J. Am. Chem. Soc. **1991**, *113*, 5299–5304

Concluding Remarks. The results obtained in this study lead to the following conclusions: (1) Complexes with chromium(III)–alkyl σ bonds that have a hydroxyl substituent on the β-carbon undergo fast, acid-catalyzed β-elimination reactions. (2) The product of the latter reaction is a $d \rightarrow \pi$ complex between chromium(III) and the olefin formed. This complex is relatively long-lived when at least two methyl substituents are bound to the olefin. (3) The rate of the β-elimination reaction is considerably affected by the number, and site of binding, of methyl substituents. (4) The stereospecificity in the decomposition of $(H_2O)_5Cr^{III}–CH(CH_3)CH(CH_3)OH^{2+}$ stems from the acid-catalyzed hydrolysis of the $d \rightarrow \pi$ complex, intermediate II, and not from the β-elimination reaction. (5) The formation of the $d \rightarrow \pi$ complex, intermediate II, catalyzes in acid solutions the formation of the thermodynamically less stable *cis*-2-butene vs the more stable *trans*-2-butene.

Acknowledgment. We wish to thank Professors J. H. Espenson, R. Breslow, and M. Sprecher for helpful discussions. This study was supported by the U.S.-Israel Binational Science Foundation (BSF), Jerusalem, Israel and the Israel Atomic Energy Committee. We thank the late Mr. Haim Klein and Mr. David Carmi for technical assistance. D.M. expresses his thanks to Mrs. Irene Evens for her continuous interest and support.

Geometric Conformations of Intermediates of B_{12} Catalysis by X-ray Edge Spectroscopy: Co(I) B_{12}, Co(II) B_{12}, and Base-Off Adenosylcobalamin

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Abstract: Geometric conformations for the cobalt(I) and cobalt(II) B_{12} intermediates in solution are examined by X-ray edge spectroscopy. Integration of 1s-3d and 1s-4p + SD (shakedown) transitions of edge spectra facilitates a direct comparison of relative preedge intensities of the lower valence B_{12} intermediates to model compounds of known structure. Our results indicate that the structure of the cobalt(I) and cobalt(II) forms of B_{12} in solution are distorted square-planar and distorted square-pyramidal, respectively. Base-off Co(III) adenosylcobalamin, generated by acidification, is shown to be a distorted octahedron where the axial 5,6-dimethylbenzimidazole (DMB) ligand is replaced by a water molecule at low temperature. The demonstration that Co(I) B_{12} has a detached DMB axial ligand indicates that cleavage of this bond is required for enzyme reactions like those catalyzed by methionine synthetase (Frasca et al. *Biochemistry* **1988**, *27*, 8458). Such a mechanism must be distinct from those of enzymes involved in homolytic cleavage, where EXAFS studies show a strengthening of the bond from cobalt to DMB for the Co(II) B_{12} species (Sagi et al. *J. Am. Chem. Soc.* **1990**, *112*, 8639). Shifts in the cobalt absorption threshold (main peak of the first derivative spectrum) are shown to be characteristic of the effective charge on the cobalt ion. Edge shifts to lower energy for the reduction of Co(III) to Co(II) to Co(I) cobalamins and cobaloximes follow a linear relationship between the absorption threshold position and the effective charge. The σ donor effects of alkyl ligands bonded to cobalt are also evidenced by shifts in the threshold. A reduced positive charge for these compounds may be important in lowering the barrier to formation of lower oxidation states upon cleavage of the cobalt–carbon bond.

Introduction

B_{12} coenzymes are recognized for playing a key role in a number of important biochemical reactions of metabolism and providing a model system for cis–trans effects in coordination chemistry. To better understand these enzyme reaction mechanisms it is necessary to know the structure of lower valence cobalt(I) and cobalt(II) B_{12} intermediates. Also, cleavage of the 5,6-dimethylbenzimidazole (DMB) base trans to the Co–C bond in alkylcobalamins, and its possible replacement by protein ligands, has been proposed as a possible mechanism for influencing cobalamin reactivity. However, this structure has not been fully characterized.

X-ray crystallographic studies have reported accurate structures for dicyanocobinamide,[1] adenosylcobalamin,[2] and methylcobalamin.[3] Dicyanocobinamide is a B_{12} analogue that contains cyanide groups in the fifth and sixth (axial) positions. Adenosyl- and methylcobalamin both contain a DMB base in the fifth axial position and 5′-deoxyadenosyl and methyl groups in the sixth position. Recently, the structure of cobalt(II) B_{12} was reported by both X-ray crystallography[4] and extended X-ray absorption fine structure (EXAFS) spectroscopy.[5] However, the structures of a number of metabolically and enzymatically important forms, including cyanocobalamin,[6,7] have not been solved satisfactorily due to difficulty in obtaining high quality crystals. Also, some concerns about possible differences in structure for crystal and solution conditions have been raised for cobalamins.[4,5,8] The advantage of X-ray absorption spectroscopy is that it provides direct structural information in solution.

