Sulfur-Containing Cobalamins: X-ray Absorption Spectroscopic Characterization†

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ABSTRACT: Sulfur-containing cobalamins are thought to have a special role in the intracellular conversion of cyanocobalamin to its coenzyme forms through a Co(I) intermediate. Glutathionylcobalamin is especially interesting as a possible precursor of cobalamin coenzymes [Wagner et al. (1969) *Ann. N.Y. Acad. Sci. 112,* 580; Pezacka et al. (1990) *Biochem. Biophys. Res. Commun. 169,* 443]. Recent NMR data [Brown et al. (1993) *Biochemistry 32,* 8421] strongly support the hypothesis that glutathione coordinates to the cobalt through the sulfur atom in glutathionylcobalamin. In this study three-sulfur containing cobalamin derivatives (glutathionylcobalamin, sulfitocobalamin, and cysteinylcobalamin) have been characterized by X-ray absorption spectroscopy. We give evidence for the sulfur coordination in these compounds and present the corresponding structural information. The Co–N_{eq} distances in the sulfur-containing cobalamins are very close to one another (1.90 ± 0.01 Å). The Co–S and Co–N_{ax} distances are also similar (Co–S: 2.28–2.35 Å and Co–N_{ax}: 2.13–2.16 Å) and in the expected range. The X-ray edge positions for the sulfur derivatives shift to lower energies with respect to cyanocobalamin. This indicates strong electron donation from the sulfur to the cobalt and suggests that the effective charge on the cobalt ion in sulfur cobalamins is largely reduced from +3.

It has been proposed that newly internalized cobalamin must be reduced to Co(I) B_{12} prior to coenzyme biosynthesis and that the early enzymatic steps required for both adenosyl- and methylcobalamin (AdoCbl,[1] MeCbl) formation are shared (Walker et al., 1969; Fenton et al., 1978; Mellman et al., 1979). Aquocobalamin (AqCbl) and glutathinylcobalamin (GSCbl; Figure 1) have been suggested as possible precursors (Mellman et al., 1979; Wagner & Bernhauer, 1969). Pezacka and co-workers showed (Pezacka et al., 1990) that GSCbl is a naturally occurring intracellular form of cobalamin in mammalian cells and the formation of AdoCbl from GSCbl was accelerated compared to AqCbl using cell extracts. Decyanation of CNCbl results in sulfitocobalamin (HSO_3-Cbl) and requires the presence of reduced glutathione (GSH). This indicates that HSO_3Cbl might be a degradation product of GSCbl. They proposed that intracellular CNCbl must first undergo a CN-elimination reaction followed by GSCbl synthesis and GSCbl or a closely related thiol–Cbl adduct then serves as an intermediate in the biosynthesis of MeCbl and AdoCbl.

The purpose of the present paper is (1) to determine which atom is coordinated to cobalt in these biologically important sulfur-containing cobalamins by using optical and X-ray edge spectroscopy, (2) to describe them structurally by using extended X-ray absorption fine structure (EXAFS) analysis, and (3) to compare the results with those of other cobalamins.

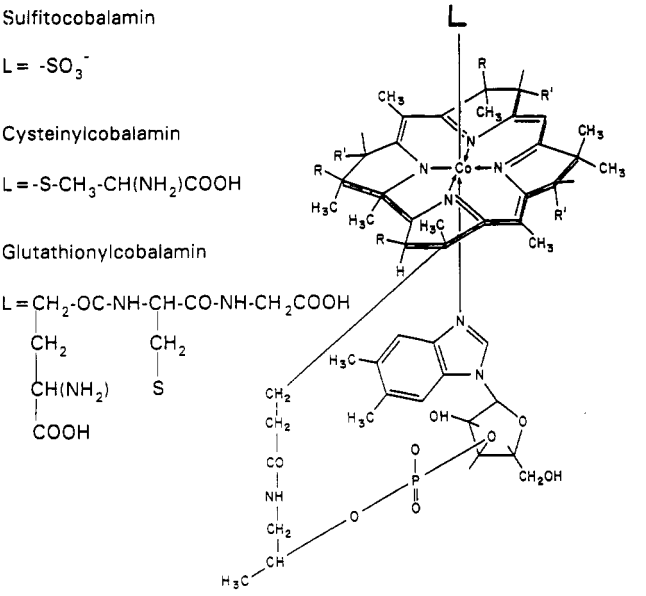

FIGURE 1: Schematic view of the cobalamins. R = acetamido group; R′ = propionamido group.

