Extent of Trans Effects in (Nonalkyl)cobalamins: Steric Effects Control the Co-N Distance to 5,6-Dimethylbenzimidazole

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Abstract: Although a number of (nonalkyl)cobalamins are considered essential to human nutrition, providing starting materials in the synthesis of alkylcobalamin coenzymes, their structures are either not available or are very old and unreliable.[1,2] We present cobalt-ligand distance information in solution for methylcobalamin, cyanocobalamin, and aquocobalamin using extended X-ray absorption fine Structure (EXAFS) spectroscopy. Methylcobalamin was examined as a control to verify our ability to accurately assign cobalt-ligand distances, since this compound has a known X-ray structure.[3] The structures of cyanocobalamin and aquocobalamin show a remarkable and unexpectedly long cobalt-ligand bond distance of 2.14-2.15 ± 0.03 Å to the 5,6-dimethylbenzimidazole (DMB) ligand, while the cobalt-ligand distances to CN and H_2O fall in the expected range of 1.90 ± 0.03 Å. The structural trans effects of CN and H_2O are reasonably considered to be weak; therefore, the relatively long Co-N(DMB) bond must be the result of steric repulsions between the DMB ligand and the corrin equatorial ligand.

Introduction

Experimental evidence available on the trans effects of axial ligands for a number of cobalt compounds, many used as models for cobalamins, shows a consistent trend of steric and electronic effects that are well-established benchmarks for coordination chemistry.[4] These observations argue that similar effects should be present in cobalamin compounds. Yet it seems that more attention has been given over the years to the structures of alkylcobalamins over (nonalkyl)cobalamins in order to probe the mechanism of Co-C bond cleavage in B_{12}-dependent enzyme systems. To date, accurate structures are available for (5′-deoxyadenosyl)cobalamin,[5] methylcobalamin,[3] and (adeninylpropyl)cobalamin.[6] However, a number of (nonalkyl)cobalamin structures important to cobalamin chemistry are not well-understood.[7] Accurate structural information for aquocobalamin and cyanocobalamin is either unavailable or unreliable.[1,2] Two important questions that are addressed and answered here. (1) What are the cobalt-ligand distances of these nonalkylcobalamin compounds? (2) How do these distance parameters fit in the family of trans effects that are clearly observed in alkylcobalamin analogues?[4,5]

In order to obtain structural information at the cobalt atom, we have carried out extended X-ray absorption fine structure (EXAFS) studies of aquocobalamin and cyanocobalamin. Also, we report an analysis of methylcobalamin EXAFS data to test our ability to accurately reproduce cobalt-ligand distances derived from crystallography. We find that the Co-C (to CN) and the Co-O (to H_2O) distances in cyanocobalamin and aquocobalamin are in good agreement with data from relevant model compounds. An unexpected result is that the distance from cobalt to the 5,6-dimethylbenzimidazole nitrogen ligand (DMB) in both structures is longer than expected based *solely* on differences in electronic trans effects compared to those of alkylcobalamins. Although the structural trans effects of the CN and H_2O ligands in these compounds are relatively small, there is a significant steric repulsion between the corrin plane and the bulky DMB ligand that results in a relatively long distance to the DMB ligand.

Experimental Section

Materials. Methylcobalamin was obtained from Sigma Chemical Co. Hydroxocobalamin and cyanocobalamin were generously provided by Dr. Paul Dowd and also were purchased from Sigma Chemical Co. Methylcobalamin, cyanocobalamin, and hydroxocobalamin were characterized by optical absorption spectroscopy. Aluminum oxide and (5,10,15,20-tetraphenyl-21*H*,23*H*-porphyrinato)cobalt(II) (CoTPP) were purchased from Aldrich Chemical Co. Potassium cobalt(III) hexacyanide was obtained from Alfa Morton Thiokol Inc., and glycerol was purchased from Fisher Scientific. Tris(acetylacetonato)cobalt(III) (CoAcAc) was obtained from Dr. Louis C. W. Baker. All reagents were used without further purification.

Sample Preparation. Methylcobalamin was dissolved in 35% glycerol to obtain an 8-mM solution. An optical spectrum taken before and after exposure to the X-ray beam showed that sample integrity was maintained. Cyanocobalamin and aquocobalamin were dissolved in a 1:2 mixture of ethylene glycol and water at a concentration of 8-9 mM. A reflectance spectrum of the frozen solutions was recorded before and after data collection and showed no damage. Glycerol and ethylene glycol were used as solvents in order to reduce sample cracking upon freezing. Solid samples of cyanocobalamin, methylcobalamin, CoTPP, cobalt(III) hexacyanide, and CoAcAc were prepared by diluting the pure sample in aluminum oxide powder and grinding the mixture to a fine powder in a mortar and pestle. All solid samples were diluted 1:10 (by weight), resulting in overall metal wt % of ca. 0.03-0.07%. The mixed powder was packed in 25- × 2.5- × 2-mm leucite sample holders and covered with Mylar tape (10- × 5-mm sample holders used for the focused beam experiments). Liquid samples were injected into the sample holders and frozen in liquid nitrogen prior to data collection.

