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X-ray Absorption Spectroscopy of the Corrinoid/Iron–Sulfur Protein Involved in Acetyl Coenzyme A Synthesis by *Clostridium thermoaceticum*

Michael D. Wirt,[†] Manoj Kumar,[‡] Stephen W. Ragsdale,[*,‡] and Mark R. Chance[*,†]

Contribution from the Department of Physiology and Biophysics, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, New York 10461, and Department of Biochemistry, East Campus, University of Nebraska, Lincoln, Nebraska 68583-0718. *Received September 25, 1992*

Abstract: The corrinoid/iron–sulfur protein (C/Fe-SP) from *Clostridium thermoaceticum* is an 88 kDa $\alpha\beta$-dimer that cycles between a Co(I) and methyl-Co(III) form as it transfers a methyl group from methyltetrahydrofolate (methyl-H$_4$folate) to carbon monoxide dehydrogenase. Extended X-ray absorption fine structure (EXAFS) and X-ray edge spectroscopy of the as-isolated C/Fe-SP indicates that the inactive Co(II) state of the corrinoid has a four-coordinate distorted square-planar structure. To our knowledge, this is the first observation of a four-coordinate Co(II) corrinoid. This unusual coordination state would be expected to poise the Co(II) state of the C/Fe-SP for facile reduction to four-coordinate Co(I), which is the active nucleophile that acts as the acceptor of the methyl group of methyl-H$_4$folate. Additionally, the first-derivative X-ray edge spectra for both the methyl-Co(III) and Co(II) forms of the C/Fe-SP show an unexpected shift to lower energy when compared to analogous free cobalamins indicating a potential role for the protein in mediating electronic as well as structural properties of the enzyme bound methylcobamide.

Introduction

The B_{12}-cofactor, methylcobalamin (MeCbl, Figure 1), is known to participate in enzyme reactions involving methyl group transfers from methyltetrahydrofolate (methyl-H$_4$folate) to the cobalamin with subsequent transfers to homocysteine in methionine synthase[1] and to carbon monoxide dehydrogenase (CODH) for the synthesis of acetyl-CoA by *Clostridium thermoaceticum*.[2] Early observations by Poston et al.[3] and Ljungdahl et al.[4] recognized that a corrinoid or corrinoid protein was involved in acetate biosynthesis by acetogenic bacteria. For the acetogenic bacterium, *C. thermoaceticum*, which completely converts sugars as well as several one-carbon compounds to acetate (via acetyl-CoA as an intermediate), the methyl-corrinoid protein is required (see ref 5 for a review).

The corrinoid/iron–sulfur protein (C/Fe-SP) from *C. thermoaceticum* is an 88-kDa $\alpha\beta$-dimer containing a cobalt corrinoid that is methylated by methyl-H$_4$folate to form base-off methyl-Co(III) cobamide in a reaction catalyzed by methyltransferase.[6] The C/Fe-SP was purified to homogeneity by Ragsdale et al., who identified two redox-active metal sites: 5-methoxybenzimidazoylcobamide and a [4Fe-4S]$^{2+/+}$ cluster.[7] The cobamide was found to be in the unusual base-off state in both the methyl-Co(III) and Co(II) forms.[7] Base-off cobamides are generally observed only at pH values below 3.0.[8] The (II)/(I) redox couples for the cobamide and the cluster are nearly isopotential with midpoint reduction potentials of ~ -510 mV vs SHE and are ca. 100 mV more positive than that of free Co(II)/Co(I) cobalamin couples.[9] Both cofactors appear to change oxidation state as the corrinoid cycles between methyl-Co(III) and Co(I) states.[6] Electron spin resonance[7] (ESR) and redox[9] studies indicate that the cobamide and cluster do not strongly interact.

To better understand the mechanism of methyl transfer performed by the C/Fe-SP and to attempt to relate structural changes of the corrin system to changes in ligation and oxidation state, we have examined the Co(II) and methyl-Co(III) forms of the C/Fe-SP using cobalt $K\alpha$ extended X-ray absorption fine structure (EXAFS) and X-ray edge spectroscopy. Both X-ray edge and EXAFS studies indicate that the Co(II) state has a base-off four-coordinate distorted square-planar structure. Comparison of edge data on methyl-Co(III) with temperature-dependent data collected previously on base-off methyl-B_{12}[10] suggests that the methyl-Co(III) form of C/Fe-SP may be six coordinate at low temperature (where a water ligand may occupy the position vacated by the benzimidazole base). UV–visible spectra of the methyl-Co(III) species indicate that the benzimidazole base is unligated to cobalt. Our results provide strong evidence for protein-induced modification of the corrinoid coordination state to facilitate heterolysis of the Co–C bond of the methylated C/Fe-SP by CODH and to facilitate reduction of the inactive Co(II) state, in both cases, forming four-coordinate Co(I).