EXPERIMENTAL PROCEDURES

Materials. AqCbl, L-cysteine hydrochloride, sodium sulfite, and GSH were purchased from Sigma Chemical Co. Aluminum oxide and (5,10,15,20-tetraphenyl-21*H*,23*H*-porphine)cobalt(II) (CoTPP) were purchased from Aldrich Chemical Co., cobalt(II) sulfide (CoS) was obtained from Alfa Morton Thiokol Inc., and sodium acetate was purchased from Fischer Scientific. All chemicals were used without further purification. We tested the glycerol and the aluminum oxide for the presence of cobalt by using the X-ray spectrometer. No detectable edge jump was observed for any of

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[1] Abbreviations: AdoCbl, 5′-deoxyadenosylcobalamin; MeCbl, methylcobalamin; GSCbl, glutathionylcobalamin; GSH, reduced glutathione; CNCbl, cyanocobalamin or vitamin B_{12}; HSO_3Cbl, sulfitocobalamin; CysCbl, cysteinylcobalamin; DMB, 5,6-dimethylbenzimidazole; CoTPP, (5,10,15,20-tetraphenyl-21*H*,23*H*-porphinato)cobalt(II); XAS, X-ray absorption spectroscopy; EXAFS, extended X-ray absorption fine structure; CoS, cobalt(II) sulfide.

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the test samples; therefore, the cobalt concentration in these samples was less than 0.5% of that in the experimental samples and could be ignored.

Sample Preparations. Sulfur cobalamins were synthesized as described by Pezacka et al. (1990). For the X-ray spectroscopic measurements the sulfur cobalamins were not further purified and were measured in a mixture of 35% glycerol and 50 mM sodium acetate buffer at pH 5.0. The final sample concentration was 9 mM. The purpose of using glycerol is to reduce sample cracking upon freezing. The solid CoTPP and CoS were prepared by diluting the pure sample in aluminum oxide powder and grinding the mixture to a fine powder in a mortar and pestle. The resulting metal content was 1.3 wt % for CoS and 0.9 wt % for CoTPP. The samples were packed in 25- × 2.5- × 2-mm lucite sample holders and covered with Mylar tape. Liquid samples were injected to the sample holders and frozen in liquid nitrogen prior to data collection. For the liquid samples an optical spectrum was taken before and after exposure to the X-ray beam, which showed no sample damage.

Data Collections. EXAFS and X-ray edge data were collected at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (Upton, NY), on beam line X-10C using a Si(220) crystal monochromator. Higher-order harmonic contamination was rejected by using a Rh mirror. All experiments were carried out at 170–180 K, and the sample temperature was maintained by flowing cooled nitrogen gas through a low-temperature lucite cryostat (Powers et al., 1981). EXAFS data were recorded by counting at a specific energy for 4 s and incrementing the energy by 10 eV from 100 eV below the cobalt edge to 30 eV below the edge, then in 2.0-eV steps to 30 eV above the edge, and finally by 3.0-eV steps from that point to 700 eV above the edge. X-ray edge data were recorded by counting at a specific energy for 2 s and incrementing the energy by 10 eV from 100 eV below the cobalt edge to 20 eV below the edge, and then in 0.5-eV steps to 100 eV above the edge. A total of six to nine EXAFS and three edge scans were collected for each compound. Data were generally taken in the range of 100–200 mA. K-α cobalt fluorescence was detected with a zinc sulfide-coated photomultiplier tube, and incident photon scattering was rejected by an iron oxide filter. Output signals were amplified with a Keithley amplifier, converted to frequency, and counted in a scaler interfaced to a Micro-VAX II computer. For reference signals, Mylar tape was mounted at a 45° angle to the X-ray beam to scatter photons counted by a similar photomultiplier tube positioned perpendicular to the X-ray beam. This method provided excellent linearity between the sample and reference detectors.