Data Collection. Data were collected at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, on beam lines X-9A and X-10C, and at Stanford Synchrotron Radiation Laboratory (SSRL) on beam line II-2. Methylcobalamin data were collected at NSLS on beam line X-9A, aquocobalamin and cyanocobalamin were collected at SSRL on beam line II-2, and aquocobalamin data were also collected at NSLS on beam line X-10C. CoTPP, CoAcAc, and cobalt hexacyanide model compounds were collected at NSLS on both beam lines, and CoTPP data were also collected at SSRL. All experiments were carried out at 115-120 K, and the sample temperature was maintained by flowing cooled nitrogen gas through a low-temperature leucite cryostat.[8] NSLS data were collected using a double flat Si(111) crystal

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monochromator (unfocused beam). A mirror was used to reject harmonics before the monochromator. EXAFS data with 3-eV resolution were collected as described previously.[9,10] SSRL EXAFS data were collected using Si(111) monochromator crystals (focused beam) and V slits were adjusted to give 3-eV resolution at the cobalt K-edge.[9] A mirror was used to focus the beam to ca. 4- × 8-mm dimensions. X-ray fluorescence data were collected with a phototube array shielded by an iron oxide filter and positioned at a right angle to the incident beam.[10]

Data Analysis and Errors. First-shell EXAFS data were manipulated and analyzed using the University of Washington EXAFS package on the Georgetown University 8700 VAX computer. Data were processed using a linear pre-edge fit, cubic polynomial spline background (isolated atom) substraction, wave-vector cubed weighting, Fourier transformation, Fourier filter, and back-transform as described previously.[9,12] Fourier-filtered data were analyzed by a nonlinear least-squares fitting procedure in the standard manner.[13] All EXAFS scans were checked for edge position and noise prior to data processing. Sharp glitches caused by nonstatistical events were removed before any further processing by fitting the data on both sides of the glitch with a polynomial interpolation.

All unknowns were fitted to model compounds containing covalent-type metal–ligand bonds that have accurate crystallographic structures. CoAcAc, cobalt hexacyanide, and CoTPP model compound data were collected and treated in the same way as the unknowns in manipulations and in the different fits. We also compared CoTPP data collected at NSLS to data collected at SSRL. NSLS CoTPP data were fitted to SSRL data in a one-atom-type fit where the coordination number (N), distance (r), and Debye–Waller factor ($\Delta\sigma^2$) were allowed as free parameters. The results of these fits show four nitrogen ligands at 1.94 ± 0.01 Å, $\Delta\sigma^2 = 0.0007$, $N = 3.4 \pm 0.6$, and $\chi^2 = 0.4$.

CoTPP was used as a standard in the fitting procedure for methylcobalamin, cyanocobalamin, and aquocobalamin, cobalt hexacyanide was used in the fitting of cyanocobalamin and methylcobalamin, and CoAcAc was used in the fitting of aquocobalamin. CoTPP has four nitrogen atoms at an average distance of 1.949 (3) Å,[14] cobalt hexacyanide has six carbon ligands at an average distance of 1.893 (11) Å,[15] and CoAcAc has six oxygen ligands at an average distance of 1.897 (8) Å.[16] The average distances were calculated using a $1/r^2$ average.

Background-subtracted data were Fourier-transformed using tailed windows.[10] Various Fourier-transform windows were examined by fitting each set of data using different windows. The window function selecting the k-range for the background-subtracted data can be a square window with sharp cutoffs or a smooth one that tails to 0 at the window edges. Based on our examination of window shapes and ranges, we chose a window that gave the best fit while maintaining sufficient degrees of freedom in the fitting procedure. The tailing was centered at 1.5 (K_1) and 10.5 Å^{-1} (K_2) in k-space for cyanocobalamin and methylcobalamin and 1.5 (K_1) and 10.2 Å^{-1} (K_2) for aquocobalamin. For the Fourier-filter back-transform window, the tail centers were set at 0.8 (r_1) and 2.1 Å (r_2) for aquocobalamin and at 0.9 (r_1) and 2.3 Å (r_2) for cyanocobalamin and methylcobalamin. The window tails were provided by applying cosine-squared tapers on the Fourier-transformed data. At $k_1 - Dk_1/2$ and at $k_2 + Dk_2/2$ the window function tails to 0. The back-transformed window or r function is of the same shape as the k window. For all three fits, $Dk_1 = Dk_2 = 3$ and $Dr_1 = Dr_2 = 0.3$, this produces a window that tailed to 0 at $k = 0$ and at $k = 11.7$ Å^{-1} (for aquocobalamin). The width and the position of the windows were chosen to isolate the first-shell contributions of the model and unknown data.

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 contributions to noise in the data, we analyzed independent partial sums of scans and noted the differences.[17] 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 χ^2 (the sum of residuals squared). The fitting errors for the first-shell distances are obtained by changing (or "stepping") the r parameter in the curve fitting, while least-squares refining the other parameters, until the χ^2 of that particular fit is doubled. A plot of χ^2 vs r resulted in a well-defined minimum from which the fitting error may be deduced.[17] 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), which is the square root of the sum of the squares of these errors. For example, the error deduced from the partial-sums error analysis on both axial ligands in methylcobalamin was 0.01 Å, and the error bars obtained from mapping χ^2 were 0.03 Å; therefore, the reported errors for these ligands' distances are both 0.03 Å.

