Equilibrium and Kinetic Studies on the Reactions of Alkylcobalamins with Cyanide

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Ligand substitution equilibria of different alkylcobalamins (RCbl, R = Me, CH_2Br, CH_2CF_3, CHF_2, CF_3) with cyanide have been studied. It was found that CN^- first substitutes the 5,6-dimethylbenzimidazole (Bzm) moiety in the α-position, followed by substitution of the alkyl group in the *β*-position trans to Bzm. The formation constants K_{CN} for the 1:1 cyanide adducts (R(CN)Cbl) were found to be 0.38 ± 0.03, 0.43 ± 0.03, and 123 ± 9 M^{-1} for R = Me, CH_2Br, and CF_3, respectively. In the case of R = CH_2CF_3, the 1:1 adduct decomposes in the dark with CN^- to give $(CN)_2$Cbl. The unfavorable formation constants for R = Me and CH_2Br indicate the requirement of very high cyanide concentrations to produce the 1:1 complex, which cause the kinetics of the displacement of Bzm to be too fast to follow kinetically. The kinetics of the displacement of Bzm by CN^- could be followed for R = CH_2CF_3 and CF_3 to form CF_3CH_2(CN)Cbl and CF_3(CN)Cbl, respectively, in the rate-determining step. Both reactions show saturation kinetics at high cyanide concentration, and the limiting rate constants are characterized by the activation parameters: R = CH_2CF_3, $\Delta H^{\ddagger} = 71 \pm 1$ kJ mol^{-1}, $\Delta S^{\ddagger} = -25 \pm 4$ J K^{-1} mol^{-1}, and $\Delta V^{\ddagger} = +8.9 \pm 1.0$ cm^3 mol^{-1}; R = CF_3, $\Delta H^{\ddagger} = 77 \pm 3$ kJ mol^{-1}, $\Delta S^{\ddagger} = +44 \pm 11$ J K^{-1} mol^{-1}, and $\Delta V^{\ddagger} = +14.8 \pm 0.8$ cm^3 mol^{-1}, respectively. These parameters are interpreted in terms of an I_d and D mechanism for R = CH_2CF_3 and CF_3, respectively. The results of the study enable the formulation of a general mechanism that can account for the substitution behavior of all investigated alkylcobalamins including coenzyme B_{12}.

Introduction

The coenzymatic forms of vitamin B_{12} are well-known to be involved in the catalysis of about 15 enzymatic reactions in various organisms, including the 1,2-intramolecular rearrangements, catalyzed by 5′-deoxyadenosylcobalamin (AdoCbl)-requiring enzymes,[2−5] and the methyl transfer reactions, catalyzed by methylcobalamin-requiring enzymes.[2,5,6] The thermolysis of AdoCbl demonstrates that such enzymes can increase the rate of thermal homolysis by a factor of at least 10^9 at 25 °C,[7,8] and it is now thought that steric distortion and crowding at the *β*-site which contains the alkyl group may play a role. There is a need to study ligand substitution reactions trans to the axial alkyl ligand in coenzyme B_{12} and some other *β*-alkylcobalamins in comparison to all the work carried out on substitution reactions trans to a non-alkyl ligand, since it is known that methylcobalamin and coenzyme B_{12} undergo substitution of their axial benzimidazole ligand by a protein histidine residue during complexation to the enzymes methionine synthase, methylmalonyl coenzyme A mutase, and glutamate mutase.[9,10]

The corrin ring does not possess a plane of horizontal symmetry, and consequently, there is a possibility of axial ligand diastereomerism when the two axial ligands are different.[11−17] The upper or *β*-face, with the upward projecting a, c, and g acetamide side chains, is less sterically hindered than the lower or α-face, which is bracketed by the downward projecting b, d, and e propionamides and the secondary amide f side chain. Reductive alkylation of Co(III) cobalamin with alkyl halides results in the formation of pairs of diastereomeric α- and *β*-isomers, the ratio of which varies widely with the nature of the alkyl group.

Equilibrium constants for binding the cyanide ion to eight alkylcobinamides (four *β*-alkylcobinamides and four α-alkylcobinamides) have been determined.[18] These eight complexes were sufficiently stable toward cleavage by cyanide to permit accurate measurements of the binding constant. The equilibrium

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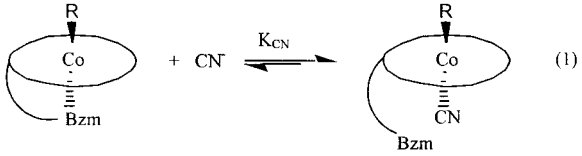
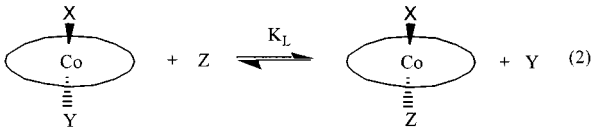
constants obtained vary by (6.4×10^4)-fold from 4.33 M^{-1} for β-ethylcobinamide to 2.79×10^5 M^{-1} for β-cyanomethylcobinamide. This binding is, in many cases, sufficiently strong to displace the axial 5,6-dimethylbenzimidazole (Bzm) ligand of β-alkylcobalamin (RCbl), with the values of K_{CN} (eq 1) varying from 0.665 M^{-1} in the case of $CH_3CH_2CH_2Cbl$ to 63.8 M^{-1} in the case of $NCCH_2Cbl$.[19] A number of organocobalt corrinoids, including β-AdoCbl, other 5′-deoxyadenosylcobalt corrinoids,[20−22] (carbomethoxymethyl)-, (carbomethoxyethyl)-, and (2-cyanoethyl)cobalamins,[23,24] and α- and β-CF_3CH_2Cbi, as well as others,[18] are labile toward dealkylation by cyanide in the dark.