First-shell EXAFS and X-ray edge data were manipulated and analyzed using a PC-based version of the AT&T Bell Labs EXAFS package on an IBM-compatible machine with coprocessor. The programs were adapted from Fortran IV UNIX-based programs supplied by Dr. Brian Kincaid to Fortran 77 language and compiled with a Microsoft Fortran 77 (v. 4.0) compiler. The fitting routine was modified to allow three-atom consistency tests. The data were also analyzed with the University of Washington EXAFS package on a VAX-VMS computer and gave indistinguishable results. Data manipulation with use of a linear pre-edge fit, cubic polynomial spline background (isolated atom) subtraction, wave-vector cubed weighting, Fourier transformation, filter, and back-transform have all been described previously (Chance et al., 1986a–c). Fourier-filtered data were analyzed by a nonlinear least-squares fitting procedure in the standard manner (Lee et al., 1981). All EXAFS and X-ray edge scans were checked for edge positions and noise prior to data processing. The corrected edge positions were determined by calibrating the edge position of the sample to the edge position of the cobalt foil, which was recorded simultaneously with the sample spectrum. Sharp glitches caused by nonstatistical events were removed before further data processing by fitting a polynomial in the appropriate region.

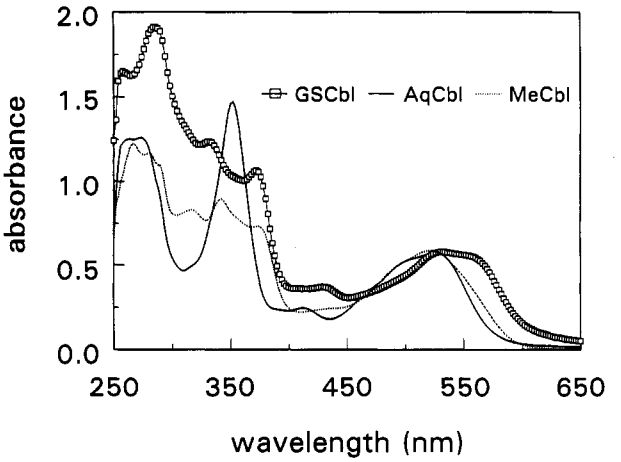

FIGURE 2: Absorption spectra of GSCbl, AqCbl, and MeCbl in 50 mM sodium acetate buffer at pH 5.0. The shape of the GSCbl spectrum is very similar to that of MeCbl, and the position of the γ-peak indicates σ-electron donation from the sulfur to the cobalt.

Table 1: γ-Peak Positions in the Absorption Spectra of Cobalamin Compared to Literature Values (in Parentheses)

compounds	peak position (nm)	compounds	peak position (nm)
MeCbl	374 (~374)[a]	HSO_3Cbl	364 (364)[b]
GSCbl	372	CNCbl	361 (360.5)[b]
CysCbl	371 (370)[b]	AqCbl	351.5 (350)[b]

[a] Hill et al., 1970. [b] Firth et al., 1969.

The errors introduced into the EXAFS analysis are of two kinds: statistical or random errors that can be reduced by signal averaging and systematic errors that can result from sample inhomogeneity, sample degradation, beam fluctuations, and other sources. To estimate the statistical contribution to noise in the data, we analyzed independent partial sums of scans and noted the differences (Lytle et al., 1989). Errors due to sample preparation or other related nonrandom errors were measured by analyzing scans from independently prepared samples. We mapped out the best fits by examining the χ^2 (the sum of residuals squared). The distance errors are obtained by changing one parameter in the curve fitting, while least-squares refining the other parameters, until the χ^2 doubled (Lytle et al., 1989). Nonlinear least-squares fitting was carried out from 4.0 to 11.5 Å^{-1} in k space. The various methods of error analysis described above lead to the reported error in our data (see tables).