Materials and Methods

Materials. Aluminum oxide and (5,10,15,20-tetraphenyl-21H,23H-porphine)cobalt(II) (CoTPP) were purchased from Aldrich Chemical Co. and were used without further purification.

Sample Preparation. *C. thermoaceticum* strain ATCC 39073 was grown with glucose as the carbon source at 55 °C under CO$_2$[11] and cells harvested as described.[12] The C/Fe-SP was purified to homogeneity[7] in an anaerobic chamber (Vacuum Atmospheres) at $\sim$16 °C. Purity after each chromatographic step was determined by SDS-PAGE followed by silver staining (Bio-Rad) and by Western hybridization to polyclonal antibodies.[13]

To methylate the C/Fe-SP, the purified protein was concentrated by ultrafiltration using an Amicon concentrator with a YM 30 membrane. The as-isolated Co(II) form of the C/Fe-SP was similarly concentrated to 60 mg/mL for X-ray absorption studies. Methylation of the C/Fe-SP (15 mg in 1 mL of 50 mM Tris-HCl, pH 7.5) was achieved by electrochemical reduction at −720 mV vs SHE (−530 mV vs NHE) as described earlier[2] followed by addition of 10 μL of 0.2 mM methyl iodide. The methylated C/Fe-SP solution was then centrifuged through a Sephadex

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[*] Author to whom correspondence should be addressed.

[†] Albert Einstein College of Medicine of Yeshiva University. Phone 718-430-4136; Facsimile 212-828-1377. Internet: MRC@AECOM.yu.edu

[‡] University of Nebraska. Phone 402-472-2943; Facsimile 402-472-7842; Bitnet. sragsdale@crcvms.unl.edu

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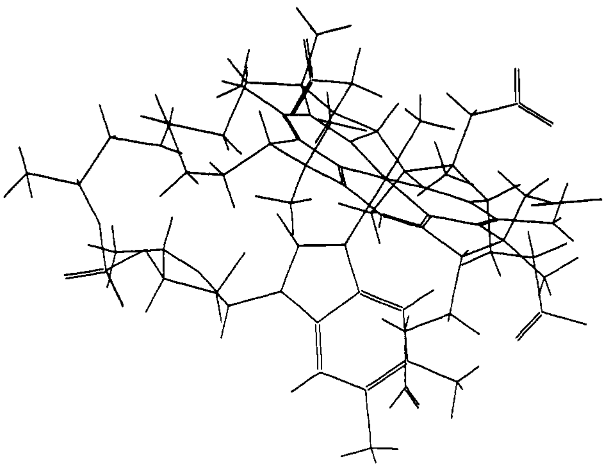

Figure 1. Structure of methylcobalamin (MeCbl) in a "base-on" configuration.[39] The base-off form of methylcobalamin is generated by cleavage of the axial cobalt–nitrogen bond to the benzimidazole base. The MeCbl structure was obtained from the Cambridge Crystallographic Data Base version 4[41] and plotted using the Chem-x molecular modeling program.[42]

G-50 column twice to remove unreacted methyl iodide and concentrated to 250 μL (50 mg/mL) using an Amicon macrosolute concentrator. Both the as-isolated Co(II) and methylated C/Fe-SP solutions (250 μL) were then transferred anaerobically to 25 × 2.5 × 2 mm deep lucite sample holders (approximate volume 200 μL) and sealed with kapton tape. EXAFS model compounds were prepared as described previously.[14]

ESR spectra were recorded on a Bruker ECS106 spectrometer equipped with an Oxford ITC4 temperature controller and automatic frequency counter (Hewlett Packard, Model 5340A). Spin concentrations were measured by comparing the double integrals (using supplied Bruker software) of the spectra with those of a 1 mM copper perchlorate standard. Spectroscopic parameters are given in the figure legends.