Analysis of 1s-3d and 1s-4p + SD transitions as well as the cobalt absorption edge provides detailed information about the central metal of coordination compounds. Our results indicate

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that the structure of the cobalt(I) form of B_{12}, in solution, is distorted square-planar. "Base-off" adenosylcobalamin, generated by acidification, has a water molecule at the sixth coordination position in place of the DMB ligand at low temperature. In addition, alkyl ligands reduce the effective charge on the central metal. This is demonstrated by cobalt absorption edge shifts to lower energy for methylcobalamin, adenosylcobalamin, and base-off adenosylcobalamin when compared to cyanocobalamin and aquocobalamin. These results are compared to data on the related cobaloximes, which are cobalt compounds with two dimethylglyoxime (DMG) groups as equatorial ligands.

This paper concerns our attempts to use the X-ray edge technique, which in some respects is phenomenological in its application, to obtain structural information on a number of B_{12} compounds of general interest. In order to justify our structural conclusions, we must examine two well-known ideas currently used in the analysis of X-ray spectra and examine the extent to which they are well-founded theoretically and experimentally. The first is the relationship of charge on the central metal and the position of various edge and preedge features. The second is the extent to which certain edge features are correctly assigned or are characteristic of particular molecular geometries. Our results suggest that the X-ray edge technique can be employed to address certain structural questions but must be constrained by chemical reasonableness and appropriate models.

Materials and Methods

Materials. Cyanocobalamin, adenosylcobalamin, hydroxocobalamin, dicyanocobinamide, and sodium borohydride were obtained from Sigma Chemical Co. and were verified by optical spectroscopy. Aluminum oxide and 5,10,15,20-tetraphenyl-21*H*,23*H*-porphinecobalt(II) (CoTPP) were purchased from Aldrich Chemical Co. Glycerol was obtained from Fisher Scientific. All were used without further purification. *trans*-Bis(dimethylglyoximato)methylpyridinecobalt(III) ($Co^{III}DMGMe(py)$) was obtained as a gift from Dr. Martin Engelhard. Cobalt(III) hexamine trichloride was synthesized by standard methods[9] and verified by EXAFS analysis.[5] *trans*-Bis(dimethylglyoximato)diaminecobalt(III) bromide ($Co^{III}DMG(NH_3)_2$) was synthesized according to the methods of Heeg and Elder[10] and Nakatsuka.[11] ^1H NMR spectra of $Co^{III}DMGMe(py)$ in $CDCl_3$ gave chemical shifts from tetramethylsilane (TMS) of 0.82, 2.14, and 8.65 ppm for the axial methyl group, the DMG methyl groups, and the pyridine protons, respectively, and were consistent with previous studies.[12] ^1H NMR spectra of $Co^{III}DMG(NH_3)_2$ in D_2O gave a single peak at 2.37 ppm from 3-(trimethylsilyl)propionic acid, sodium salt (TSP). We tested the glycerol, borohydride, and aluminum oxide in solution or solid form for the presence of cobalt by X-ray absorption prior to sample preparation. No detectable edge jump was observed for 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 Preparation. Cobalt(II) B_{12} was prepared by reduction of 1.5 mL of 12 mM cyanocobalamin with 0.5 mL of 0.8 M $NaBH_4$ under argon as described by Dolphin.[13] The cyanocobalamin and $NaBH_4$ solutions were degassed with argon for 30 min prior to reduction. Cobalt(I) B_{12} was prepared by adding an additional 0.2 mL of 0.8 M $NaBH_4$ solution, under argon, to the yellow-brown solution of cobalt(II) B_{12} with constant stirring. The cobalt(II) B_{12} solution immediately turned to the dark blue-green cobalt(I) B_{12} species.[13]

Bis(dimethylglyoximato)pyridinecobalt(II) ($Co^{II}DMG(py)$) was prepared by reduction of $Co^{III}DMGMe(py)$ with $NaBH_4$ following the same procedure as for the cobalt(II) B_{12}, with the exception that 0.05 mL of degassed 6 M HCl was added to the reaction, giving a neutral solution of $Co^{II}DMG(py)$, which prevented disproportionation to the Co^I- or $Co^{III}DMG$ species.[14] Reduction of a 20 mM solution of $Co^{III}DMGMe(py)$ to the blue-green bis(dimethylglyoximato)pyridinecobalt(I) ($Co^IDMG(py)$) species was accomplished in the same way as for cobalt(I) B_{12}. Base-off adenosylcobalamin was prepared by acidification of a 12 mM solution of adenosylcobalamin in 500 mM phosphate buffer with 6 M HCl to a final pH of 2.0. All samples were characterized by optical absorption spectroscopy and were prepared as solutions in 35% glycerol to reduce sample cracking upon freezing, with the exception of the $Co^{III}DMGMe(py)$, $Co^{III}DMG(NH_3)_2$, CoTPP, and cobalt(III) hexamine which were solids. All samples were placed in 25 × 2.5 × 2 mm deep lucite sample holders covered with mylar tape (approximate volume 200 μL). In order to minimize fluorescence saturation effects, solid samples were diluted with aluminum oxide and ground to a fine powder in a mortar and pestle. The mixed powder was packed in a lucite sample holder. All solid samples were diluted 1:10 in aluminum oxide (by weight). The liquid samples were anaerobically transferred into the sample holders under argon and immediately submerged in liquid nitrogen.