(1)

Another important factor to consider is the trans influence/effect that a ligand (X) bound to a metal ion has on the properties of the coordinated trans ligand (Y) and on the kinetics and thermodynamics of the displacement of Y by an incoming ligand (Z) as shown in eq 2. This effect is a well-known phenomenon in the chemistry of vitamin B_{12} operating at three levels, viz., structure, thermodynamics, and kinetics.[25] It has been established, for example, that the value of K_L, which represents the association constant (eq 2), declines across the series X = H_2O > OH^- > CN^- > vinyl > methyl,[25] as X becomes a better σ-donor. A similar order in trans influence occurs for the energy of the γ-band transition (around 360 nm) in XCbl compounds.[25]



(2)

Ligand substitution reactions of vitamin B_{12}, in general, follow a dissociative (I_d or D) mechanism.[26,27] In the case of coenzyme B_{12} (AdoCbl), however, evidence for an associative substitution mechanism was reported, and it was postulated that the attack of the first nucleophile (cyanide) occurred at the β-(5′-deoxy-5′-adenosyl) site rather than at the α-dimethylbenzimidazole site.[28] More recently, it was reported that when the reaction between AdoCbl and cyanide is carried out in 92% DMF/8% D_2O, an intermediate (β-Ado)(α-cyano)cobalamin species can be identified by ^1H NMR spectroscopy.[29] Thus, the rate-determining heterolytic cleavage of the Co−C bond is preceded by the rapid addition of cyanide to the α-position. The associative mechanism obtained for the reaction of AdoCbl and CN^- is rather surprising since it is generally expected that the introduction of a metal−carbon bond will induce a dissociative substitution reaction in the trans position and not an associative reaction as reported for the reaction of the coenzyme with cyanide.[28] To gain further insight into the reason for this unexpected mechanistic changeover, we have studied the substitution behavior of a series of alkylcobalamins in which the nature of the alkyl group was varied systematically along the series Me, CH_2Br, CH_2CF_3, and CF_3. The kinetics of the substitution reactions with cyanide were studied as a function of nucleophile concentration, temperature, and pressure. The reported results enable us to draw a consistent mechanistic picture for such ligand displacement reactions and to account for the unexpected mechanistic changeover found in the case of the coenzyme.

Experimental Section

Materials. All the chemicals used were P.A. grade and used as received without further purification. CAPS buffer (3-(cyclohexylamino)-1-propanesulfonic acid) was purchased from Sigma. $NaClO_4$ and NaCN were purchased from Merck. Ultrapure water was used for the kinetic measurements. The preparation and measurements were carried out in diffuse light since all the alkylcobalamins are known to be very light sensitive.[25]

Methylcobalamin was supplied by Sigma. The other alkylcobalamins (alkylCbls) were prepared as described in the literature[11,30,31] by reacting Co(II) cobalamin with suitable alkylating agents. In a typical reductive alkylation, H_2OCbl (10 mg, ca. 0.01 mmol) in 5.0 mL of 10% acetic acid or 5% NH_4Cl was purged with argon for 1 h, zinc wool (0.01 mol), quickly freshened with 1.0 N HCl, was added, and the reduction was allowed to proceed for 30 min. Alkyl halide (ca. 1 mmol) was introduced, and the reaction was allowed to proceed for 30 min (where R = CH_2CF_3, CF_3, and CH_2Br as alkylating agents required for the preparation of CF_3CH_2Cbl, CF_3Cbl, and $BrCH_2Cbl$, respectively). β-CF_2HCbl is a byproduct in the preparation of β-CF_3Cbl as described previously.[32] The reaction mixtures were desalted by chromatography on Amberlite XAD-2,[32] and the β-isomers were separated by high-performance liquid chromatography (HPLC).[11,33,34]

Instrumentation and Measurements. The pH of the solutions was measured using a Mettler Delta 350 pH meter with a combined glass electrode. It was calibrated with standard buffer solutions at pH = 7 and 10. UV−visible (UV−vis) spectra were recorded on Shimadzu UV-2101 or Cary 5 spectrophotometers.

Analytical HPLC was performed on a 4.6 × 250 mm Beckman C_8 ultrasphere column while semipreparative HPLC was performed on a 10 × 250 mm Beckman C_8 ultrasphere column, using 50 mM of aqueous ammonium phosphate buffer (pH = 3.0) and acetonitrile as described previously.[11,33,34]

Kinetic measurements were carried out on an Applied Photophysics SX 18MV stopped-flow instrument coupled to an online data acquisition

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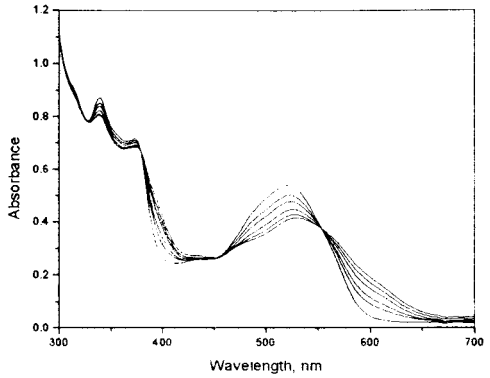


Figure 1. UV−vis spectra of MeCbl (6×10^{-5} M) recorded for different concentrations of CN^- (1−6 M) at pH = 11.0 and 25 °C.

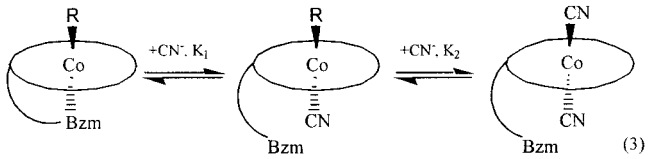
system. At least eight kinetic runs were recorded under all conditions, and the reported rate constants represent the mean values. All kinetic measurements were carried out under pseudo-first-order conditions, i.e., the ligand concentration was in 10-fold excess at least. Measurements under high pressure were carried out using a homemade high-pressure stopped-flow instrument.[35] Kinetic traces were analyzed with the OLIS KINFIT program.

All the instruments used during the course of this work were thermostated to the desired temperature of ±0.1 °C. Values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were calculated from the slopes and intercepts, respectively, of plots of $\ln(k/T)$ vs $1/T$, and values of $\Delta V^{\ddagger}$ were calculated from the slope of plots of $\ln(k)$ vs pressure.

Equilibrium measurements were obtained for $(3-6) \times 10^{-5}$ M RCbl dissolved in CAPS buffer (I = 0.5 M using $NaClO_4$) placed in a 1.0 cm path length cuvette in the thermostated cell block of the spectrophotometer for 20−30 min. This solution was titrated by the addition of small volumes of a concentrated stock solution of sodium cyanide, using a Hamilton syringe. The cyanide solution was prepared in the same buffer, and the ionic strength was also adjusted to 0.5 M using $NaClO_4$, except in the case of MeCbl and $BrCH_2Cbl$ where it was difficult to adjust the ionic strength since the binding constant is small. Hence, a high CN^- concentration had to be used. The titrations were carried out in duplicate and were monitored at several wavelengths where the largest changes in absorbance took place. The values of the equilibrium constant, K, were obtained by fitting the absorbance vs concentration curves, after correction for dilution (see Results and Discussion for further details).