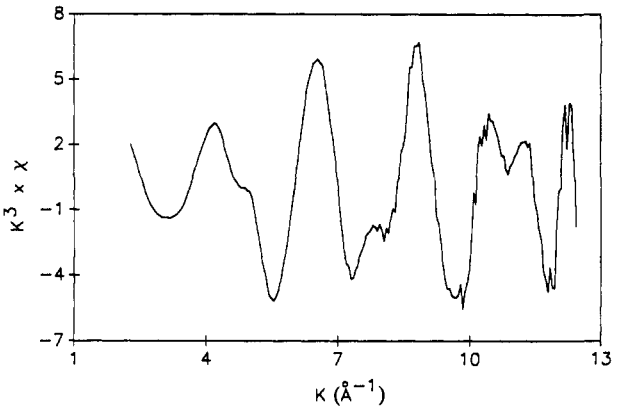

Figure 1. Wave-vector cubed, background-subtracted data for cyanocobalamin. Monochromator glitches have been removed from data by replacement of cubic polynomial fits across the glitches. Data were Fourier-transformed from 1.5 to 10.5 Å^{-1} in k-space with cosine-squared tapered windows as described in the text.

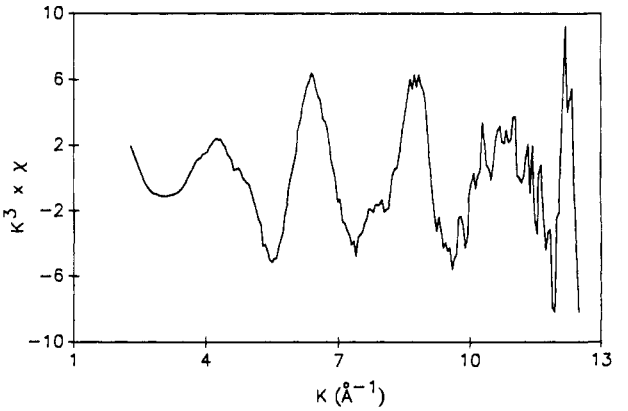

Figure 2. Wave-vector cubed, background-subtracted data for aquocobalamin. Data were Fourier-transformed from 1.5 to 10.2 Å^{-1} in k-space with cosine-squared tapered windows.

Results

Figures 1 and 2 show the k^3-weighted background-subtracted data for cyanocobalamin and aquocobalamin. The Fourier transforms of Figures 1 and 2 are very similar for both the first-shell peaks and the higher shells (data provided in supplementary material). All compounds were analyzed in the same manner by performing one- and two-atom-type fits and a three-atom consistency test. The fits allow free parameters of distance (r) and Debye-Waller factor ($\Delta\sigma^2$) shift (both with respect to the model compound), where the coordination number is fixed at six ligands in the one-atom-type fit and at 5:1 and 4:2 ratios (totaling

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Table I. Nonlinear Least-Squares Fitting Solutions for Cyanocobalamin EXAFS Spectra[a]

fit type	fit	model	r	N	ΔE_0	$\Delta\sigma^2$	χ^2
one-atom	1	Co–N	1.89 ± 0.05	6	0	0.002	23.9
two-atom	2	Co–N	1.90 ± 0.02	5	−2	0.003	2.3
		Co–N	2.15 ± 0.03	1	−5	0.002	
	3	Co–N	1.93 ± 0.02	5	−1	0.002	1.73
		Co–C	1.90 ± 0.04	1	−5	0.007	
	4	Co–N	1.88 ± 0.01	5	0	0.003	3.99
		Co–C	2.22 ± 0.03	1	−5	0.002	
	5	Co–N	1.89 ± 0.01	4	−3	0.002	1.97
		Co–N	2.08 ± 0.02	2	−5	0.004	
three-atom	6	Co–N	1.89	4	0	−0.001	1.27
		Co–N	2.15	1	−4	0.003	
		Co–C	1.90	1	−4	0.002	

[a] Parameters: r, distance in Å; N, coordination number; ΔE_0, energy shift relative to model compound; $\Delta\sigma^2$, Debye–Waller factor shift relative to model; χ^2, sum of residuals squared. CoTPP and cobalt hexacyanide were used as model compounds for these distance solutions.