RESULTS

The γ-peak position in the absorption spectra of cobalamins is indicative of the donor strength of the axial ligands. The γ-band moves to increasingly longer wavelength as the σ-donor strength of the axial ligands increases (Pratt, 1972). The spectra of GSCbl, MeCbl, and AqCbl are shown in Figure 2, and Table 1 lists the peak positions. The absorption spectra of sulfur cobalamins have methylcobalamin-like γ-peaks both in shape and in position. These spectra are clearly distinct

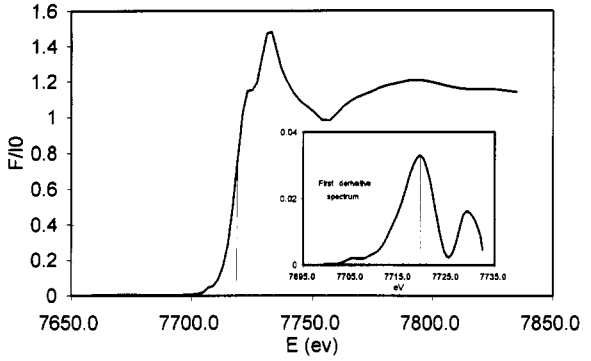

FIGURE 3: X-ray fluorescence edge spectrum of GSCbl. The edge position is defined as the maximum of the first derivative (see insert).

Table 2: The Calculated Edge Positions for the Sulfur-Containing Cobalamin Corrected to the Cobalt K-Edge Position (7709.0 eV) Using the Simultaneously Measured Edge Position of a Cobalt Foil in the Calibration Channel

compound	edge position (eV)	Co foil edge (eV)	corrected edge (eV)
glutathionylcobalamin	7719.9	7707.1	7721.8 ± 0.25
sulfitocobalamin	7719.5	7707.2	7721.3 ± 0.2
cysteinylcobalamin	7719.0	7707.0	7721.0 ± 0.2

from that of AqCbl. In the sulfur derivatives the σ-donor strength as indicated by the optical spectra decreases in the order of GSCbl, CysCbl, and HSO_3Cbl. We carried out charge calculations using the Chem-X molecular modeling package.[2] The calculations were performed on free sulfur ligands, replacing the cobalamin moiety with a hydrogen atom. The calculated charges on the sulfur atom are as follows: GSH, –0.18; CysH, –0.08; and HSO_3^-, +1.18. As the charge becomes more positive on the sulfur, the σ-donor strength of the ligand decreases. This shows the same trend found in the absorption spectra for the γ-band.

Kunzl's law proposes a linear relationship between the absorption edge position and the valence state of the central atom (Kunzl, 1932). This has been confirmed in a number of transition metal complexes (Wong et al., 1984; Belli et al., 1980), and we have also confirmed the significance of this relation for a number of cobalt and cobalamin compounds (Wirt et al., 1991). Using Kunzl's law, Boehm et al. (1954) arrived to the important conclusion that the valency of cobalt is three in CNCbl. It appears that the chemical shift is not only governed by the valency. The concept of the effective charge on the ion has been developed, and several methods are given to calculate it (Barinskii & Nadzhakov, 1960; Böke, 1957; Suchet, 1962; Suchet & Baily, 1965). The effective charge takes into account various parameters such as valency, coordination number, ionicity, and the nature of bond in the molecule. As the effective charge increases, the potential of the nucleus on the 1s core electron also increases and the repulsive interaction with other core electrons decreases. This leads to the fact that the excitation of the 1s electron happens at lower energy. A negative shift of the edge position with respect to a reference means larger electron density, and a positive shift can be interpreted as reduced electron density on the central atom.