Optical characterization was accomplished by transferring concentrated samples under argon to stoppered cuvettes containing deionized water that were previously degassed for 15 min. Immediately following sample transfer, the cuvette was sealed, and an optical spectrum taken. The cobalt(I) and cobalt(II) B_{12} spectra were consistent with earlier absorption spectra of Beaven and Johnson.[15] Percent conversion from cyanocobalamin to cobalt(II) B_{12} was calculated at 93% ± 3%. Percent conversion to cobalt(I) B_{12} was calculated at 93% ± 2%. The presence of cyanocobalamin in the cobalt(I) and cobalt(II) B_{12} samples was measured by observing the remaining parent compound as described previously.[5] $Co^{II}DMG(py)$ and $Co^IDMG(py)$ were characterized similarly. Percent conversion of $Co^{III}DMGMe(py)$ to $Co^{II}DMG(py)$ was 90% ± 3% and to $Co^IDMG(py)$ was 94% ± 3%. Optical absorption spectra for the DMG series were consistent with earlier works of Schrauzer.[16–18]

Data Collection. Data were collected at the National Synchrotron Light Source, Brookhaven National Laboratory, on beam line X-9A, with a double flat Si (111) crystal monochromator with fixed exit geometry. All experiments were carried out at 115–120 K, and sample temperature was maintained by flowing cooled nitrogen gas through a low-temperature cryostat as described previously.[19] X-ray edge data having 3-eV resolution were recorded by counting at a specific energy for 2 s and incrementing the energy by 0.5 eV from 20 eV below the cobalt edge to 50 eV above the edge. Photon flux was 3.75×10^{11} photons/s at 100 ma beam current; data were generally taken in the range of 90–180 ma. Cyanocobalamin was used as a standard to account for any shifts in the monochromator. 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 PDP 11/23 + computer via CAMAC. 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 the reference detectors.

Data Analysis and Errors. Data were converted to two-column ASCII format, and the files were processed with Galactic Industries SPECTRA CALC (1988) software package. First, linear fits were separately applied to the preedge and EXAFS regions of the spectra to determine the absorption step-jump for each spectrum. This method has been described in detail by a number of previous investigators[20–22] and allows samples of different concentrations to be normalized to each other, so that the integrated intensities of preedge features can be compared. The step-jump for a particular sample is the numerical difference between the preedge and EXAFS region's linear fits. In order to compare spectra under different conditions, each spectrum is normalized to the step-jump of the other spectrum by multiplying one spectrum by the ratio of the two. This process allows for direct comparison of preedge intensities of any pair of spectra. Integration of the 1s-3d and 1s-4p + SD peak areas was accomplished by fitting the preedge region containing the 1s-3d or 1s-4p + SD transition with a cubic polynomial to establish a baseline. The resulting baseline corrected peak was then integrated to determine the peak area. Data are presented in ratio form referenced to the area of the cobalt hexamine 1s-3d peak which was arbitrarily given the value of 1. The actual area of the Co(III) hexamine 1s-3d transition is 2.0 ×

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Table I. Integrated 1s-3d and 1s-4p + Shakedown (SD) Transition Intensities for Cobalamin and Model Compounds[a]

compound	1s-3d	1s-4p + SD	comments
Co(III) hexamine	1.0	<0.3	octahedral, no distortions[32]
Co^{III} DMG $(NH_3)_2$ (**1**)	2.6	<0.1	octahedral, slight distortion[10]
Co^{III} DMG methyl pyridine (**2**)	9.2	<0.2	distorted octahedron, Co–C bond present[38]
Co^{II} DMG pyridine (**3**)	20.5	<0.1	square-pyramidal*
Co^{I} DMG pyridine (**4**)	25.8	<0.3	square-pyramidal, no 1s-4p + SD present*
dicyanocobinamide Co(III)	3.6	<0.3	distorted octahedron, 8.0° tilt in corrin ring[1]
aquocobalamin Co(III)	6.3	<0.5	distorted octahedron*
adenosylcobalamin Co(III)	14.9	<0.6	distorted octahedron, 14.6° tilt in corrin ring, Co–C bond present[2]
methylcobalamin Co(III)	12.5	<0.8	distorted octahedron, 15.8° tilt in corrin ring[3]
cyanocobalamin Co(III)	5.1	<0.1	distorted octahedron, ca. 19° tilt in corrin ring[6,7]
cobalt(II) B_{12}	8.3	<0.6	distorted square-pyramidal 16.3° tilt in corrin ring[4,5]
cobalt(I) B_{12}	<0.6	13.3	distorted square-planar*
base-off adenosylcobalamin, Co(III)	13.4	<0.6	distorted octahedron, Co–C bond present*
Co^{II}TPP	<0.3	59.4	square-planar[36]

[a] Errors are described in text. Geometries determined in this study are indicated by an asterisk.

10^{-5} eV with the step-jump normalized to 1.

Errors in the analysis were estimated from a combination of two factors: errors due to statistical noise and errors introduced due to the method of analysis. Statistical noise was measured in the region prior to the 1s-3d transition. Contributions of the statistical noise to the integrated 1s-3d or 1s-4p + SD peak area were determined by calculating the percent contribution of the statistical noise to the total peak area. This provides a statistical error measure for each and every scan. Statistical error varied from 0.5 to 30% depending on the sample preedge intensity, sample concentration, beam conditions, and signal averaging. Errors introduced 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 generally ≤5%. For example, for dicyanocobinamide in Table I, the 1s-3d intensity is 3.6 times Co(III) hexamine. The 1s-4p + SD intensity is quoted as <0.3. This represents the expected error for this measurement taking into consideration the statistical and systematic errors discussed above. For compounds with 1s-4p + SD transitions, the noise level is seen in the 1s-3d column.