six ligands) for the two-atom-type fit. The three-atom consistency test, however, has fixed coordination numbers (N) at 4:1:1 ligands in addition to fixed distances (r_1, r_2, r_3) that are deduced from the two-atom-type fit. All data are treated and fitted by the following logic: since cyanocobalamin, aquocobalamin, and methylcobalamin are all octahedral complexes, a one-atom-type fit, with six fixed ligands, is performed first in order to give a rough estimate of the average distance around the cobalt atom for the different compounds. This type of fit is not intended to be accurate since the distances around the metal ion are not expected to be of the same length and the ligands are not the same in nature. The two-atom-types fits are used in order to resolve the various distances around the metal, with the 5:1 fit resolving possible axial ligand distances opposite the fixed coordination number of one (Table I, fits 2–4) while a 4:2 fit resolves the Co–N equatorial distances opposite the fixed coordination number of four (Table I, fit 5). The two-atom-type fits often suggest more than one solution for the structure, and these are considered as hypotheses at this stage. Finally the hypotheses (a combination of axial and equatorial distances) are examined by the three-atom consistency test. We performed this test in two ways. The first way involved fixing both coordination numbers and distances (taken from the two-atom-type fit) for all bond distances, while $\Delta\sigma^2$ remains as a free parameter. The second way involved having one additional distance parameter float. The final distance solution is chosen by the best fit obtained from the three-atom consistency test that is consistent with the two-atom results.

The two-atom fits, used for EXAFS data of the k-range in this paper, are generally unable to resolve distances separated by less than 0.15 Å,[13] in this case equatorial ligand distances from axial ligand distances. However, factors present here give a slight advantage over this theoretical estimate. This calculation is for the resolution of identical backscattering atoms at room temperature. In this case we are attempting to resolve Co–C or Co–O contributions from the equatorial nitrogens (the Co–N distance to the DMB ligand is well-resolved by distance alone), and although the (backscattering) phase differences between adjacent atoms in the periodic table are small, they increase at lower temperatures. Also, we are certain of the identity of the ligands in these compounds. Thus, we can search for solutions to the data set that are consistent with particular combinations of ligands we know to be there.

The results of nonlinear least-squares fitting for cyanocobalamin are summarized in Table I. The E_0 shift was stepped for −8 to 8 in order to derive the best correlation between the phase-shift functions of the unknown and the standard. These fits are performed by allowing the distance (r) and Debye–Waller (σ^2) parameters to vary, and the solution with the best χ^2 that forms a stable minimum is chosen within each fit. All reported solutions had a minimum with respect to the E_0 shift, and had E_0 and Debye–Waller factors that were chemically reasonable.[9-12]

Solution 1 for the cyanocobalamin data (Table I) represents a one-atom-type fit using a CoTPP model to fit the average of all six ligands. The high χ^2 and the relatively high error associated with the average distance is not unexpected. Solution 1 gives a rough estimate of the average distance for the six ligands since the Co–N corrin ring distances have been previously found to be close to 1.90 ± 0.02 Å.[10] In order to possibly resolve the Co–N distance to the DMB ligand and the Co–C distance to the CN axial ligand, we attempted two-atom-type fits represented by solutions 2 and 3. Solution 2 shows five nitrogens at 1.90 ± 0.02 Å and one well-resolved distance at 2.15 ± 0.03 Å. This solution is found to be very stable considering our ability to easily reproduce the same distances starting from many different initial conditions. For example, changing the initial distance parameters for the nonlinear least-squares fitting from 1.8 to 2.3 Å or fixing the E_0 shift at different values (chemically reasonable) did not influence the minimum ultimately found. The Co–C axial distance of solution 3, however, is less stable but suggests a Co–C distance of 1.90 ± 0.04 Å. The difficulty in obtaining a stable solution for the axial cyanide ligand is not unexpected since the four averaged corrin distances (solution 5) are virtually identical to the Co–C bond distance. However, the reported Co–C axial ligand distance in combination with the reported average of the other Co–N distances is consistent with the data. As stated above, the phase differences of the carbon and nitrogen ligands are small but measurable, especially at low temperature. When it is known that both carbon and nitrogen are present, even a poorly resolved ligand contribution (with respect to distance) may be observed as a minimum solution. An alternative solution for the Co–C bond (solution 4) suggested a cobalt–cyano distance of 2.22 ± 0.03 Å. We ruled out this solution for two reasons: the two-atom fit with fixed coordination number of 4:2 (see solution 5) has an average of the axial distances inconsistent with two long axial distances and a three-atom consistency test, where the Co–C distance is fixed at 2.22 Å, cannot provide a reasonable minimum solution of any kind (not shown). Solution 5 shows the very stable averaged corrin ring distance at 1.89 ± 0.01 Å and also suggests that if, indeed, the Co–N(DMB) distance is at 2.15 Å, then a Co–CN ligand distance on the order of 1.9 Å, as suggested by solution 3, is reasonable.

With this in mind we further examined these solutions by performing a three-atom consistency test (solution 6) that held fixed four equatorial ligands and fixed axial nitrogen and carbon ligands. The E_0 shifts were stepped from −8 to 8 on the two axial ligands and were fixed at 0 for the equatorial nitrogen ligands. Solution 6 clearly suggests that the cobalt–ligand distances in cyanocobalamin consist of the distance combination of the two-atom fits described in solutions 2, 3, and 5. Any attempt to switch the fixed distances on both axial ligands (not shown) resulted in a worse fit and a high $\Delta\sigma^2$ associated with the Co–C distance. Although we could not trust the assignment of a Co–C distance so close to the equatorial distances based solely on Table I, solution 3, the three-atom consistency test demonstrates that the possible range for the Co–C distance must be very close to 1.90 Å. Thus, the cobalt–ligand distance results for cyanocobalamin in solution are 1.89 ± 0.01 Å for the equatorial corrin distance, 2.15 ± 0.03 Å for the Co–N(DMB) ligand, and 1.90 ± 0.03 Å for the Co–CN ligand.