Data Collection. Data were collected at the National Synchrotron Light Source, Brookhaven National Laboratory, on beam line X-9A, using a double flat Si(111) crystal monochromator with fixed exit geometry. Harmonics were rejected by a Ni mirror positioned downstream of the monochromator. All experiments were carried out at 115–130 K, and sample temperature was maintained by flowing cooled nitrogen gas through a low-temperature cryostat.[15] EXAFS and X-ray edge data having 3 eV resolution were collected as described previously.[14] A total of 47 EXAFS scans were collected from three different 60 mg/mL Co(II) C/Fe-SP samples. To limit radiation damage and minimize X-ray exposure on the sample, the horizontal beam aperture was reduced (1.8 mm H, 2.0 mm V) and the position of the beam along the sample was incremented every 4 scans. Photon flux was 9.40×10^9 photons s^{-1} mm^{-2} at 100 ma beam current.[16] Data were generally taken in the range of 100–200 ma. $K\alpha$ cobalt fluorescence was collected using a 13-element energy resolving Germanium detector. For reference signals, mylar tape was mounted at a 45° angle to the X-ray beam to scatter photons counted by a photomultiplier tube positioned perpendicular to the X-ray beam. For an internal standard, cobalt foil calibration spectra were collected simultaneously with each scan using the same collection configuration as the reference signal. This method accounts for shifts in the monochromator.

Data Treatment and Error Analysis. EXAFS data for samples of the C/Fe-SP were manipulated and analyzed using the University of Washington EXAFS package. EXAFS background removal, k^3-weighting, Fourier filtering, and nonliner least-squares fitting followed standard procedures.[17-19] All scans were examined for edge position and sharp nonstatistical noise glitches prior to data processing. Obvious noise glitches were removed prior to further processing by fitting the data on each side of the glitch with a polynomial interpolation. Background subtracted data were Fourier transformed using cosine-squared tapered windows to isolate first shell contributions as described previously.[14,17,20] The tapers had centers of 1.5(k_1) and 11.0(k_2) Å^{-1} in wave-vector-(k) space. The Fourier filter back-transform window taper centers were set to 1.0(r_1) and 2.0(r_2) Å. At $k_1 - Dk_1/2$ and $k_2 + Dk_2/2$, the window function tailed to zero, and at $k_1 + Dk_1/2$ and $k_2 - Dk_2/2$, the window function was completely square and left unchanged (where D is an arbitrary value that simply selects the degree of taper). The back-transform (r-window function) was the same type as the k-window function. The taper functions were set at $Dk_1 = 3.0$, $Dk_2 = 2.0$ Å^{-1}. The Fourier filter window functions were set at $Dr_1 = Dr_2 = 0.2$ Å. All data were fit from 4.0 to 11.0 in k-space. The data collected for CoTPP were treated in exactly the same manner as those for the C/Fe-SP.

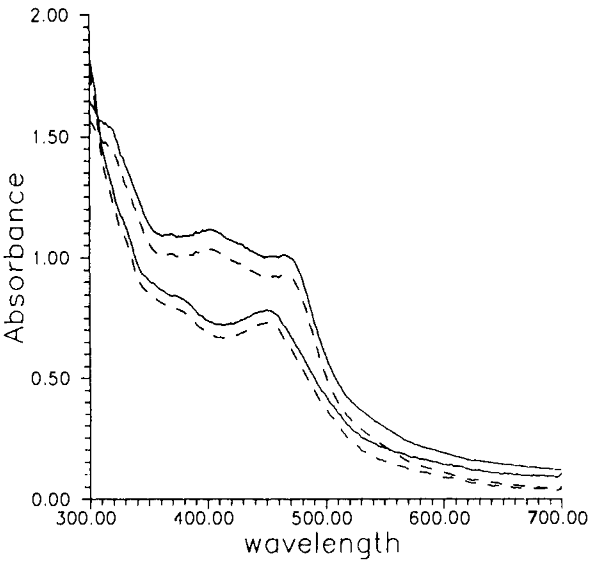

Figure 2. UV-visible spectra of the C/Fe-SP. Top 2 traces: as-isolated Co(II) form of the C/Fe-SP before (solid line) and after (dashed line) X-ray exposure. Bottom 2 traces: methylated C/Fe-SP before (solid line) and after (dashed line) X-ray exposure. The spectra of the C/Fe-SP before X-ray exposure (solid lines) have been positively displaced for ease of comparison.

