding of Co(II)Cbl to *L*PduO complexed with ATP or UTP are indicated by arrows.

Figure 8.
X-ray crystal structure of Co(II)Cbl bound to the active site of the *LrPduO*/MgATP complex (PDB code: 3CI1⁹).

Table 1

Corrin fold angles and axial ligand bond distances for various Co(II)rrinoid species predicted computationally and observed by X-ray crystallography

	Method	θ ($^{\circ}$) ^a	ϕ ($^{\circ}$) ^b	Co–Axial ligand
Co(II)Cbl, Fig. 4a	DFT	10.4	8.0	2.21 Å
Co(II)Cbi ⁺ , Fig. 4b	DFT	4.5	4.6	2.33 Å
Four-coordinate Co(II)rrinoid, Fig. 4c	DFT	1.8	1.0	
free Co(II)Cbl ^c	X-ray	16.2	3.6	1.93 Å
Co(II)Cbl + <i>Lt</i> PduO/ATP ^d	X-ray	7.3	10.8	

^a θ corresponds to the LA fold angle and is defined as the angle between the plane that is composed of N21, C4, C5, C6, and N22 and the vector connecting the Co center and C15.

^b ϕ corresponds to the SA fold angle and is defined as the angle between the plane that is composed of N22, C9, C10, C11, and N23 and the vector connecting the Co center and the midpoint between C1 and C19.

^cFrom ref 33.

^dFrom ref 9.

Table 2

Experimental and DFT-computed frequencies (in cm^{-1}) of the relevant corrin-based vibrational modes depicted in Fig. 5. The isotopic shifts for H/D exchange at C10 are given in parentheses; their estimated error is $\pm 1 \text{ cm}^{-1}$.

		ν_s (#1)	ν_s (#2)	ν_{as}	ν_s (#3)
Free Co(II)Cbl	Exp.	1482 (– 6)	1529 (– 3)	1555 (– 9)	1587 (– 2)
	Calc.	1487.4 (– 4.9)	1530.0 (– 2.3)	1556.8 (– 5.7)	1584.9 (– 0.6)
Free Co(II)Cbi ⁺	Exp.	1486 (– 4)	1533 (– 6)	1564 (– 6)	1594 (0)
	Calc.	1485.2 (– 4.7)	1528.1 (– 2.0)	1557.1 (– 5.5)	1584.2 (– 0.9)
Four-coordinate Co(II)rrinoids	Co(II)Cbl	1493	1533	1561	1592
	Co(II)Cbi ⁺	1491	1535	1564	1591
	Calc.	1490.6 (– 5.0)	1528.1 (– 2.2)	1558.2 (– 5.1)	1584.6 (– 1.1)

Table 3

Calculated displacements δ_n of the excited state associated with the LA-polarized “ α -band-type” transition along the $\nu_s(\#n)$ modes ($n=1, 2, 3$) and relative rR intensities of the $\nu_s(\#n)$ modes obtained using the relationship $I_n \propto \delta_n^2 \nu_s(\#n)^4$, along with the corresponding experimental intensity ratios

Species	1	2	3	$I_1 : I_2 : I_3$	
				Calc.	Exp.
Co ²⁺ Cbl	0.023	0.014	− 0.012	100 : 41 : 35	86 : 16 : 100
Co ²⁺ Cbi ⁺	0.031	0.015	0.001	100 : 26 : 0	100 : 24 : 60
Four-coordinate	0.030	0.013	− 0.020	100 : 21 : 57	100: 11 : 43 (Cbl)
Co ²⁺ corrinoide					100 : 18 : 39 (Cbi ⁺)