The same logical analysis described in Table I for cyanocobalamin is applied in analyzing the data from aquocobalamin and methylcobalamin (see supplementary material for the details of fitting). The cobalt–ligand distances for aquocobalamin, derived by EXAFS, are summarized in Table II. The Co–N(DMB) ligand was resolved by a two-atom-type fit in 5N:1N ligand ratio and resulted in a very stable solution at 2.13 ± 0.03 Å. Resolving the cobalt–oxygen ligand (using CoAcAc as a standard) required specific conditions since nitrogen and oxygen are very similar backscatterers. The Co–O(H_2O) distance solution at 1.89 ± 0.03 Å is not as stable as the distance solution obtained for the Co–N(DMB); however, it was confirmed by a three-atom consistency test, and it is well-supported by examination of appropriate model compounds.

Previous EXAFS studies have clearly demonstrated that octahedral transition-metal compounds can yield accurate metal–

Table II. Comparison of Crystallographic and EXAFS Solutions for Methylcobalamin,[8] Cyanocobalamin,[2] Adenosylcobalamin,[5,10] (Adeninylpropyl)cobalamin,[6] and Aquocobalamin

compound	metal–ligand Distance (Å)	EXAFS	X-ray
methylcobalamin	Co–N(eq)[a]	1.90 ± 0.01	1.89 (2)
	Co–N(DMB)	2.20 ± 0.03	2.19 (2)
	Co–C(CH_3)	2.00 ± 0.03	1.99 (2)
cyanocobalamin	Co–N(eq)	1.89 ± 0.01	1.89 (10)
	Co–N(DMB)	2.15 ± 0.03	1.97 (10)
	Co–C(CN)	1.90 ± 0.03	1.92 (10)
adenosylcobalamin	Co–N(eq)	1.90 ± 0.01	1.90 (3)
	Co–N(DMB)	2.19 ± 0.01	2.24 (3)
	Co–C(adenosyl)	2.03 ± 0.02	2.01 (3)
(Ade(–CH_2–)$_3$Cbl	Co–N(eq)	*b*	1.87 (1)
	Co–N(ax)		2.21 (1)
	Co–C((CH_2)$_3$-adenine)		1.96 (1)
aquocobalamin	Co–N(eq)	1.89 ± 0.01	*c*
	Co–N(DMB)	2.14 ± 0.03	
	Co–O(H_2O)	1.90 ± 0.02	

[a] Co–N(eq) is the $1/r^2$ average of the corrin equatorial distances. [b] No EXAFS data are available. [c] Crystal structure is not available. Reported errors for crystallography data are 1 standard deviation. EXAFS errors include the maximum expected range.

ligand bond distances to both equatorial and axial ligands.[10,21] In order to further determine the accuracy of our EXAFS analysis procedure and our ability to assign axial and equatorial contributions, we chose to solve the EXAFS structure of methylcobalamin as a control for our analysis. The metal–ligand distances for methylcobalamin are summarized in Table II. All distance solutions to the various ligands are well-resolved with very stable solutions at 2.20 ± 0.03 Å for the Co–N(DMB) distance, 2.00 ± 0.03 Å for the Co–CH_3 distance, and 1.90 ± 0.01 Å for the Co–N equatorial distances. These EXAFS results are in very good agreement with the methylcobalamin X-ray structure (see Table II). This demonstrates that EXAFS can attain the precision and accuracy of small-molecule crystal structures and thus provides valuable metal–ligand distance information for cyanocobalamin and aquocobalamin.

Discussion

A comparison of the data of Table II with other cobalamin structures and model compounds shows that while the corrin ring distances from cobalt to the equatorial nitrogens and the nonalkyl ligands are not surprising, the Co–N distance to the DMB ligand is relatively longer than expected. The crystal structure of cyanocobalamin shows a much shorter Co–N(DMB) distance than EXAFS shows; however, these data (as well as the structure of cyanocobalamin monocarboxylic acid[23]) suggest Co–N(DMB) distances that are within 2 standard deviations of the EXAFS results reported here and are therefore not inconsistent.

The cobalt–cyano distance at 1.90 ± 0.03 Å is consistent both with the X-ray distance of this bond in dicyanocobinamide where the Co–CN bond distances are at 1.92 and 1.93 Å[22] and also with the structure of monocarboxylic acid of vitamin B_{12}[23] at 1.88 Å. The X-ray structures of monocarboxylic acid and dicyanocobinamide are more precise than that of cyanocobalamin (ESD of 0.04 Å for monocarboxylic acid and 0.01 Å for dicyanocobinimide) and therefore can be used for comparison. Further examination of the nature of the cobalt–cyano bond in (nonalkyl)cobalamin model compounds[24–26] leads to the same range of distances. The structures of cyanoaquobis(dimethylglyoximato)cobalt(III),[24] *trans*-cyanobis(dimethylglyoximato)(trimethyl phosphite)cobalt(III),[25] and *trans*-cyanobis(dimethylglyoximato)(pyridine)cobalt(III)[26] show Co–CN bond distances at 1.906 (5), 1.903 (9), and 1.937 (2) Å, respectively. The Co–C distance, determined by EXAFS, for cyanocobalamin at 1.90 ± 0.03 Å is in very good agreement with both reliable cobalamin X-ray structures and cobaloxime model compounds.