Results and Discussion

Equilibrium Measurements. This paper describes the ligand substitution reaction between different organocobalamins and cyanide, for which the general reaction can be represented by eq 3.



(3)

Preliminary experiments at pH = 9−11, in which the UV−vis spectrum was scanned in the range between 300 and 700 nm, showed that CN^- reacted rapidly with all the RCbl complexes studied and that the equilibria were established within the mixing and measurement time.

We started our work by reinvestigating the reaction between MeCbl and CN^- at pH = 11 and 25 °C. Figure 1 shows the UV−vis spectra for the reaction of MeCbl with 1−6 M CN^-. It shows that good isosbestic points are observed at 325, 380, 455, and 553 nm, and the absorbance increases significantly at 580−600 nm. Spectrophotometric titrations were carried out by following the absorbance at 580−600 nm, and the value of K was found to be 0.38 ± 0.03 M^{-1} (see further discussion and Figure S-1 in Supporting Information). It is important to note that there is a discrepancy in the literature regarding the axial ligand substitution reaction of MeCbl by CN^-. In earlier work, Dolphin et al.[36] reported that no reaction occurs between MeCbl and CN^-, but Hayward et al.[37] reported log K of 0.1 for the binding of CN^- to MeCbl. It was also reported in the literature that CN^- can displace Bzm from MeCbl with $k = 2.8 \times 10^{-2}$ s^{-1} for 0.1 M KCN, 0.1 M buffer ($NaHCO_3$), pH = 10.5, and 25 °C.[38] In later work it was reported that MeCbl forms an association complex with CN^- with no evidence for displacement of Bzm, but this study was carried out at $[CN^-]$ between 0.1 and 1.2 M.[39] It was reported that K for the formation of the association complex (MeCbl−CN) in CD_3OD equaled 2.5 M^{-1}.[39] However, the selected concentration range was not high enough for the substitution of Bzm from the α-position since the binding constant for CN^- to MeCbl is indeed very low, and a high $[CN^-]$ is required to observe any significant change in the UV−vis spectrum. The intermediate obtained after reacting MeCbl with ca. 6 M CN^- is light sensitive, and $(CN)_2Cbl$ is formed upon photolysis. This suggests that CN^- substitutes α-Bzm under these conditions. It is known from the literature that substitution of α-Bzm by CN^- is accompanied by an increase in absorbance at 580−600 nm[19] due to a new d−d transition band common to all complexes of this type (see Figure 1).

Spectrophotometric titration of $BrCH_2Cbl$ with cyanide showed spectral changes similar to that of MeCbl (Figure 1); the value of K was found to be 0.43 ± 0.03 M^{-1} (see further discussion and Figure S-2 in Supporting Information). It is clear that the more electronegative Br atom has no influence on the value of K. The limited number of available bromoalkylcobalamins has led us to investigate fluoroalkylcobalamins, since it is known that the high electronegativity of fluorine creates an opportunity to study the thermodynamic equilibria and rate constants for the cyanation reactions of these compounds and to elucidate the mechanism of these reactions. We found that β-CF_3CH_2Cbl, β-CF_2HCbl, and β-CF_3Cbl are ideal for such a study. Unfortunately, it was difficult to determine the value of K_{CN} in the case of β-CF_3CH_2Cbl since it decomposes rapidly in the dark to give $(CN)_2Cbl$. Many organocorrinoids have been found to be labile toward dealkylation by excess cyanide in the dark to give $(CN)_2Cbl$, including β-AdoCbl and other 5′-deoxyadenosylcobalt corrinoids, (carbomethoxymethyl)-, (carbomethoxyethyl)-, and (2-cyanoethyl)cobalamins, and α- and β-CF_3CH_2Cbi, as well as others.[18,20−24]

Figure 2a shows the UV−vis spectra recorded for the equilibrium between β-CF_3Cbl and CN^- (pH = 11, 25 °C, and I = 0.5 M ($NaClO_4$)). This figure also shows a significant increase in the absorbance at 580−600 nm, good isosbestic points at 361, 390, and 554 nm, a significant shift in the absorption wavelengths, and new bands at 361, 538, and 577

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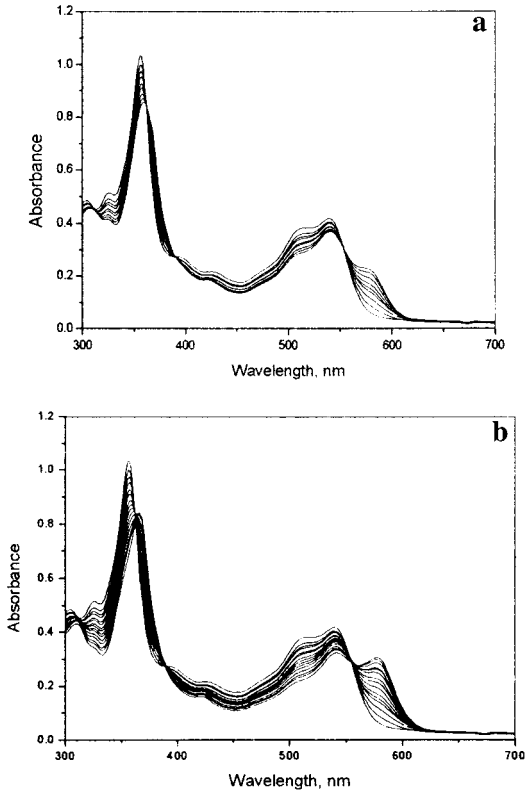

Figure 2. (a) UV−vis spectra of β-CF_3Cbl (4×10^{-5} M) recorded for different concentrations of CN^- (0.001−0.025 M) at pH = 11.0, 25 °C, and I = 0.5 M ($NaClO_4$). (b) UV−vis spectra of β-CF_3Cbl (4×10^{-5} M) recorded for a larger concentration range of CN^- (0.001−0.2 M) at pH = 11.0, 25 °C, and I = 0.5 M ($NaClO_4$).