Errors in the EXAFS analyses were estimated by three methods. To determine the degree of statistical or random noise, partial sums of the total number of scans were independently fit. The differences in fit distances provide an estimate of random noise. Second, systematic errors due to variations in sample preparation, radiation damage, and beam fluctuations are estimated by separate analysis of independently prepared samples. Third, the method of mapping out the minimum solution by examination of χ^2 was applied.[14,21] These error estimates gave similar results of ±0.01 Å for distances.

X-ray edge data for the Co(II) and methyl-Co(III) states of the C/Fe-SP samples were collected and processed as described in detail previously.[22] X-ray edge energy calibrations are obtained by comparison of the X-ray fluorescence and cobalt foil transmission data collected simultaneously. The cobalt edge is assigned as the maximum of the first-derivative fluorescence spectrum, and as the minimum of the corresponding first-derivative cobalt foil transmission spectrum. Cobalt edge data are calibrated to the absolute cobalt edge (7709.0 eV). Integrated pre-edge transition intensities (Table II) are presented in ratio form referenced to the integrated area of a cobalt hexamine 1s-3d peak (arbitrarily given the value of 1.0). The actual area of the cobalt hexamine 1s-3d transition is 2.0×10^{-2} eV with the step jump normalized to 1.[22] Cobalt hexamine provides an excellent reference for pre-edge transition comparisons due to its almost perfect octahedral geometry[23] and correspondingly low 1s-3d transition intensity. Errors associated with integrated intensities of pre-edge features were estimated from a combination of statistical noise and errors introduced due to the method of analysis. Contributions of statistical noise (measured in the region prior to the 1s-3d transition) to 1s-3d or 1s-4p shake-down (SD) peak areas were

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Table I. Nonlinear Least-Squares Fitting Solutions for EXAFS Spectra of the Co(II) C/Fe-SP from *C. thermoaceticum*[a]

solution	model	r (Å)	N	ΔE_0 (eV)	$\Delta\sigma^2$ (Å^{-2})	X^2
A	Co-N	1.87	3.0	0	-4×10^{-3}	5.0
B	Co-N	1.87	3.5	0	-3×10^{-3}	1.3
C	Co-N	1.88	3.8	−2	-2×10^{-3}	0.8
D	Co-N	1.88	4.0	−2	-1×10^{-3}	1.1
E	Co-N	1.88	4.5	−2	-1×10^{-3}	3.5
F	Co-N	1.88	5.0	−4	2×10^{-4}	8.0

[a] Simulated solutions A–F represent fitting results at various values of fixed coordination number. Parameters: r, distance in Å; N, coordination number; ΔE_0, energy shift relative to model compound; $\Delta\sigma^2$, Debye–Waller shift relative to model compound; X^2, sum of residuals squared. All solutions, except solution C where N was floated, result from fixing N, letting r and σ^2 float, and stepping E_0 from −8 to +8 eV to minimize X^2. EXAFS solutions for Co(II) /Fe-SP are obtained from Fourier filtered data. The back-transformed data are fit to CoTPP (4 nitrogens at 1.949 Å average distance)[30] by a nonlinear least-squares fitting procedure.

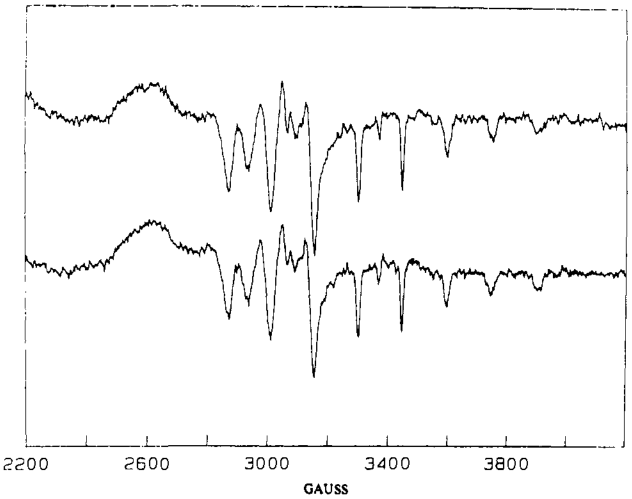

Figure 3. EPR spectra of the Co(II) form of the C/Fe-SP. The C/Fe-SP was in 50 mM Tris/HCl, pH 7.5. EPR conditions were the following: temperature, 77 K; field set, 3300 G; sweep width, 2000 G; gain, 1×10^4; power, 20 mW; and frequency, 9.451 GHz. (Top) EPR spectrum of the as-isolated C/Fe-SP taken before X-ray exposure. (Bottom) EPR spectrum of the as-isolated C/Fe-SP taken after X-ray exposure. Note the absence of superhyperfine coupling indicating a base-off configuration.