Table III. Influence of the Trans X Ligand on the Co–N(py) Distance in Cobaloximes

X ligand	Co–N(py) distance (Å)
N_3[28]	1.97 (3)
Cl[29]	1.97 (2)
CH_3[30]	2.068 (3)
CH_2CH_3[31]	2.084 (7)
i-C_3H_7[32]	2.099 (5)
CH_2NO_2[33]	2.028 (3)
CH_2CMe_3[34]	2.081 (4)
i-C_3H_7[35]	2.194 (4) for (NH_2py)

Since very little structural information exists for aquocorrin compounds that are trans to a nonalkyl ligand, a direct comparison of the Co–O distance in aquocobalamin to the same wide range of appropriate model compounds is not possible. However, a few structures can serve as models, including the structures of *trans*-aquabromobis(ethanedialdioximato(1–)-*N*,*N*′)cobalt(II),[18] (cumylperoxo)(pyridine)cobaloxime,[19] *trans*-aquobis(dimethylglyoximato)(pyridine)cobalt(III),[27] and the recent structure of superoxocobalamin.[20] The Co–OH_2 bond distance in *trans*-aquabromobis(dimethylglyoximato)cobalt(III) is at 1.955 (11) Å, the Co–O bond of the peroxide ligand at (cumylperoxo)(pyridine)cobaloxime is at 1.909 (3) Å, and the Co–O to the H_2O ligand in *trans*-aquobis(dimethylglyoximato)(pyridine)cobalt(III) is at 1.916 (3) Å. The X-ray structure of superoxocobalamin suggests a Co–O distance to the peroxide ligand at 1.930 (15) Å with the cobalt center 0.09 Å out of the corrin plane toward the DMB ligand. Therefore, the EXAFS solution for the Co–O distance at 1.90 ± 0.03 Å in aquocobalamin falls in the range suggested by these model compounds.

It is well-established that the Co–N(py) ligand, which is used as a Co–N(DMB) ligand analogue in cobaloxime compounds, is sensitive to trans effects attributable mainly to the σ-donor power of the axial ligand in alkylcobaloximes.[4] As the σ-donor power increases, it places more electron density on the cobalt ion which results in an increase in bond distance for the opposite axial ligand. Examination of cobaloxime compounds containing pyridine and several trans X ligands (Table III) shows that the Co–N(py) bond distances are shorter (1.973 (5) Å for X = N_3[28] and 1.97 (2) Å for X = Cl[29]) in (nonalkyl)cobaloximes and clearly elongated (2.068 (3) Å for X = CH_3,[30] 2.084 (7) Å for X = CH_2CH_3,[31] and 2.099 (5) Å for X = *i*-C_3H_7 Å[32]) in alkylcobaloximes. The sensitivity of the Co–N(py) bond distance to the electronic nature of the X ligand is also evidenced by more elegant comparisons that clearly negate any steric effects. For example, when X = CH_2NO_2, the Co–N(py) distance (2.028 (3) Å[33]) is shorter than when X = CH_2CMe_3 (2.081 (4) Å[34]). Alternatively, steric effects are also significant. Replacement of py by the bulkier 2-NH_2-py results in a significant lengthening of the Co–N distance from 2.099 (2) Å[32] to 2.194 (4) Å when X = *i*-C_3H_7.[35]

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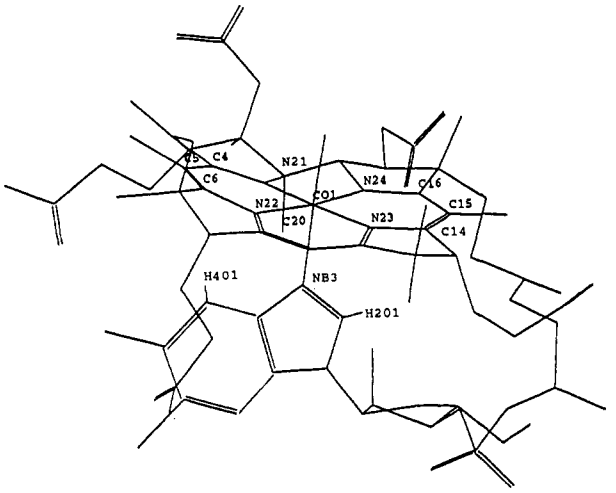

Figure 3. View of selected nonbonded contact distances for methylcobalamin. Note the short distances from the two hydrogens (H401, H201), projected from the benzene and the imidazole moieties of the DMB ligand to various corrin ring atoms. The nonbonded distances between H(401) and N(22), N(21), C(4), C(5), C(6) are 2.68, 3.01, 2.84, 2.63, and 2.56 Å, while the nonbonded distances between H(201) and N(23), N(24), C(14), C(15), C(16) are 2.76, 3.03, 2.65, 2.83, and 3.00 Å, respectively.