Results and Discussion

As the energy region near the X-ray absorption edge is scanned, the excitation of a core electron to bound states, below the ionization threshold, reveals substantial chemical and structural information. K-edge absorption spectra of octahedral transition-metal complexes containing empty 3d orbitals generally exhibit a low intensity peak approximately 10 eV prior to the metal absorption threshold that has been assigned to symmetry forbidden transitions of 1s core electrons to 3d empty states.[23,24] The most reasonable sources of this intensity for symmetrical compounds, i.e., those having a center of inversion, are contributions from quadrapole allowed transitions, 3d-4p orbital mixing due to vibronic coupling, and overlap of the metal 3d orbitals with 2p ligand orbitals.[23] The intensity of the 1s-3d transition also increases with a decrease in coordination number.[24] This has been attributed to loss of the inversion center in lower symmetry complexes as well as an increase in 3d-4p orbital mixing.[22] Shorter metal-to-ligand distances, which increase the degree of metal 3d-ligand 2p orbital mixing, also increase the intensity of the 1s-3d absorption due to the "molecular cage" size effect on the oscillator strength of the transition.[21,25] 1s-3d transitions are also observed in both square and trigonal-pyramidal complexes as well as in tetrahedral geometries. However, only extremely weak 1s-3d transitions have been observed in pure square-planar complexes through the use of plane polarization techniques.[26] Although these are a somewhat complicated set of structural dependencies, coordination number is the most influential effect on the intensity due to the substantial changes in 3d,4p mixing.[21,22]

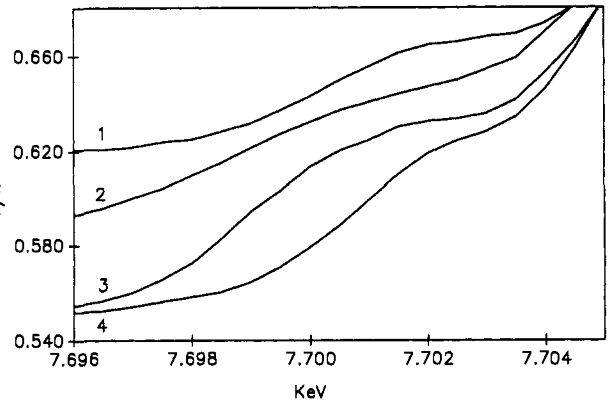

Figure 1. Comparison of X-ray fluorescence preedge data of 1s-3d transitions observed in model compounds: (1) $Co^{III}DMG(NH_3)_2$, (2) Co(III) hexamine; (3) cyanocobalamin; and (4) dicyanocobinamide.

The sharply rising slope of the 1s-4p transition also contains important geometrical information. Here, the spectral feature that has often been assigned to the symmetry forbidden 1s-4s transition[23] actually corresponds to a 1s-4p + SD (shakedown) phenomenon in complexes where charge transfer is present.[27,28] Shakedown is the transfer of an electron, concurrent with the 1s-4p transition, from the ligand orbitals to the metal atom as a result of core hole creation. The outcome is a transition in addition to and lower in energy than 4p due to the screening effect of the ligand-metal charge transfer. The intensity of the 1s-4p + SD transition is maximal for square-planar configurations and is essentially absent in tetrahedral and all five- and six-coordinate complexes studied.

The cobalt absorption threshold position (determined as the main peak of the first derivative spectrum) as well as the 1s-3d and 1s-4p transitions have been used to detect changes in the oxidation state of the central metal. For example, for a series of vanadium oxides, Wong et al. found that all three of these quantities varied linearly with formal oxidation state of vanadium.[21] Bianconi studied a series of manganese oxides and found a similar effect.[29] However, the threshold position was slightly more reliable in predicting the oxidation state accurately in the latter study. All these charge indicators follow Kunzl's law,[30,31] which proposes a linear relationship between the energy of absorption edge features and the oxidation state of the absorbing metal atom. This shift can be rationalized by considering the increasing potential of the nucleus on 1s core electrons and reduction of the core repulsive Coulumbic interaction with other electrons in the compound as valence state increases.

Geometric Distortions and Intensities of Preedge Transitions. Knowledge of the geometric and electronic environment of atoms in lower valence states of cobalt corrin compounds is fundamental toward understanding the catalytic processes of the B_{12} system. X-ray edge spectroscopy can contribute to our understanding of

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these processes by providing both geometric and electronic information. We have compared the integrated areas of 1s-3d and 1s-4p + SD transitions for intermediates of B_{12} enzyme catalysis and model compounds of various geometries in Table I. Compounds containing primarily nitrogen ligands in the first coordination sphere are used as models for the B_{12} intermediates. The structure of cobalt hexamine is almost a perfect octahedron having a maximum deviation of only 2.1° from ideal octahedral bond angles.[32] Consequently, cobalt hexamine has the smallest integrated 1s-3d transition area for the octahedral compounds. $Co^{III}DMG(NH_3)_2$ is slightly more distorted from ideal octahedral geometry having an 8.5° variation in equatorial nitrogen bond angles[10] and exhibits a 1s-3d intensity of 2.6 times that of cobalt hexamine (Figure 1). $Co^{III}DMG(NH_3)_2$ contains six nitrogens in the first coordination sphere, like hexamine, maintaining consistency in the ligand system to demonstrate the sensitivity of the technique to structural distortions.