The edge position has been identified as the maximum in the first derivative spectrum (shown in Figure 3 for GSCbl). The edge positions for the sulfur derivatives are summarized in Table 2, showing the measured edge values and the corrected edge positions calibrated to a cobalt foil measured simultaneously. These measured edge values were used as E_0 values in the conversion to k space. Table 3 lists the edge shifts of the sulfur-containing cobalamins and other important compounds with respect to CNCbl, showing a significant shift to lower energies for the sulfur-containing derivatives.

[2] Chem-X, developed and distributed by Chemical Design Ltd., Oxford, England.

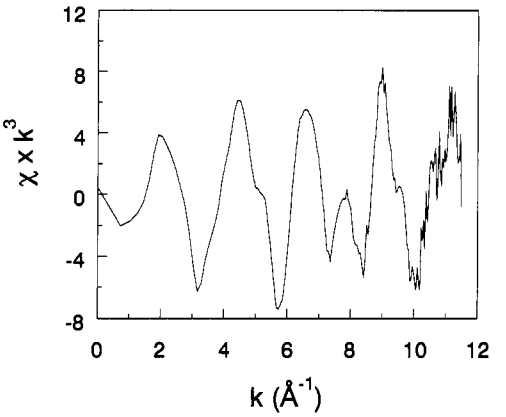

FIGURE 4: Wave-vector cube-weighted and background-subtracted χ data of gluthationylcobalamin.

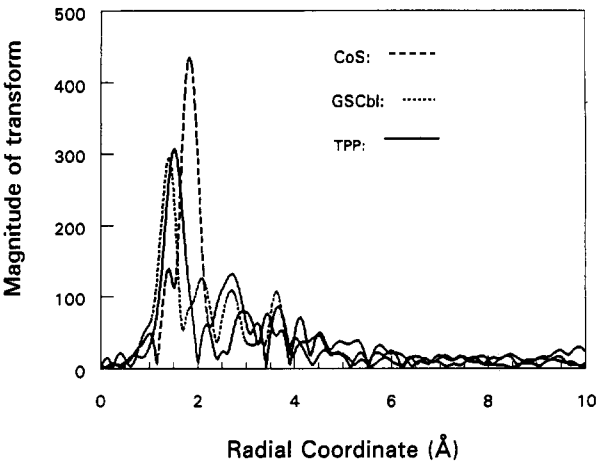

FIGURE 5: Fourier transforms of CoS, CoTPP, and GSCbl $k^3\chi$ data. The transform magnitude is plotted versus the relative radial distance from the central atom in Å. The CoTPP (dashed line) shows a main peak from the four-coordinated nitrogens at 1.52 Å. The GSCbl shows a main peak at 1.41 Å. The CoS model compound has a main peak from the six sulfur ligands at 1.83 Å. Fourier filter windows from 0.9 to 2.5 Å were applied for the CoTTP and CoS model data to provide the nitrogen and sulfur contributions for fitting the sulfurcobalamin first-shell data. The same window was applied for the GSCbl data.

In the EXAFS analysis all unknowns were fitted to model compounds containing covalent-type metal–ligand bonds that have accurate crystallographic structures. The CoTPP and CoS model compound data were collected and treated in the same manner as the cobalamin samples in manipulations and in the different fits. CoTPP was used as a standard in order to determine the Co–N distances, and the CoS model was used for the Co–S distance calculation. CoTPP has four nitrogen atoms at an average distance of 1.949(3) Å (Kruger, 1978), and Co–S has six sulfur atoms at an average distance of 2.330 Å (Gmelins, 1961).