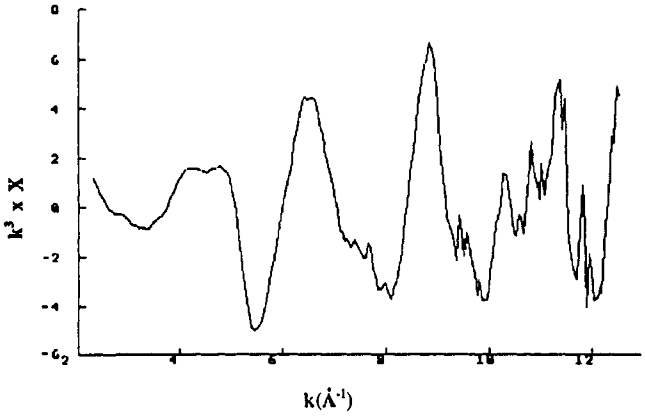

Figure 4. Plot of raw data of Co(II) C/Fe-SP in k-space after cubic weighting, background subtraction, and removal of obvious noise glitches.

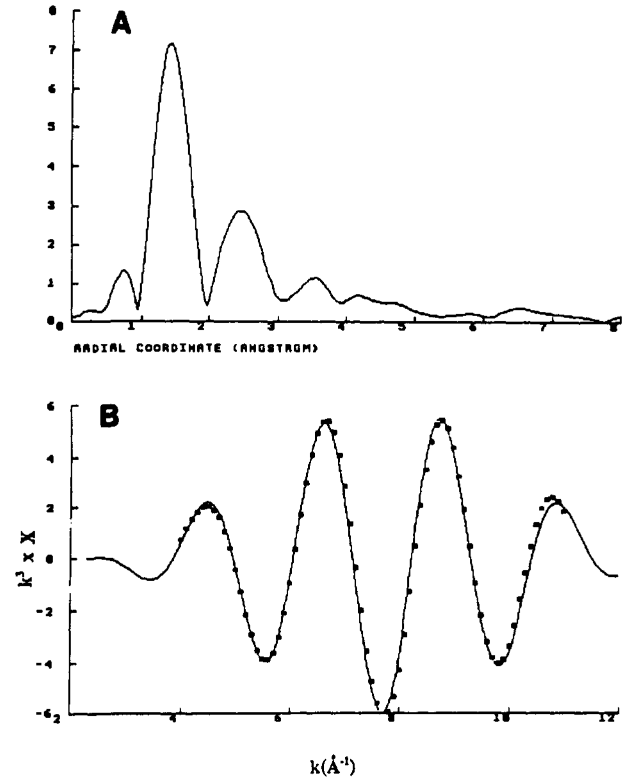

Figure 5. (A) Fourier transform of Co(II) C/Fe-SP data presented in Figure 4. (B) Back-transformed data from the Co(II) form of the C/Fe-SP after removal of glitches (solid lines) compared with the 1-atom fit simulated solution C (squares); $x^2 = 0.8$. Data are fit from 4.0 to 11.0 in k-space.

determined by calculating the percent contribution to the total peak area. Statistical errors varied between 10 and 15% for protein samples. Systematic errors due to the method of data analysis were calculated by comparison of the integrated peak areas of different scans and from duplicate or triplicate samples. These systematic errors were ≤5%. Errors seen in Table II represent the expected error for this measurement taking into consideration both the systematic and statistical errors discussed above.