The lengthening of about 0.1 Å for Co–N(py) in the alkylcobaloximes structures (compared to the weaker trans influencing ligands) seems to be twice as much as observed for the change in the Co–N(DMB) distance in cobalamins based on crystallographic and EXAFS structural data for alkylcobalamins[3,5,6,10] and our EXAFS results for the (nonalkyl)cobalamins reported here. The σ-donor power of the alkyl ligands in addition to the rehybridization of the normal sp^3-hybridized bond[36] (in order to form a σ bond), in combination with the steric bulk of the benzimidazole ligand, accounts for the unusually long Co–N(DMB) distance in alkylcobalamins.[3,5,6,10] However, these arguments cannot be used for nonalkyl ligands such as CN and OH_2 since their σ-donor power and hence trans influence is much less. This suggests that the long distance of the Co–N(DMB) ligand in these structures is a result of a steric effect caused by crowding of the corrin ring and the DMB ligand.

In order to better quantify the nature of the nonbonded interactions, we used the CHEMX molecular modeling package (developed by Chemical Design Ltd., Oxford, England) to identify points of close, nonbonded approach for atoms in the methylcobalamin[5] crystal structure, which is available in the Cambridge Crystallographic Data Bank. Although the crystal structure cannot place the hydrogen atoms, there are two hydrogens that project upward from the DMB ligand for which the positions can be reasonably estimated. These are the hydrogens seen in Figure 3 that project upward from the benzene and imidazole moieties. Assuming standard C–H distances (1.08 Å), we can use CHEMX to estimate the distances from various corrin ring atoms to these hydrogens. The results show a large number of unfavorable contacts that predict significant repulsions for the DMB ligand as a whole and a significant effect on the cobalt–N(DMB) distance, tending to lengthen the bond.

The nonbonded distances from H(401) to N(22), N(21), C(4), C(5), and C(6) are 2.68, 3.01, 2.84, 2.63, and 2.56 Å, while the nonbonded distances from H(201) to N(23), N(24), C(14), C(15), and C(16) are 2.76, 3.03, 2.65, 2.83, and 3.00 Å, respectively. This suggests 10 contact distances of 3.0 Å or less despite the uncertainty in placing the C–H distances and bond angles correctly. Shortening the Co–N(DMB) distance in the methylcobalamin structure from 2.19 to 2.14 Å in order to primitively model the repulsions for the aquocobalamin structure results in nonbonded distances about 0.04 Å shorter for each of these contacts. Without reorganization of the corrin plane, this would result in four distances from 2.5 to 2.65 Å. It is clear that the van der Waals potential as a function of Co–N(DMB) distance is steeply sloping for cobalamins, as there is strong competition between the tendency to form a strong Co–N bond and the tendency of steric forces to prevent it.

The necessity for the long Co–N(DMB) distance, dictated by the steric forces described above, has important implications for coenzyme-B_{12}-dependent enzyme mechanisms. Several enzyme systems are known to precipitate cobalt–carbon cleavage resulting in Co(I) or Co(II) forms of the B_{12} coenzyme.[10,37,38] We have recently established that the Co(I) B_{12} species is four-coordinate and square planar,[38] emphasizing that Co(I) B_{12} intermediates involve the cleavage of both the Co–C and the Co–N(DMB) bonds. Indeed, of the two well-characterized Co(I) B_{12}-dependent enzyme systems, the corrinoid protein from *Clostridium thermoaceticum* exists as a five-coordinate coenzyme[39,40] while the methionine synthetase coenzyme is six-coordinate in the free enzyme state.[41] Controversy over the structure of Co(II) B_{12} has arisen as crystallographic data suggest a Co–N(DMB) distance of 2.13 (2) Å with the cobalt atom displaced 0.12 (1) Å from the corrin plane toward the DMB ligand,[37] while EXAFS data in solution are consistent, only with a much shorter bond of 1.99 ± 0.03 Å.[10] The results here suggest that steric factors are critical in controlling the Co–N(DMB) distance and that in the presence of innocent trans ligands the DMB ligand cannot approach much closer than 2.11–2.12 Å to cobalt (the lower bound of the EXAFS data). It is also presumed that the cobalt ion remains in the corrin plane for six-coordinate Co(III) species of this type.[22,23] However, for Co(II) cobalamin the crystallographic data suggest that the steric pressure may be reduced since the cobalt atom moves toward the DMB ligand and the tilt angle of the corrin plane is relatively unchanged.[37] Thus, it is not unreasonable that the Co–N(DMB) distance could shorten significantly and in an amount commensurate with the displacement (i.e., to ca. 2.0 Å). A recent cobalamin structure, that of superoxo Co(II) B_{12},[20] reports a Co–N(DMB) distance of 2.06 (2) Å for this six-coordinate species with the cobalt atom displaced toward the DMB ligand by 0.09 (1) Å. In comparing the two Co(II) crystal structures, it is surprising that both the Co–N(DMB) distance and the cobalt displacement would increase in going from the six- to five-coordinate species.