Since the corrin ring system is not flat, a second set of model compounds consisting of dicyanocobinamide and cyanocobalamin is used for comparison to the lower valence B_{12} intermediates. A tilt in the corrin ring, giving it an appearance of mostly open butterfly wings, occurs at the intersection of two distinguishable planes. Plane 1 contains N_{23} and N_{24} of the tetrapyrrole rings and bisects the corrin ring, passing through C_{10} and cobalt.[1] Plane 2 contains N_{21} and N_{22} and shares the axis with plane 1 passing through C_{10} and cobalt.[1] Changes in this tilt angle may be critical to the dynamics of B_{12} enzyme mechanisms. Dicyanocobinamide is a distorted octahedral compound that contains an 8.0° tilt in the corin ring plane.[1] When compared to the more distorted cyanocobalamin (ca. 19° tilt), the integrated 1s-3d intensity increases from a value of 3.6 for dicyanocobinamide to 5.1 for cyanocobalamin (Figure 1), although this may be related to the change in the fifth ligand.

The preceding paragraph makes clear that the integrated areas of the 1s-3d peak are very sensitive to small structural changes that are known to exist from crystallographic data. However, such small changes may not be useful in deducing the structure of unknown compounds, especially if the identity of the ligands are not known. For example, comparison of "molecular cage" effects[21] on the 1s-3d intensity of Co(III) hexamine vs $Co^{III}DMG(NH_3)_2$ predicts an increase on the order of 10% in 1s-3d intensity, since the average of the first coordination sphere bond lengths for $Co^{III}DMG(NH_3)_2$ are decreased from those of Co(III) hexamine. However, the observed increase in 1s-3d intensity is much greater than predicted solely by the "molecular cage" effect. This result demonstrates how changes in the symmetry of the ligand field effect the 1s-3d intensity. Further perturbation of the centrosymmetric ligand environment occurs when a carbon atom is added to the octahedral environment in compounds such as $Co^{III}DMGMe(py)$. The "molecular cage" effect would predict no change in 1s-3d intensity due to no change in average first coordination sphere bond distances between $Co^{III}DMGMe(py)$ and $Co^{III}DMG(NH_3)_2$. However, we see a substantial 3.5-fold increase in 1s-3d intensity demonstrating the primacy of 3d-4p mixing in the determination of 1s-3d intensities.[22] Compounds containing a Co–C bond and five nitrogen ligands, such as adenosylcobalamin, methylcobalamin, and $Co^{III}DMG(py)$, have significantly greater 1s-3d intensities than compounds of similar geometry (octahedral) that do not contain a Co–C bond (Figure 2). When a σ-bonding alkyl group is present in the octahedron, a noncentrosymmetric ligand field is created, where the alkyl carbon is known to reduce the rigorous symmetric-(g) character of the 1s-3d transition and increases the degree of 3d-4p orbital mixing.[33]

The structure of cobalt(II) B_{12} has been examined by both EXAFS[5] and X-ray crystallography.[4] It is a five-coordinate square-pyramidal species with cobalt ca. 0.13 Å out of the mean plane of the corrin nitrogens toward the DMB ligand. The presence of a strong 1s-3d transition, 8.3 times the intensity of cobalt hexamine, as well as the absence of a 1s-4p + SD peak in the edge spectrum is expected. This intensity difference compared to all-nitrogen-containing octahedral complexes is within the expected range for pure coordination number effects.[22] The spectrum of base-off adenosylcobalamin (1s-3d intensity 13.4 times that of Co(III) hexamine) is similar to that of methyl- and adenosylcobalamin. A five-coordinate structure for base-off adenosylcobalamin has been predicted for room temperature; however, at lower temperatures the equilibrium favors the six-coordinate form.[34,35] The minimal change from the 1s-3d intensity of adenosylcobalamin strongly supports six coordination at ca. 110 K, presumably with a water molecule replacing the DMB ligand (Figure 3). The substitution of water is not expected to influence the degree of 3d,4p mixing.

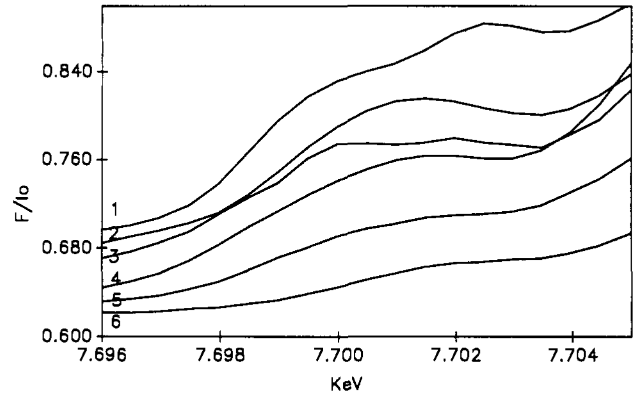

Figure 2. X-ray fluorescence preedge data of 1s-3d transitions comparing model compounds to compounds containing Co–C bonds: (1) base-off adenosylcobalamin (2) adenosylcobalamin, (3) methylcobalamin, (4) $Co^{III}DMGMe(py)$, (5) cyanocobalamin, and (6) $Co^{III}DMG(NH_3)_2$.