Results and Discussion

Optical characterization of both the Co(II) and methyl-Co(III) forms of the C/Fe-SP was completed before and after sample exposure to the X-ray beam (Figure 2). ESR spectra of the Co(II) form of the C/Fe-SP form were also collected before and after X-ray analysis to detect oxidation of the Co(II) species and to confirm the base-off configuration (Figure 3). By both methods of analysis, it is clearly demonstrated that the C/Fe-SP was not damaged or altered during X-ray exposure. Raw Co(II) C/Fe-SP data, after cubic weighting, background subtraction, and removal of obvious noise glitches are presented in Figure 4. Figure 5A shows the Fourier transform of Co(II) C/Fe-SP data presented in Figure 4. The Co(II) C/Fe-SP data were subsequently back-transformed and analyzed using a one-atom fit in which distance (r) and Debye–Waller factor shift ($\Delta\sigma^2$) were floated, and the coordination number (N) was held fixed for all solutions except solution C, where it was floated. Edge energy shift (ΔE_0) was stepped from −8 to +8 eV in 1-eV increments for all solutions. Reported values for $\Delta\sigma^2$ and ΔE_0 were with respect to the model compound. The best fit gave a solution of $r = 1.88 \pm 0.01$ Å, $N = 3.8 \pm 0.4$ ligands, $\Delta\sigma^2 = -2 \times 10^{-3} \pm 0.0006$ Å^{-2}, $\Delta E_0 = -2.0$ eV, and x^2(sum of the residuals squared) = 0.8. Solution C (Table I) and the experimental data are compared in Figure 5B. A complete series of one-atom fits were conducted by fixing N at various values and examining the effects on x^2 and the Debye–Waller factors (Table I). When N is held fixed at 3.0, x^2 is more than six times that of the $N = 3.8$ solution. When N

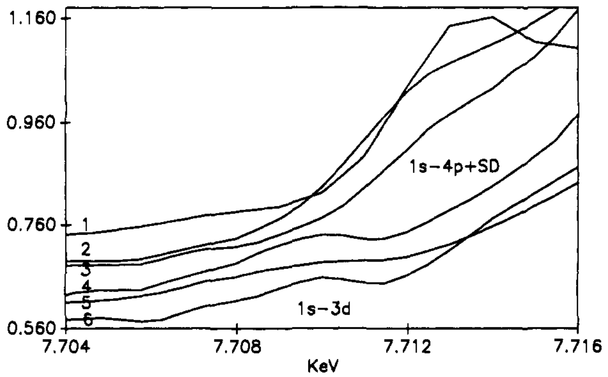

Figure 6. Comparison of X-ray fluorescence pre-edge data of transitions observed in cobalamin models, free cobalamins, and C/Fe-SP compounds: (1) CoTPP, 1s-4p + SD (4-coordinate, not to scale compared to other spectra); (2) Co(I) B_{12}, 1s-4p + SD (4-coordinate); (3) Co(II) C/Fe-SP, 1s-4p + SD (4-coordinate; (4) Co(II) B_{12}, 1s-3d (5-coordinate); (5) cyanocobalamin, 1s-3d (6-coordinate); (6) methyl-Co(III) C/Fe-SP, 1s-3d (6-coordinate). X-ray edge spectra are calibrated for energy and intensity comparisons. Spectra have been arbitrarily offset along the *Y* axis for ease of comparison.

Table II. Integrated X-ray Fluorescence Pre-edge Transition Intensities and Edge Positions for Co(II) and Methyl-Co(III) C/Fe-SP from *C. thermoaceticum*, Free Cobalamins, and Cobalamin Model Compounds

compd	coord no.	1s-3d	1s-4p + SD	edge position (eV)
Co(III) hexamine[22]	6	1.0 ± 0.3		7723.5 ± 0.2
Co(II) B_{12}[22]	5	8.3 ± 0.6		7722.0 ± 0.2
Co(II) TPP[22]	4		59.4 ± 0.3	7721.5 ± 0.2
Co(I) B_{12}[14]	4		13.3 ± 0.6	7721.0 ± 0.2
Co(II)/Fe-SP	4		12.4 ± 1.8	7721.0 ± 0.3
methyl-Co(III) C/Fe-SP (125 K)	6	12.8 ± 2.1		7721.5 ± 0.3
Co(III) methylcobalamin[22]	6	12.5 ± 0.8		7722.5 ± 0.2
Co(III) base-off methylcobalamin (180 K)[10]	6	15.8 ± 0.8		7723.0 ± 0.2
Co(III) base-off methylcobalamin (298 K)[10]	5	22.9 ± 0.2		7723.0 ± 0.2