Conclusion

EXAFS studies of cyanocobalamin and aquocobalamin report Co–N(DMB) distances of ca. 2.15 ± 0.03 Å. These distances suggest that the steric repulsion of the DMB ligand and the corrin plane is greater than has been previously supposed. Although electronic trans effects are critical in maintaining the long (ca. 2.20 Å) Co–N(DMB) distance in alkylcobalamins, this distance is reduced by only half of what would be expected for nonalkyl ligands based on examination of electronic trans effects observed in cobaloxime models. The importance of these steric forces in regulating cobalt–carbon bond cleavage in B_{12} enzymes is reflected in the different structures of the Co(I) and Co(II) intermediates. The long Co–N(DMB) distance of alkylcobalamins makes for a low barrier to formation of the square-planar Co(I) B_{12}. For the Co(II) species, movement of the cobalt ion out of the corrin plane toward the DMB ligand reduces the steric repulsion, allowing for a shortening of the Co–N(DMB) bond and the production of a five-coordinate species. Thus, appropriate enzyme interaction with

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the DMB ligand and its nucleotide loop can easily influence the chemical mechanism.

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Supplementary Material Available: Detailed description of the data analysis for aquocobalamin and cyanocobalamin, along with two data analysis tables and Fourier transforms of the background-subtracted data of cyanocobalamin and aquocobalamin (7 pages). Ordering information is given on any current masthead page.

A Stable Dinitrogen Complex of a Ruthenium Cofacial Diporphyrin

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Abstract: An unusually stable, bridged dinitrogen complex of a biphenylene bridged diporphyrin is described. The complex $(\mu\text{-}N_2)Ru_2DPB(1\text{-}tert\text{-butyl-}5\text{-phenylimidazole})_2$ (DPB = diporphyrinatobiphenylene tetraanion) is proposed as a model for a dinitrogen reduction catalyst based on cofacial metallodiporphyrins. The differences in binding by bridged and nonbridged ligands to the two coordination sites between the porphyrin rings are discussed. The complex's preparation, structural characterization, ligand substitution reactivity, electrochemistry, and acid-base properties are reported. It is proposed that the complex's unusual stability is due to a chelation of the dinitrogen by the two tethered metal centers. Loss of dinitrogen from the complex occurs only through replacement of dinitrogen by an added ligand. The rate of replacement is dependent on the concentration of the added ligand. Advantages of cofacial diporphyrins as dinitrogen electroreduction catalysts compared to monomeric or untethered binuclear dinitrogen complexes are noted.

The discovery of an electrode catalyst for the reduction of dinitrogen is a challenging and unsolved problem. The problem is interesting primarily from a fundamental rather than a technological point of view because the transformation of dinitrogen to ammonia by the Haber-Bosch process is fairly efficient and economically well-entrenched in spite of the high temperature and pressure required.[1]

The difficulty in reducing (or activating) dinitrogen derives from the strength of the N-N triple bond and the high ionization energy of dinitrogen. Over half the bond strength of the N-N bond (about 130 out of 225 kcal/mol)[2] is required to rupture the first π-bond. The first ionization potential of dinitrogen (15.6 eV)[2] rivals that of argon. The key to reducing nitrogen is to activate the LUMO's of N_2 with transition metals of the proper (low) d-electron configuration.

Even though dinitrogen is quite inert, its reduction to ammonia can be carried out at ambient temperature, atmospheric pressure, and neutral pH by the nitrogenase enzymes contained in anaerobic bacteria. Mo/Fe, V/Fe, and "Fe only" nitrogenase enzymes have been extensively studied,[3] and it is generally accepted that the site of dinitrogen reduction in these enzymes is at one or more of the metal centers. Although it has been shown that dinitrogen can be reduced stoichiometrically at a single metal center,[4] it is still unclear whether the binding sites of the enzymes include one or more metal atoms. A recent, low-resolution X-ray crystallographic study of the molybdoprotein of the *Clostridium pasteurianum* indicates that in the solid state the two molybdenum centers are 70 Å apart.[5] This evidence suggests that only one molybdenum center can be associated with each active site, but it does not preclude bridging complexes between molybdenum and iron, for example.

Chemists have tried for many years to develop systems that mimic the nitrogenase enzyme. The first chemical systems found to reduce dinitrogen were discovered by Vol'pin and Shur in the early 1960's. Similar systems involving early transition metals and strong reducing agents in ethereal solvents were developed independently by VanTamelen et al. These reduction systems involve forcing conditions, and little mechanistic information about them has been obtained. The work by both of these groups, including a report of a catalytic system, has been reviewed.[6]

Reports of catalytic reduction systems in aqueous solutions primarily include the work of Shilov or Schrauzer and involve either molybdenum or vanadium catalysts.[6a,7] These reduction systems are too complicated to allow elucidation of the mechanisms

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