(sh) nm. This suggests that CN^- displaces α-Bzm to form *trans*-CF_3(CN)Cbl, since the product of this reaction is a light-sensitive complex. The value of K_{CN} in this case was found to be 123 ± 9 M^{-1}, i.e., considerably larger than those for MeCbl and $BrCH_2$-Cbl reported above. However, addition of excess CN^- shifts the isosbestic points, and new bands at 367, 540, and 580 nm were observed as shown in Figure 2b. This indicates that the excess CN^- displaces the alkyl group located in the β-position, and $(CN)_2$Cbl is formed with characteristic bands at 367, 540, and 580 nm.[25]

The spectrophotometric titrations for all RCbls were monitored by following the increases in absorbance at 580−600 nm where the largest changes in absorbance occurred. Selected data are shown in Figure 3 for the reaction of β-CF_3Cbl with cyanide. The solid line represents the fit of eq 4 to the experimental data.

$$A_x = A_0 + A_\infty K[CN^-]/(1 + K[CN^-]) \qquad (4)$$

The values of A_0 and A_∞ represent the absorbances at 0 and 100% formation of *trans*-R(CN)Cbl, respectively, and A_x is the absorbance at any cyanide concentration. The values of K and A_∞ were calculated from eq 4. The analysis of these data by plotting $\log(A_x - A_i)/(A_\infty - A_x)$ vs $\log[CN^-]$ gave a good linear plot with a slope of 1.03 ± 0.03, which indicates that one CN^- ligand has been coordinated to the cobalt atom. Similar log−log plots were obtained in the case of MeCbl and $BrCH_2$Cbl, and the slopes were 0.99 ± 0.04 and 0.97 ± 0.03, respectively. The intercepts of these linear plots give the values of log K, which are in good agreement with those obtained from eq 4.

The values of K_{CN} obtained during the course of this work and those from the literature are summarized in Table 1. This table shows that the values of K_{CN} follow the sequence CH_3 < CH_3CH_2 < $CH_3CH_2CH_2$, which agrees with an increase in the inductive effect of the organic alkyl group and will be accompanied by the increased labilization of Bzm and a more efficient binding of CN^-.

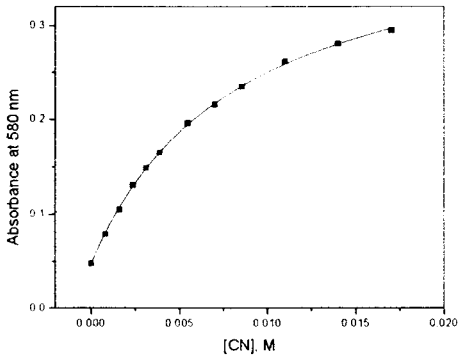

Figure 3. Variation in absorbance at 580 nm on addition of CN^- to β-CF_3Cbl. The solid line is a fit to eq 4 in the text and gives a value of K_{CN} = 123 ± 9 M^{-1}.

Table 1. Formation Constants K_{CN} and Related Thermodynamic Constants at 25 °C for the Reaction of RCbl with CN^-

R	$pK_{base-off}$[a]	K_{CN}[b]	K_{Co}[c]	fraction (base-off)[d]
CH_3CH_2	4.16	0.665 ± 0.066[e]	2.00×10	4.7×10^{-2}
$CH_3CH_2CH_2$	4.10	1.3 ± 0.14[e]	2.37×10	4.05×10^{-2}
Ado	3.67[f]	*g*	7.25×10	1.3×10^{-2}
CH_3	2.9	0.38 ± 0.03[h], 1.2[i]	4.52×10^2	2.1×10^{-3}
$BrCH_2$		0.43 ± 0.04[h]		
CF_3CH_2	2.6	*g*	9.23×10^2	1.1×10^{-3}
CF_2H	2.15	3.27 ± 0.11[e]	2.60×10^3	3.84×10^{-4}
$NCCH_2$	1.81	63.8 ± 1.4[e]	5.62×10^3	1.78×10^{-4}
CF_3	1.44	123 ± 9[h]	1.32×10^4	7.7×10^{-5}
CN	0.1	5×10^3 and 10^4 [i,j]	2.88×10^5	3.4×10^{-6}
H_2O	−2.13		4.90×10^7	2.0×10^{-8}

[a] Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3548. See eq 12 in the text. [b] K_{CN} for the substitution of Bzm by CN^- trans to the alkyl group (R) to produce R(CN)Cbl. See eq 1 in the text. [c] Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3548. See eq 11 in the text. [d] Fraction (base-off) = $1/(1 + K_{Co})$. [e] Reference 19. [f] Brown, K. L.; Hakimi, J. M.; Jacobsen, D. W. *J. Am. Chem. Soc.* **1984**, *106*, 7894. [g] Decomposes with CN^- in the dark. [h] This work: [RCbl] = (4−6) × 10^{-5} M, pH = 11, 25 °C, and I = 0.5 M $NaClO_4$ except in the case of MeCbl and $BrCH_2$Cbl (see text). [i] Reference 43. [j] Reference 42.

The affinity of CN^- toward CF_3Cbl is ca. 37 times higher than that for CF_2HCbl. The values of K_{CN} in the case of fluorocobalamins allow us to insert the organofluoro ligands in the trans effect order obtained previously.[25] It was found that $K_{imidazole}$ equals 301, 700, and 1.36×10^4 M^{-1} for substituting H_2O located in the trans position to $NCCH_2$, CF_3, and CN^-, respectively.[40,41] It was also found that K_{CN} equals 2.79×10^5, 1.6×10^6, and 10^8 M^{-1} for substituting H_2O trans to $NCCH_2$, CF_3, and CN^-, respectively.[18,42] This shows that the CF_3 group is located between CN^- and $NCCH_2$ with respect to the value of K. K_{CN} equals 63.8, 123, and 3×10^3 M^{-1} for substituting Bzm trans to $NCCH_2$, CF_3, and CN^-, respectively.[19,43]

Kinetic Measurements. Preliminary experiments (pH = 9 and 11, 10 °C) on the stopped-flow instrument showed that the reactions of MeCbl and $BrCH_2$Cbl with CN^- are too fast to be followed (deadtime of the stopped-flow instrument is 2−4 ms).