Figure 4 shows the wave-vector cube-weighted and background-subtracted χ data of glutathionylcobalamin. Figure 5 shows the Fourier-transformed data for CoS, GSCbl, and CoTPP. A Fourier-filter back-transform window was chosen to isolate the first-shell contributions of the unknown and the model data. The first-shell peak of CoS is shifted to higher R value with respect to the other compounds. In order to

Table 3: Relative Edge Shift Values for Sulfur-Containing Cobalamin and for Other Structurally Relevant Compounds Using Cyanocobalamin Standard

compound	charge on cobalt	shift from CNCbl (7724.4 eV) in eV	compound	charge on cobalt	shift from CNCbl (7724.4 eV) in eV
cyanocobalamin	+3	0.0	adenosylcobalamin[a]	+3	−1.5
glutathionylcobalamin	+3	−2.6	cobalt hexamine[a]	+3	0.0
sulfitocobalamin	+3	−3.1	tris(acetylacetonato)cobalt(III)	+3	−1.4[c]
cysteinylcobalamin	+3	−3.4	Co(II) B_{12}[a]	+2	−1.5
aquocobalamin[a]	+3	0.0	Co(II) oxymyoglobin[b]	+2	−0.4
methylcobalamin[a]	+3	−1.0	Co(I) B_{12}[a]	+1	−2.5

[a] Wirt et al., 1991. [b] L. M. Miller, unpublished results. [c] Data points were taken at every 2 eV across the edge.

Table 4: Nonlinear Least-Squares-Fitting Solution for Glutathionylcobalamin EXAFS Spectra

fit type	fit	model	r	N	ΔE_0	$\Delta\sigma^2$	χ^2
one-atom	1	Co–N	1.85	6	9.3	−0.006	103
two-atom	2	Co–N	1.89	4	−0.4	−3E−5	2.0
		Co–N	2.11	2	−5.1	−0.002	
	3	Co–N	1.90	5	1.2	−0.002	2.3
		Co–N	2.14	1	−4.2	0.003	
	4	Co–N	1.89	5	1.8	−0.003	2.1
		Co–S	2.33	1	6.8	0.006	
three-atom	5	Co–N	1.89 ± 0.01	4	0.6	−3E−4	1.1
		Co–N	2.15 ± 0.03	1	4.3	−0.001	
	Co–S	2.28 ± 0.05	1	4.3	0.003		

include the sulfur contribution in the unknown compounds, we had to open a wide enough window, and so the limits for the models and for all the unknown sulfur derivatives were chosen to be 0.9 and 2.5 Å.