is fixed at 4.5, x^2 more than quadruples that of the minimum solution. Coordination numbers of less than four are not chemically reasonable for the corrin ring system. Solutions with coordination numbers greater than four had rapidly increasing x^2 values (Table I). The well-defined minimum observed at 3.8 ligands provides evidence for a coordination number of approximately four. Additionally, two different two-atom fits were attempted to examine the possibility of extracting a unique Co–N contribution. Neither a 3(Co–N):1(Co–N) nor a 2(Co–N):2(Co–N) fit gave reasonable solutions. Both of the two-atom fits had unreasonable Debye–Waller factors as well as distance solutions with differences less than the resolution of the data.[21] As with earlier ESR[7] and redox[9] studies, the X-ray absorption spectra indicate that the cobalt and the iron of the [4Fe-4S] cluster are too distant to communicate, since Figure 5A shows no evidence of a strong backscatterer at 3–5 Å.

Since the back-scattering amplitude between the nearest neighbor to the absorber is susceptible to non-transferability,[24] coordination number assignments based on EXAFS data alone are not recommended. Integration of pre-edge features of X-ray edge spectra, followed by comparison to model compounds of known structure, provides another basis for predicting coordination number and geometry.[22,25,26] The edge spectrum of Co(II) C/Fe-SP (Figure 6) contains a moderate intensity 1s-4p + shakedown transition and no 1s–3d peak. Shake-down (SD) transitions, which are indicative of transfer of charge from the ligand to the metal, occur in compounds with square-planar geometries.[22,27–29] The presence of the 1s-4p + SD transition and absence of a 1s-3d transition in the edge spectrum of the Co(II) C/Fe-SP are inconsistent with five-coordinate or tetrahedral geometries.[25,26] Comparison of the 1s-4p + SD transition intensity for CoTPP, a nearly perfect square-planar complex,[30] to that of the Co(II) C/Fe-SP shows a much greater intensity for CoTPP (Table II). A reduction in intensity of the Co(II) C/Fe-SP 1s-4p + SD transition, compared to CoTPP, is also observed in our recent X-ray edge and EXAFS study of the distorted square-planar Co(I) B_{12} intermediate.[14] This reduction in 1s-4p + SD intensity is anticipated due to deviation from a pure square-planar geometry resulting from fusion of corrin tetrapyrrole rings A and D (Figure 1). Thus, both our X-ray edge data and EXAFS results provide direct evidence of a four-coordinate (distorted) square-planar configuration for the Co(II) state of the C/Fe-SP. To our knowledge, this is the first report of a four-coordinate Co(II) corrinoid. Generally, Co(II) corrinoids are five- and sometimes six-coordinate. That the C/Fe-SP has imposed a distorted square-planar geometry on the cobamide constitutes a destabilization that would be expected to facilitate the important one-electron reductive activation to form the active Co(I) state.

To investigate changes in the protein structure associated with changes in oxidation state, we examined the X-ray edge spectrum of the methyl-Co(III) state of the C/Fe-SP. In a previous study, Ragsdale et al. presented evidence based on optical spectroscopy for a base-off conformation of the methyl-Co(III) state of the C/Fe-SP.[7] Our X-ray edge spectra, collected at 125 K, also are consistent with a base-off configuration except it appears that a weak donor ligand (likely a water molecule) may replace the 5-methoxybenzimidazole base at the sixth coordination position. We have recently completed a study of the dependence of coordination number on temperature for base-off adenosyl- and methylcobalamins over temperatures ranging from 180 to 295 K.[10] As the temperature was raised, an increase in 1s-3d intensity was observed consistent with a change in coordination number from six-coordinate (where a water molecule is believed to occupy the coordination position vacated by the benzimidazole base) to a five-coordinate square-pyramidal structure. A similar optical study of base-off phosphitocobalamins was performed by Chemaly who found an analogous shift in coordination number from six to five as the temperature increased from 288 to 328 K.[31,32] In light of these studies, it is a reasonable hypothesis to propose a five-coordinate structure at room temperature for the methyl-Co(III) state. Additional X-ray edge and EXAFS studies of the base-off methyl-Co(III) protein at different temperatures will address the question of coordination number in more detail. The benzimidazole base has been found to have a major effect on stabilizing the methyl-Co(III) bond against heterolysis.[33] Therefore, a base-off structure for methyl-Co(III) C/Fe-SP would be expected to facilitate a heterolytic Co–C bond cleavage mechanism in the transfer of the methyl group from methylcobamide to CODH, forming a methyl-metal intermediate on CODH. It would also be expected to protect methyl-Co(III) from homolytic cleavage that would form two disadvantageous products, a methyl radical and Co(II).[34]