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(41) Hanania, G. I. H.; Irvine, D. H.; Irvine, M. V. *J. Chem. Soc. A* **1966**, 296.
(42) George, P.; Irvine, D. H.; Glauser, S. C. *Ann. N. Y. Acad. Sci.* **1960**, *88*, 393.
(43) Reenstra, W. W.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 5780.

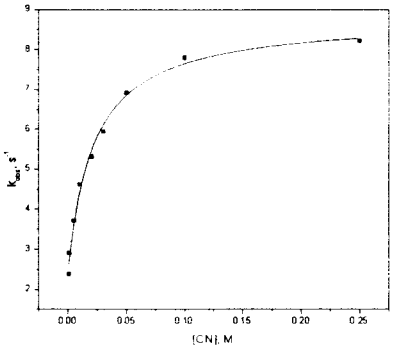


Figure 4. k_{obs} vs [CN^-] for the reaction between β-CF_3Cbl and CN^- at pH = 11, 10 °C, and I = 0.5 M ($NaClO_4$). The solid line is a fit to eq 6 in the text and results in $k_1 = 8.8 \pm 1.6$ s^{-1}, $k_{-2} = 2.6 \pm 0.2$ s^{-1}, and $k_2/k_{-1} = 42 \pm 4$ M^{-1}.

The second-order rate constant for the substitution of α-Bzm by CN^- in β-CF_2HCbl was found to be ca. $(1 \times 10^3) \pm 50$ M^{-1} s^{-1} at 10 °C. However, it was difficult to investigate this reaction in more detail because of the small change in absorbance and the high reaction rate. In this case, the intermediate *trans*-CF_2H(CN)Cbl complex was obtained, since a significant increase in absorbance at 580−600 nm was observed. This intermediate is stable in the dark for more than 100 h. We, therefore, concentrated our kinetic studies on the two remaining fluorocobalamin complexes (β-CF_3Cbl and β-CF_3CH_2Cbl), which could be studied in more detail.

Figure 4 shows a plot of k_{obs} vs [CN^-] for the reaction of β-CF_3Cbl with excess CN^- at pH = 11, [CN^-] = 0.0005−0.25 M, I = 0.5 M ($NaClO_4$), and 10 °C. This plot shows saturation kinetics, and a limiting value of k_{obs} ($k_{obs} = k_1$) is reached at high [CN^-]. The intercept can be assigned to a contribution of the reverse reaction, whereas the observed curvature can be considered as evidence in favor of a limiting D or an I_d mechanism. The kinetic data are for the first step which involves substitution of α-Bzm by CN^- as shown in eq 3. The product spectrum obtained in this case suggests the formation of an intermediate CF_3(CN)Cbl, since there is an increase in absorbance at 580−600 nm and since this intermediate is also light sensitive (see Figure S-3 in Supporting Information).

On the basis of all the available evidence, the suggested mechanism for the reaction between β-CF_3Cbl and CN^- can be represented by eq 5, which involves dissociative dechelation of Bzm to form a six-coordinate intermediate aqua complex. If k_{-2} has a significant value, as seen in Figure 4, then the observed rate law is expressed by eq 6. The data in Figure 4 were fitted to eq 6 and resulted in $k_1 = 8.8 \pm 1.6$ s^{-1}, $k_{-2} = 2.6 \pm 0.2$ s^{-1}, and $k_2/k_{-1} = 42 \pm 4$ M^{-1}, from which an overall equilibrium constant $k_1k_2/k_{-1}k_{-2} = 142 \pm 15$ M^{-1} can be calculated. This value is in good agreement with the spectrophotometric value of 123 ± 9 M^{-1} determined above. The value of k_2/k_{-1} represents the efficiency of cyanide, compared to the Bzm of the nucleotide loop, to scavenge the six-coordinate intermediate, which will depend on the selected cyanide concentration. Further evidence for the suggested mechanism comes from the direct measurement of the dechelation rate constant of Bzm (k_1) through acidification of β-CF_3Cbl to produce the protonated base-off species. Kinetic measurements indicated a linear acid concentration dependence for this reaction; i.e., the dechelation process is catalyzed by acid. The intercept of a plot of k_{obs} vs acid concentration resulted in a rate constant of 32 s^{-1} at 15 °C for the spontaneous dechelation process, which is ca. twice the value of the saturation rate constant ($k_1 = 15.4 \pm 1.2$ s^{-1}) measured at 15 °C (Table 2). However, at low acid concentration the dechelation process does not go to completion; i.e., the observed rate constant for dechelation includes a contribution of the reverse chelation reaction. We conclude from these data that the limiting rate constant observed for reaction 5 is indeed the dechelation step (k_1).

Table 2. Kinetic Data for the Reaction of β-CF_3Cbl and β-CF_3CH_2Cbl with CN^- as a Function of Temperature

	k_{obs} (s^{-1})	
T (°C)	R = CF_3[a]	R = CF_3CH_2[b]
10.0	8.2 ± 0.2	$(2.9 \pm 0.09) \times 10^{-2}$
15.0	14.4 ± 1.2	$(5.2 \pm 0.04) \times 10^{-2}$
20.0	27.5 ± 1.0	$(8.5 \pm 0.09) \times 10^{-2}$
25.0	43.2 ± 2.5	$(13.6 \pm 0.1) \times 10^{-2}$
30.0		$(21.9 \pm 0.07) \times 10^{-2}$
35.0		$(37.7 \pm 0.3) \times 10^{-2}$
$\Delta H^{\ddagger}$ (kJ mol^{-1})	77 ± 3	71 ± 1
$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})	$+44 \pm 11$	-25 ± 4

[a] Experimental conditions for CF_3Cbl: [CF_3Cbl] = $(2-4) \times 10^{-5}$ M, [CN^-] = 0.25 M, pH = 11.0, and I = 0.5 M $NaClO_4$. [b] Experimental conditions for CF_3CH_2Cbl: [CF_3CH_2Cbl] = $(2-4) \times 10^{-5}$ M, [CN^-] = 0.4 M, pH = 11.0, and I = 0.5 M $NaClO_4$.