Fourier-filtered, back-transformed data were compared to combinations of model compound simulations by performing various one- and two-atom-type fits. The fits allow the distance (r), the Debye–Waller factor ($\Delta\sigma^2$), and the energy (ΔE_0) to vary with respect to the model compound. Since all the sulfur-containing cobalamins are octahedral, at first a one-atom fit with six fixed ligands is performed to give a rough estimate of the average distance around the cobalt atom. The two-atom-type fits are used in order to resolve the various distances around the metal, with the 5:1 fit resolving the axial distances opposite the fixed coordination number of one while a 4:2 fit resolves the Co–N equatorial distances opposite the fixed coordination number of four. Finally, a three-atom consistency test was performed, including the contribution from all the different shells and using the two-atom-fitting results as starting values. The results of nonlinear least-squares fitting for glutathionylcobalamin are summarized in Table 4. The one- and two-atom fits are performed by allowing the distance (r), the Debye–Waller factor (σ^2), and the energy (E_0) to vary but by keeping the coordination number (N) fixed, and the solution with the best χ^2 that forms a stable minimum is chosen within each fit. The three-atom consistency test, however, has fixed distances in addition to the coordination number. All reported solutions had E_0 and Debye–Waller factors that were chemically reasonable (Chance et al., 1986a–c; Sagi et al., 1990). The one-atom-type fit for the GSCbl data (fit 1 in Table 4) is performed using the CoTPP model to fit the average of all six ligands. The high χ^2 value associated with the average distance is not surprising. In order to resolve the axial Co–N distance to the DMB and the Co–S distance to the sulfur ligands, we used two-atom-type fits represented by solutions 3 and 4. Solution 3 shows a Co–N distance to the DMB at 2.14 Å, and solution 4 gives a Co–S distance at 2.33 Å. Solution 2 shows a stable averaged Co–N distance to the equatorial nitrogens at 1.89 Å. These values were then used as starting parameters for the three-atom consistency test. The test was performed in two ways. The first way involved both fixing the coordination number and all distances, while ΔE_0 and $\Delta\sigma^2$ remained free parameters. The second way involved having an additional distance parameter float. Solution 5 shows the final results based on the three-atom consistency test. The Co–N distances are in close agreement with the two-atom-fitting results. The Co–S distance is shorter but agrees with the two-atom-fitting result within the error. By use of the same strategy, similar results were obtained for the other two sulfur derivatives. Table 5 contains the overall fitting results for the sulfur-containing cobalamins. The Co–N_{eq} distances in the sulfur cobalamins are very close to one another (Co–N_{eq}: 1.90 ± 0.01 Å) and to those found in other cobalamins. The Co–N_{ax} distances are well resolved, and they are also close to each other (2.13–2.16 Å) and to data obtained for (nonalkyl)cobalamins (Sagi & Chance, 1992). The Co–S distances are similar for the three compounds within the error. Crystallographic data (Doppelt et al., 1984) for bis(2,3,5,6-tetrafluorobenzenethiolato)(*meso*-tetraphenylporphyrinato) cobalt(III) show a 2.35-Å Co–S distance. We searched the Cambridge Crystallographic Database (Allen et al., 1983) for six-coordinate cobalt compounds with sulfur and nitrogen ligands. We examined 242 structures, and the Co–S distances were in the range of 2.165–2.716 Å, with an average of 2.27 ± 0.08 Å, showing that the Co–S distances we obtained for the sulfur-containing cobalamins are in the expected range.

DISCUSSION

The absorption spectra and the X-ray edge results of the sulfur cobalamins provide convincing evidence that sulfur, and not oxygen, is coordinated to the cobalt atom. Recently, Brown et al. (1993) came to the same conclusion by using NMR spectroscopy to determine the site of GSH coordination in GSCbl. Firth et al. (1969) described the formation of an intermediate with a 355-nm γ-peak when they reacted hydroxocobalamin with sodium sulfite at pH 14. The intermediate slowly yielded sulfitocobalamin (γ-peak at 364 nm). They speculated that the intermediate is the O-bounded sulfitocobalamin, which is less stable, and so through isomerization it becomes the S-bounded derivative. An O-coordinated species would be expected to have an aquo- or cyanocobalamin-like spectrum (Pratt, 1972) on the basis of the behavior of a number of well-characterized cobalt coordination complexes.

The X-ray edge values are also strongly supporting the sulfur coordination as they significantly differ from that of any oxygen-coordinated compound, such as aquocobalamin, Co(II) oxymyoglobin (L. M. Miller, unpublished results), and tris(acetylacetonato)cobalt(III) (see Table 3). The fact that the X-ray edge values for the sulfur-containing derivatives are lower than that of CNCbl and AqCbl (Table 3) means that the electron density is larger in sulfur cobalamins and

Table 5: Comparison of EXAFS Results for Sulfur-Containing Cobalamin and for Other Cobalamins

compound		distance (Å)	compound		distance (Å)
glutathionylcobalamin	Co–N_{eq}	1.89 ± 0.01	methylcobalamin[a]	Co–N_{eq}	1.90 ± 0.01
	Co–N(DMB)	2.15 ± 0.03		Co–N(DMB)	2.20 ± 0.03
	Co–S	2.28 ± 0.05		Co–$C(CH_3)$	2.00 ± 0.03
cysteinylcobalamin	Co–N_{eq}	1.90 ± 0.01	cyanocobalamin[a]	Co–N_{aq}	1.89 ± 0.01
	Co–N(DMB)	2.13 ± 0.04		Co–N(DMB)	2.15 ± 0.03
	Co–S	2.34 ± 0.03		Co–C(CN)	1.90 ± 0.03
sulfitocobalamin	Co–N_{eq}	1.91 ± 0.02	aquocobalamin[a]	C–N_{eq}	1.89 ± 0.01
	Co–N(DMB)	2.16 ± 0.04		Co–N(DMB)	2.14 ± 0.03
	Co–S	2.35 ± 0.02		Co–$O(H_2O)$	1.90 ± 0.02