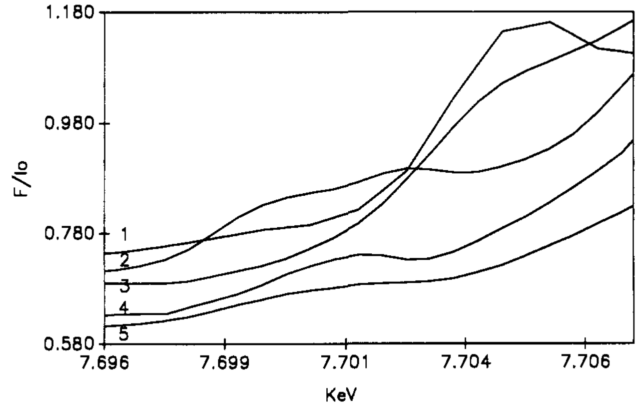

Figure 3. Comparison of X-ray fluorescence preedge data of transitions observed in cobalt model and cobalamin compounds: (1) CoTPP (not to scale compared to other spectra), (2) base-off adenosylcobalamin; (3) Co(I) B_{12}, (4) Co(II) B_{12}, and (5) cyanocobalamin.

The spectrum of the cobalt(I) B_{12} species contains a moderate intensity 1s-4p + SD peak and no 1s-3d transition. The absence of a 1s-3d transition (less than 30% of cobalt–hexamine) rules out coordination numbers five and six as well as tetrahedral geometries. The presence of the 1s-4p + SD peak confirms the square-planar assignment. Extensive studies of square-planar copper chlorides[26,27] and nickel tetracyanide complexes[28] have been used in an attempt to establish a theoretical basis for the 1s-4p + SD transition. Kosugi et al., with use of polarized synchrotron radiation, have established that 1s-4p + SD transitions have a strong *z* dependency (where the *z* axis is normal to the molecular plane).[28] The relationship between the shakedown process and square-planar geometry may lie in the interaction of the empty $4p_z$ metal orbital with ligand orbitals from which charge transfer

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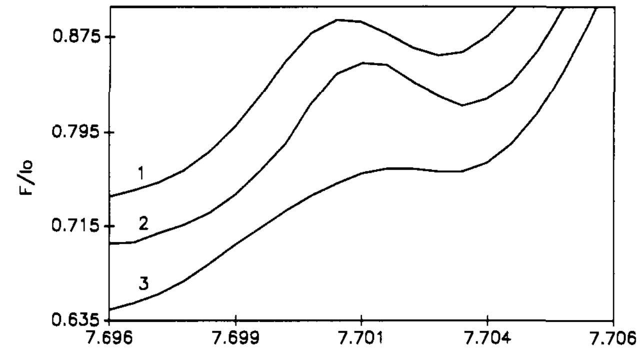

Figure 4. Comparison of X-ray fluorescence preedge data of 1s-3d transitions observed in the dimethylglyoxime (DMG) series: (1) Co^{I}DMG(py), (2) Co^{II}DMG(py), and (3) Co^{III}DMGMe(py).

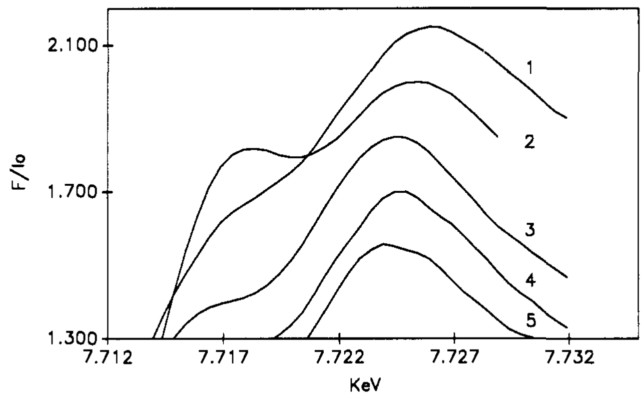

Figure 5. X-ray fluorescence edge data of shifts in 1s-4p transitions for cobalamin compounds. Spectra are calibrated by comparison to a cyanocobalamin standard; absolute energies are not calibrated: (1) base-off adenosylcobalamin, (2) cyanocobalamin, (3) adenosylcobalamin, (4) Co(II) B$_{12}$, and (5) Co(I) B$_{12}$.

to the metal can occur. The 4p$_z$ orbital that would normally be involved in σ bond formation in the presence of axial ligands in five- and six-coordinate compounds may be the key to the shakedown process. Comparison with CoTPP, a nearly perfect square-planar complex,[36] shows a smaller intensity for cobalt(I) B$_{12}$ (Figure 3). We speculate that Co(I) B$_{12}$ deviates from planar geometry due to the fusion of corrin tetrapyrrole rings A and D. This results in enough distortion to reduce the transition intensity. The square-planar configuration has been previously predicted based on electronic absorption studies.[15,37]