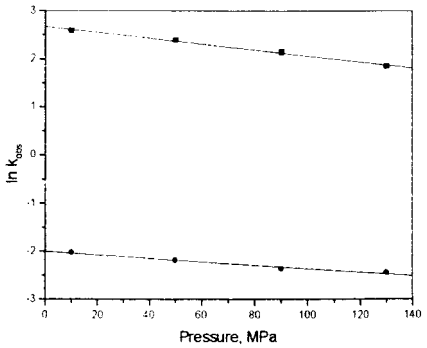
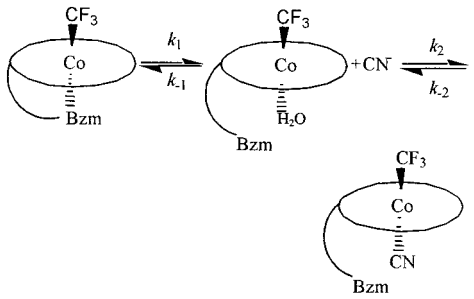


Figure 5. ln k_{obs} vs pressure for the reaction between β-CF_3CH_2Cbl and CN^- (●) and the reaction between β-CF_3Cbl and CN^- (■) measured at 0.4 and 0.25 M CN^-, respectively. The best fit of the data (solid line) gives $\Delta V^{\ddagger} = +8.9 \pm 1.0$ and $+14.8 \pm 0.8$ cm^3 mol^{-1}, respectively.



(5)

$$k_{obs} = (k_1k_2[CN^-] + k_{-1}k_{-2})/(k_{-1} + k_2[CN^-]) \qquad (6)$$

The reaction between β-CF_3Cbl and CN^- was studied as a function of temperature and pressure at a high cyanide concentration (0.25 M), i.e., where $k_{obs} = k_1$, and the results are reported in Table 2 and Figure 5, respectively. Figure 5 demonstrates a good linear correlation between ln(k) and pressure. The activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were found to be 77 ± 3 kJ mol^{-1} and $+44 \pm 11$ J K^{-1} mol^{-1}, respectively, and the activation volume $\Delta V^{\ddagger}$ was $+14.8 \pm 0.8$ cm^3 mol^{-1}. These data along with the observed rate law suggest that the first step of the reaction of β-CF_3Cbl with CN^- indeed follows a limiting D mechanism. The volume of activation is significantly positive and supports this suggestion. It was reported that $\Delta V^{\ddagger}$ for ligand

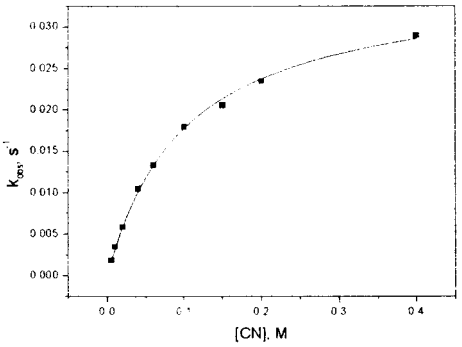
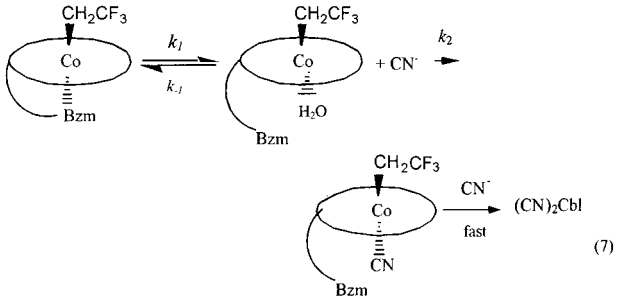


Figure 6. k_{obs} vs [CN^-] for the reaction between β-CF_3CH_2Cbl and CN^- at pH = 11, 10 °C, and I = 0.5 M ($NaClO_4$). The solid line is a fit to eq 10 in the text and results in $K_5 = 9.8 \pm 0.5$ M^{-1} and $k_6 = 0.036 \pm 0.001$ s^{-1}.

substitution on $[Co(TMPP)(H_2O)_2]^{5+}$ and $[Co(TPPS)(H_2O)_2]^{3-}$, where TMPP = *meso*-tetrakis(4-*N*-methylpyridyl)porphine and TPPS = *meso*-tetrakis(*p*-sulfonatophenyl)-porphine, is +14.4 and +15.4 cm^3 mol^{-1}, respectively,[44,45] the values of which are very close to the expected value of +13 cm^3 mol^{-1} for a limiting D mechanism.[45,46] The operation of a limiting D mechanism requires the intermediacy of a five-coordinate alkylcobalt corrinoid species. Such species are known for the R−Co(III) complexes of bis(salicylaldehyde)ethylenediamine and bis(acetylacetone)ethylenediamine prepared by Costa and coworkers,[47−49] as confirmed by the X-ray crystal structure of the methyl−cobalt derivatives of these chelates.[50,51] The temperature dependence of the UV−vis and ^1H NMR spectra of alkylcobinamides and alkylcobalamins is also consistent with the existence of five-coordinate species among the alkylcobalt corrinoids.[52]

Figure 6 reports data for the effect of [CN^-] on k_{obs} for the reaction of β-CF_3CH_2Cbl with CN^- ([CN^-] = 0.005−0.4 M, pH = 11, I = 0.5 M ($NaClO_4$), and 10 °C). Figure 6 also shows significant curvature, and a limiting rate constant is reached at high CN^- concentration, which could again be an indication of a limiting D mechanism. In this case, however, no indication of the formation of the intermediate R(CN)Cbl complex was observed from the UV−vis spectra. A comparison of the spectrum before and directly after mixing (1−10 s) gave identical results, demonstrating that negligible reaction had occurred within this time. Rapid scan and stopped-flow measurements showed that the reaction occurred through a single kinetic step. This suggests that the first reaction, which involves the displacement of α-Bzm by CN^-, is slow and can be monitored by stopped-flow as in the case of CF_3Cbl. The second step, which is the displacement of the β-CF_3CH_2 group to form the dicyano complex $(CN)_2Cbl$, is in fact a fast step. The final product was identified as $(CN)_2Cbl$,[25] since λ_{max} at 367, 540, and 580 nm were observed during the reaction.

The data in Figure 6 can be interpreted in terms of either a limiting D or a dissociative interchange (I_d) mechanism. The limiting D mechanism for the reaction between β-CF_3CH_2Cbl and CN^- can be presented by eq 7, and the corresponding expression for k_{obs} is given in eq 8.