[a] Sagi & Chance, 1992.

leads to the conclusion that the effective charge on the cobaltion is largely reduced with respect to CNCbl. The X-ray edge values do not follow the precise trend of the calculated charges on the ligand and of the γ-peak positions of the absorption spectra. This indicates that the π–π^* transitions are affected by the electron density of the ligand and are not as sensitive to the electron density of the central atom. We have previously noted that the X-ray edge position for methylcobalamin is also lower than that for aquo- or cyanocobalamin. We have attributed this to reorganization of electron density from the strongly σ-donating methyl group to cobalt. The sulfur-containing cobalamins all have the same general effect on optical or X-ray edge spectra as a methyl group, inconsistent with oxygen or nitrogen coordination and consistent with sulfur coordination.

In conclusion, on the basis of the optical data, X-ray edge results, and the NMR data we have no doubt of the sulfur coordination. Thus a known coordination environment can be assumed to analyze the EXAFS data. This makes reasonable three kinds of fits: a 5N–1N fit to estimate the axial Co–N distance, a 5N–1S fit to estimate the axial Co–S distance, a 4N–2N fit to give an estimate of the average equatorial Co–N distance, and finally a three-atom consistency test to resolve the distances simultaneously.

Because of the strong electron donation from the sulfur to the cobalt ion one might expect a large trans effect and so longer Co-N_{ax} distances for sulfur-containing cobalamins than that found in (nonalkyl)cobalamins. However, crystallographic data for [(diiodomercuriomethyl)thio]bis(dimethylglyoximato)pyridylcobalt (Co–S: 2.28 Å; Co–N_{ax}: 1.99 Å) (Kergoat et al., 1982) and for *trans*-cyanobis(dimethylglyoximato)pyridylcobalt (Attia et al., 1989) (Co–CN: 1.94 Å; Co–N_{ax}: 2.00 Å) gives almost the same axial Co–N distances, while in *trans*-methylbis(dimethylglyoximato)pyridylcobalt (Bigotto et al., 1989) the axial Co–N distance is significantly longer (Co–CH_3: 1.998 Å; Co–N_{ax}: 2.068 Å). This shows that the Co–N_{ax} distances found here for sulfur-containing cobalamins should not deviate from those for aquo and cyano derivatives. Thus, the close agreement with data of Sagi and Chance (1992) for CNCbl (2.15 ± 0.03 Å) is not surprising.

Summarizing the results for the cobalamins in Table 5, we can conclude that the choice of the axial ligand has no significant effect on the equatorial ligand distances as the average equatorial Co–N distances are the same in all cobalamins within the error. The axial Co–N distances are the result of steric and electronic effects, and the modulation of this bond is very important in regulating the cobalt–carbon bond cleavage in B_{12} enzymes. The structural description of the sulfur-containing cobalamins is a major step in the understanding of their role in the biosynthetic pathway toward B_{12} coenzymes.

ACKNOWLEDGMENT

We thank Dr. Brian Kincaid for providing the source code of the EXAFS data analysis package. We also thank the reviewers for their helpful comments.

SUPPLEMENTARY MATERIAL AVAILABLE

Data analysis tables, wave-vector cube-weighted and background-subtracted χ data, and Fourier transforms of the background-subtracted data of cysteinylcobalamin and sulfitocobalamin (7 pages). Ordering information is given on any current masthead page.

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