For the cobalt(III, II, I) DMG series, as the coordination number decreases a significant increase in integrated 1s-3d intensity is observed (Figure 4). The Co^{III}DMGMe(py) is distorted from octahedral geometry, having an average deviation of 8.8° from ideal bond angles for the equatorial nitrogens.[38] This deviation and the presence of the Co–C bond results in the observed 1s-3d area of 9.2. The Co^{II}DMG(py) species is known to be five coordinate based on ESR data.[39] The 1s-3d intensity is consistent with the ESR data. Notice, however, that this species has a higher intensity than Co(II) B$_{12}$. We speculate that the 16° tilt of the corrin ring[4] reduces the transition intensity compared to the more nearly square-pyramidal Co^{II}DMG(py) species.[40] The spectrum of Co^{I}DMG is quite different from Co(I) B$_{12}$, in that no 1s-4p + SD peak is observed and a strong 1s-3d peak is seen. Polarographic and kinetic studies by Schrauzer[14,41] support a five-coordinate Co^{I}DMG(py) species, where the pyridine ligand is presumed to be in an equilibrium with a four-coordinate species.[42] A four-coordinate tetrahedral configuration, which would have an even stronger 1s-3d transition, is considered unlikely due to restrictions imposed by the equatorial DMG ligands.

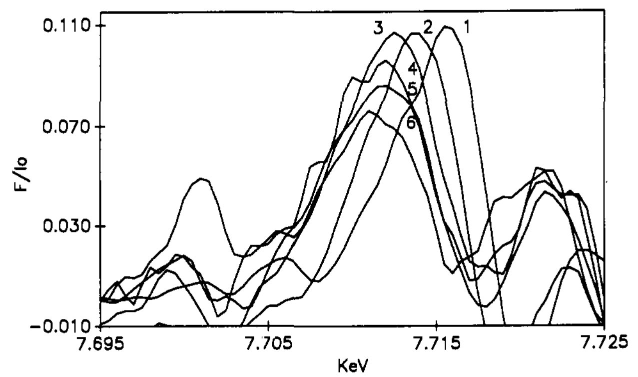

Figure 6. Comparison of shifts in first derivative X-ray fluorescence edge data of cobalamin compounds containing Co–C bonds to lower valence B$_{12}$ intermediates and model compounds. The cobalt edge is defined as the absorption maximum, generally seen as the second peak in the derivative spectrum. Spectra are calibrated to a cyanocobalamin standard; absolute energies are not calibrated: (1) dicyanocobinamide, (2) cyanocobalamin; (3) methylcobalamin, (4) Co(II) B$_{12}$, (5) adenosylcobalamin, and (6) Co(I) B$_{12}$.

Table II. X-ray Fluorescence Edge Data of Shifts in 1s-4p and Cobalt Edge Positions for Cobalamin and Model Compounds[a]

compound	formal charge on Co	1s-4p shift from cyanocobalamin	cobalt edge shift from cyanocobalamin
cyanocobalamin	3+	0.0	0.0
cobalt(II) B$_{12}$	2+	−0.5	−1.5
cobalt(I) B$_{12}$	1+	−1.0	−2.5
dicyanocobinamide	3+	+0.5	+2.0
aquocobalamin	3+	0.0	0.0
adenosylcobalamin	3+	−0.5	−1.5
methylcobalamin	3+	−0.5	−1.0
base-off adenosylcobalamin	3+	+0.5	−1.0
cobalt hexamine	3+	−1.5	0.0
cobalt DMG(NH$_3$)$_2$	3+	−3.0	+1.0
cobalt(III) DMG methylpyridine	3+	−4.0	+0.5
cobalt(II) DMG pyridine	2+	−4.0	−0.5
cobalt(I) DMG pyridine	1+	−6.0	−1.5

[a] Spectra are calibrated by comparison to a cyanocobalamin standard; absolute energies are not calibrated. 1s-4p shifts are obtained directly from the X-ray fluorescence data. Cobalt edge shifts are obtained from the first derivative of X-ray fluorescence data.

Effective Metal Charge and Shifts in the Absorption Edge. Examination of the 1s-4p transition in B$_{12}$ compounds shows a surprisingly small 0.5 ± 0.2 eV shift to lower energy for changes in the oxidation state from Co(III) (cyanocobalamin or aquocobalamin) to Co(II) to Co(I) B$_{12}$ (Figure 5), although Kunzel's law is obeyed. However, no shift is observed in the 1s-4p transition for the Co^{III}DMGMe(py) compared to Co^{II}DMG(py). A 2.0 eV shift to lower energy is observed in the comparison of Co^{III}DMGMe(py) to Co^{I}DMG(py), which is consistent with a two-electron reduction to the Co^{I}DMG(py) species.[43] Also puzzling is that the 1s-4p transitions for the cobalt dimethylglyoxime compounds are observed 4–6 eV lower in energy than the corresponding cobalamin compounds.

First derivative spectra are very useful in identifying the cobalt absorption edge.[21,29] We have identified the absorption edge as the absorption maximum in our first derivative spectra, generally seen as the second peak in the derivative spectrum (Figure 6).

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Shifts of the cobalt absorption edge are compared with results from the analysis of the 1s-4p transition (Table II). Edge shifts for the reduction of Co(III) to Co(II) B_{12} and Co(II) to Co(I) B_{12} are 1.5 ± 0.2 and 1.0 ± 0.2 eV, respectively (Figure 6). This is greater than the shifts observed for 1s-4p transitions in both cases. For the cobalt DMG series, reduction from Co^{III}DMG-Me(py) to Co^{II}DMG(py) and Co^{II}DMG(py) to Co^{I}DMG(py) show cobalt edge shifts of 1.0 ± 0.2 eV to lower energy for each one-electron reduction. These results are much more consistent with Kunzel's law. Also, the Co(DMG) compounds show only small deviations from the positions of their corresponding cobalamins.