We have examined the first-derivative X-ray edge positions of the methyl-Co(III) and Co(II) forms of the C/Fe-SP relative to

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Table III. Comparison of EXAFS Data for Co(II) C/Fe-SP from *C. thermoaceticum* to EXAFS and X-ray Crystallographic Data of Free Cobalamins[a]

compd	coord no.	EXAFS (Å)	X-ray (Å)
Co(III) methylcobalamin	6		
Co–N (eq)		1.90 ± 0.01[20]	1.89[39]
Co–N (ax)		2.20 ± 0.03	2.19
Co–C		2.00 ± 0.03	1.99
			ESD = 0.02
Co(II) C/Fe-SP	4		
Co–N (eq)		1.88 ± 0.01	
Co(II) B_{12}	5		
Co–N (eq)		1.88 ± 0.02[17]	1.89[40]
Co–N (ax)		1.99 ± 0.03	2.13
			ESD = 0.02
Co(I) B_{12}	4		
Co–N (eq)		1.88 ± 0.02[14]	

[a] The Co–N (equatorial) distances for the two X-ray solutions are the $1/r^2$ average of the four individual equatorial distances. EXAFS results for Co(II) C/Fe-SP, Co(I), Co(II) B_{12}, and methylcobalamin are from solution samples. Bond distance entries correspond to cobalt–nitrogen equatorial (eq), axial (ax), and axial cobalt–carbon coordinations. Estimated standard deviations (ESD) are noted for structures determined by X-ray crystallography.

the edge spectra of a cobalt foil as an indication of effective nuclear charge on cobalt. Edge shifts to lower energy correspond to a reduction in effective nuclear charge[35,36] (Table II). The edge positions of Co(III) MeCbl and Co(II) B_{12} are at 7722.5 ± 0.2 and 7722.0 ± 0.2 eV, respectively. A shift of 1 eV to lower energy is observed between Co(II) B_{12} and Co(I) B_{12}. Thus, there is a consistent trend for the free corrinoids to shift to lower energy upon reduction. Examination of the edge shift for Co(II) C/Fe-SP compared to Co(II) B_{12} shows a 1.0-eV shift to lower energy. In addition, comparison of the base-off methyl-Co(III) C/Fe-SP edge to absolute edge positions seen for base-off MeCbl collected at 180 K shows a 1.0-eV shift to lower energy. Thus, both the Co(II) and methyl-Co(III) C/Fe-SP forms indicate edge positions at lower energies than their respective free B_{12} analogues. This reduction in effective nuclear charge of the methyl-Co(III) and Co(II) states may be related to the mechanism the protein utilizes to facilitate conversion of the methyl-Co(III) to Co(I) in the methyl transfer reaction and of Co(II) to Co(I) in the reductive activation. Reduction of the electron donating strength of the Co–C bond in the base-off methyl-Co(III) C/Fe-SP species and/or charge distribution to the corrin ring may account for this surprising result.

Conclusion

Recent structural data collected on several B_{12} compounds[10,14,17,20,22] indicate that detachment of the benzimidazole base may be the initial step in heterolytic Co–C bond cleavage. In addition, electrochemical studies indicate that base-off Co(II) is a likely intermediate in reduction to Co(I).[37,38] Understanding the role of the protein in controlling the coordination state of the benzimidazole base, predisposing the methyl-Co(III) or Co(II) states toward formation of the Co(I) species, is a primary goal. The identification of a four-coordinate distorted square-planar Co(II) state in the C/Fe-SP provides strong evidence that the protein facilitates reduction by generation of a strained Co(II) intermediate, which would be poised for one-electron reductive activation to Co(I). In addition, we corroborate the earlier proposal of an unusual base-off methyl-Co(III) state and predict that methyl-Co(III) may be five-coordinate with the methyl group as the only axial ligand at physiologically relevant temperatures. The unexpected shift to lower energy for both the Co(II) and methyl-Co(III) C/Fe-SP as compared to their free cobalamin analogues invites further questions regarding the role of the protein in mediating cobalamin function. EXAFS and X-ray edge studies of the Co(I) form of the C/Fe-SP will provide further electronic and structural information.

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