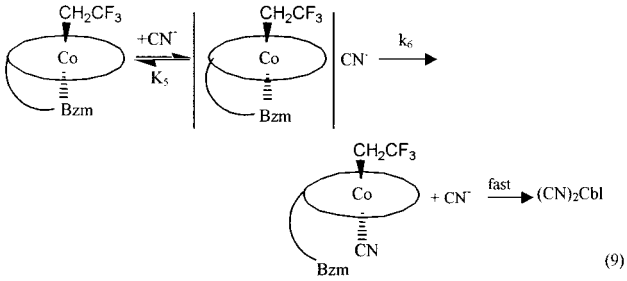


(7)

$$k_{obs} = k_1k_2[CN^-]/(k_{-1} + k_2[CN^-]) \quad (8)$$

The suggested mechanism for the reaction between β-CF_3CH_2Cbl and CN^- shown in reaction 7 involves slow dechelation of Bzm and the coordination of a water molecule, followed by displacement of the coordinated water by cyanide. The data in Figure 6 were fitted to eq 8 and resulted in $k_1 = 0.036 \pm 0.001$ s^{-1} and $k_2/k_{-1} = 9.2 \pm 0.7$ M^{-1}. The value of k_2/k_{-1} represents the efficiency of cyanide, compared to the Bzm of the nucleotide loop, to scavenge the intermediate aqua complex. In contrast to CF_3Cbl, the reaction between β-CF_3CH_2Cbl and 1 M $HClO_4$ to form the protonated base-off species was found to be very fast and impossible to measure using the stopped-flow technique. This suggests that the mechanism of the reaction with cyanide is not a limiting D mechanism, since the limiting rate constant (k_1) is smaller than the dechelation rate constant by orders of magnitude.

The I_d mechanism outlined in eq 9 along with the corresponding rate law given in eq 10 can in principle also account for the data in Figure 6.



(9)

$$k_{obs} = k_6K_5[CN^-]/(1 + K_5[CN^-]) \quad (10)$$

A fit of the data results in $K_5 = 9.8 \pm 0.5$ M^{-1} and $k_6 = 0.036 \pm 0.001$ s^{-1}. To help distinguish between the two possible mechanisms, we studied the reaction between β-CF_3CH_2Cbl and CN^- as a function of temperature and pressure at high CN^- concentration, i.e., under limiting rate conditions, for which the results are reported in Table 2 and Figure 5, respectively. $\Delta H^‡$ and $\Delta S^‡$ were found to be 71 ± 1 kJ mol^{-1} and −25 ± 4 J K^{-1} mol^{-1}, respectively, and the activation volume $\Delta V^‡$ was found to be +8.9 ± 1.0 cm^3 mol^{-1}.

The pressure dependence of the reaction of β-CF_3CH_2Cbl with CN^- shows that this reaction may occur through a

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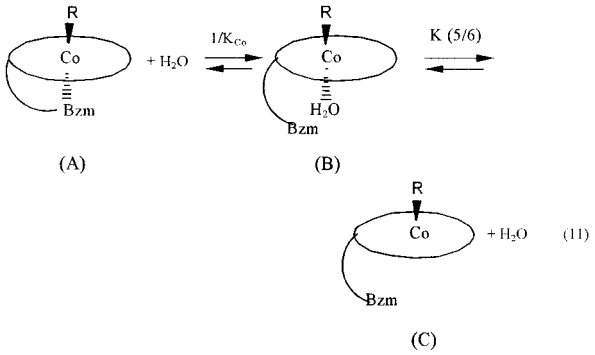
dissociative interchange mechanism, since it is reasonable to expect a significantly more positive value of $\Delta V^{\ddagger}$ for a limiting D mechanism,[46] keeping in mind that this value was measured under conditions where $k_{obs} = k_1$ (D) or k_6 (I_d). Similar results were reported for substitution reactions of aquacobalamin with different ligands such as HN_3, N_3^-, pyridine and its derivatives, and thiourea and its derivatives.[27c-e] In all these cases, $\Delta V^{\ddagger}$ was found to be in the range between +4 and +8 $cm^3\ mol^{-1}$, i.e., similar to the value found for the reaction of β-CF_3CH_2Cbl with CN^- in this study, and was interpreted in terms of an I_d mechanism. We recently studied the ligand substitution reactions of *trans*-$[Co^{III}(en)_2(Me)H_2O]^{2+}$ (a simple model for coenzyme B_{12}) with cyanide and imidazole.[53] From temperature and pressure dependence studies, activation parameters ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta V^{\ddagger}$) for the reaction of *trans*-$[Co^{III}(en)_2(Me)H_2O]^{2+}$ with cyanide were found to be 49 kJ mol^{-1}, −38 J K^{-1} mol^{-1}, and +7.0 $cm^3\ mol^{-1}$ compared to 53 kJ mol^{-1}, −22 J K^{-1} mol^{-1}, and +4.7 $cm^3\ mol^{-1}$, respectively, for the reaction with imidazole. On the basis of the reported activation volumes, it was concluded that these reactions followed a dissociative mechanism in which the entering nucleophile is weakly bound in the transition state. A direct differentiation between D and I_d could not be made since k_{obs} for these reactions did not exhibit a nonlinear concentration dependence, such that the pressure dependence could not be studied under limiting concentration conditions. In the present case, however, we favor an I_d mechanism, based on the $\Delta V^{\ddagger}$ value.

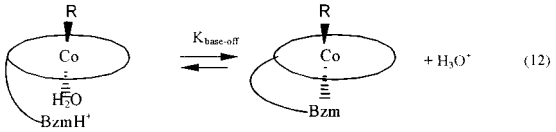
The entropy of activation for the reaction of β-CF_3CH_2Cbl with CN^- was found to be -25 ± 4 J K^{-1} mol^{-1}, whereas that for the reaction of β-CF_3Cbl with CN^- was found to be $+44 \pm 11$ J K^{-1} mol^{-1}. It is known that $\Delta S^{\ddagger}$ is usually subjected to large error limits because of the intrinsic extrapolation involved in its determination, such that these small absolute numbers are not very significant in terms of the assignment of a mechanism.[54,55] The negative activation entropy for the reaction of β-CF_3CH_2Cbl with CN^- as compared to the positive activation entropy for the reaction of β-CF_3Cbl with CN^- does support the suggestion of a less dissociative mechanism, i.e., I_d instead of D, for the former reaction. Therefore, on the basis of the volume of activation data, we propose that the reaction of β-CF_3CH_2Cbl with CN^- follows an I_d mechanism, where the entering ligand is weakly bonded in the transition state. The changes in volume during the complex-formation reaction are controlled by the lengthening of the Co−R bond, which should be independent of L.