Application of Batsanov's concept of coordination charge[44] to the 1s-4p transition as well as the cobalt edge data does not correct the apparent inconsistencies. Problems arise in the treatment of multiple atom coordinating ligands, where, for example, a carbon of an alkyl group is considered equivalent to the carbon atom of a cyanide group when calculating the coordination charge. Qualitatively, Batsanov's concept states that as coordinating ligands pull valence electrons away from the metal, due to their electronegativity, the electrons associated with the central metal (i.e., lower atomic orbitals) are pulled in closer to increase shielding of the existing nuclear charge. Thus, the energy required to remove an electron from the K-shell is increased with increased coordination charge. However, the concept does not account for other ligand-metal electronic interactions such as π back-bonding or π accepting ligands and the electron-donating properties of methyl and adenosyl groups. In summary, shifts in the cobalt threshold, as opposed to shifts in the 1s-4p transition, seem to provide a chemically reasonable picture of the effective charge on the cobalt atom (Table II). Edge shifts referenced to cyanocobalamin follow Kunzl's law closely for the reduction of Co(III) to Co(II) to Co(I) cobalamins and cobaloximes. In the following sections we use the cobalt edge results to analyze the effect of different ligands on cobalt electron density.

Examination of the edge shift in adenosylcobalamin shows a 1.5 ± 0.2 eV shift to lower energy compared to cyanocobalamin (Figure 6). The alkyl group of adenosylcobalamin is strongly electron-donating[1] and reduces the effective charge on cobalt from the expected value of Co(III). The cobalt edge for adenosylcobalamin occurs at the same energy as Co(II) B_{12}. This implies that the effective charge on cobalt in both species is similar. The localization of electron density on cobalt in adenosylcobalamin may aid in its catalytic ability, favoring conversion to the Co(II) B_{12} form. The edge shift for methylcobalamin is 1.0 ± 0.2 to lower energy, indicating that the adenosyl group may be acting as a stronger electron-donating substituent than the methyl group. This may pertain to differences in their catalytic reactivity.

The edge shift of 2.0 ± 0.2 to higher energy for dicyanocobinamide (Figure 6) may be due to the presence of two π accepting cyanide ligands in the fifth and sixth axial positions. The effect of replacing the neutral nitrogen of the DMB ligand in the sixth position, found in cyanocobalamin, with a CN^- ligand forming dicyanocobinamide is to change the distribution of electron density about the cobalt atom and to increase the polarization of the CN–Co–CN linkage, resulting in an increase in the effective nuclear charge on cobalt. Comparison of the edge positions of Co^{III}DMGMe(py) to $Co^{III}DMG(NH_3)_2$ shows that the $Co^{III}DMG(NH_3)_2$ is shifted 0.5 ± 0.2 to higher energy. The presence of an electron-donating methyl group in Co^{III}DMG-Me(py) causes the difference in effective charge on cobalt and is consistent with observations from the cobalamin series. However, the effect is not as large as that for the cobalamins. The cobaloximes are also seen to have slightly more positively charged cobalt ions than comparable cobalamins. Glusker predicted this based on the shorter axial distance to the nitrogenous base for cobaloximes, but the differences in equatorial ligand may also be involved.[1]

Conclusions

Development of the X-ray edge technique has provided a powerful tool for the analysis of local molecular geometry. Integration of preedge transitions of X-ray edge spectra has enabled us to predict the geometries of Co(I) B_{12} and the base-off form of adenosylcobalamin as distorted square-planar and distorted octahedral, respectively. The absence of a DMB ligand for the Co(I) B_{12} intermediate is quite relevant to the mechanism of heterolytic cleavage for methionine synthetase and the coronoid protein of *Clostridium thermoaceticum*.[45,46] For these enzymes to promote Co(I) formation, a weakening and/or cleavage of the Co–DMB bond is indicated. Interestingly the protein studied by Ragsdale and co-workers[46] has characteristics of a base-off methylcobalamin, which we interpret as favoring the cleavage of the cobalt–carbon bond heterolytically. Lexa et al.[47] and Ragsdale et al.[46] have demonstrated that reduction of Co(II) species for model compounds and enzyme are more facile for the base-off Co(II) forms. This suggests that removing the base eliminates a possible barrier to Co(I) B_{12} formation. We have not yet established whether base-off adenosylcobalamin is five-coordinate at room temperature; however, removal of the DMB base is a logical prerequisite for formation of a Co(I) B_{12} complex.

Acknowledgment. This research is based upon work supported in part by the Cooperative State Research Service, U.S. Department of Agriculture, under agreement No. 90-37200-5357 of the Program in Human Nutrition and supported in part by a grant from the Upjohn Company. We thank Syed Khalid and Mike Sullivan for technical assistance at beamline X-9A. We also thank Amanda Ennis for helpful translations and Linda Powers and Britton Chance for advice and encouragement. We also thank the reviewers for their helpful comments and suggestions. The construction and operation of beamline X-9A is supported by National Institutes of Health Grant RR-01633 and National Science Foundation Grant DMR-85190959. The National Synchrotron Light Source is supported by the Department of Energy, Division of Materials Sciences and Division of Chemical Sciences.

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