The present study shows that the reactions, investigated during the course of this work, of β-CF_3CH_2Cbl and β-CF_3Cbl with CN^- proceed through an I_d and a limiting D mechanism, respectively. However, for the reaction between AdoCbl and CN^-, it was previously found[28] that the reaction proceeds through an associative mechanism. So, it seems clear that the alkyl group in the trans position, which controls the equilibrium between the base-on and base-off cobalamin species, as well as the ratio between the five- and six-coordinate species shown in reaction 11 must play a crucial role in controlling the ligand displacement mechanism and account for this unexpected mechanistic changeover.

Organocobalamins (RCbl) with different R (Ado, Et, Me, $BrCH_2$, CF_3CH_2, $NCCH_2$, CF_3, CN^-) are believed to exist in aqueous solution in a base-on form (A) and two base-off forms (B and C), which can be either five or six coordinate (H_2O occupies the α-position) as shown in eq 11.



Most of the known cobalt corrinoid complexes are six-coordinate species, except when one of the axial ligands is a polarizable group such as SO_3^- or an organic ligand, thereby creating a possibility of a five-coordinate complex (C).[52] The ratio of the base-on form (A) to the base-off forms (B and C) should be related to the value of K_{Co} for the formation of the base-on species from the base-off species. Thus, when the pK_a value of the protonated base-off complex is high and close to the value for the free Bzm (5.54),[19,32] the fraction of B and C in this equilibrium will be high. It was found that the change in the spectrum on drying or heating cobalamin (a qualitative measure of the ease with which the Bzm is displaced) does depend on the nature of the axial ligand in the order expected from the pK_a values: CN^- (0.1) ∼ ethynyl (0.7) < CF_3 (1.44) < vinyl (2.4) < methyl (2.89) < Ado (3.67) < ethyl (4.14).[23,37]



The values of K_{Co} (see eq 11), the fraction of the base-off species, the values of p$K_{base-off}$ (see eq 12), and the binding constant for cyanide when it is coordinated trans to the alkyl group (K_{CN}) for some of the organocobalamins studied are summarized in Table 1. This table shows the effect of the alkyl group located in the trans position and the trans effect order for these different RCbl complexes. The value of K_{Co} varies from 20 in the case of CH_3CH_2Cbl to 4.9×10^7 in the case of H_2OCbl. Consequently, the fraction of the base-off form of RCbl decreases significantly from 0.047 in the case of CH_3CH_2Cbl to 2×10^{-8} for H_2OCbl. This table also shows that the value of K_{CN} decreases significantly from 10^4, when R = CN^-, to 0.38 M^{-1}, when R = Me. It is also known that the complex-formation constants for the substitution of water by another ligand (Z) decrease and approach zero as the trans ligand is varied in the order H_2O > cyanide > CF_3 > $NCCH_2$ > CH_2CF_3 > methyl > Ado > ethyl.[25,40,52] This weakening of the bond between cobalt and all other ligands, Z, only makes sense if the bond to H_2O is also being weakened in the same way in the above trans effect order. One would therefore expect that the Co−OH_2 bond could be weakened to such an extent that a five-coordinate complex could be formed. In the case of Co(II) complexes $[CoL_5(H_2O)]$, where L = CN^-, MeNC, and PhNC,[56] water can readily be removed to produce a five-coordinate complex, when L = PhNC, or a dimer with a metal−metal bond,

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when L = CN or MeNC, and the formation constant is indeed very low.[56,57] Also, it was found that Co−N bond lengths and Co−C bond lengths in RCbls increase in the order H_2O < CN < CF_3 < $NCCH_2$ < CF_2H < CF_3CH_2 < CH_3 < Ado.[58−60] This suggests that AdoCbl is the most labile complex for ligand substitution reactions.

Absorption spectroscopy has played an important role in the study of the nature of the cobalt corrinoids. The cobalt corrinoids provide a unique example of a system the electronic structure of which can be profoundly altered by the nature of the ligand attached to the metal. The wide variation in the spectra of corrinoids provides an opportunity to distinguish the different corrinoids easily, because the UV−vis spectra are very sensitive to the changes in the number and nature of the axial ligands. The UV−vis spectra of certain cobalamins such as AdoCbl and ethylcobalamin show reversible changes in spectrum on heating the solution, changing the solvent, or drying the solid (when a thin film was used to record the reflection spectrum).[25,52] The spectrum at high temperature is similar to that observed for cobinamides or acidified cobalamins at high temperature. This suggests that the five- and six-coordinate species are in equilibrium. The UV−vis spectra of methylcobinamide, isopropylcobinamide, and sulfitocobinamide at low temperature (−180 °C) were assigned to the six-coordinate complexes. However, the spectra of these complexes at 20 °C were assigned to the five-coordinate complexes. In the case of ethylcobalamin, the fraction of base-off cobalamin (∼5% for the two base-off forms B and C) at equilibrium is in good agreement with the values of 13 and ∼15% from NMR measurement and optical spectra, respectively.[25,52] The ratio of B and C is probably similar for ethylcobinamide (100% at high temperature for form C), so that ethylcobalamin exists as a mixture of 85% A, 0% B, and 15% C. For AdoCbl, calculation gives ∼5% (B and C) in aqueous solution at room temperature, whereas the spectrum indicates the presence of ∼90% A and ∼10% (B and C); by analogy with ethylcobalamin one may assume 0% B and 10% C. We conclude, from these arguments, that the base-off form in the case of AdoCbl and ethylcobalamin is mainly a five-coordinate species at room temperature.

The alkylcobinamides are known to be five coordinate in the gas phase under fast atom bombardment as shown by their mass spectra.[13,16,33,61] In addition to the spectroscopic evidence for the existence of a five-coordinate−six-coordinate equilibrium for alkyl(aqua)cobaltcorrinoids and the sensitivity of this equilibrium to the inductive effect of the alkyl ligand, there are thermodynamic results that suggest the same equilibrium for the base-off alkylcobalamins.[62] It was found that this equilibrium is displaced toward the six-coordinate aqua species as the organic ligand becomes more electron withdrawing, where there is an increasing compensation for the entropy loss from the entropy gain of H_2O dissociation, and the entropy change no longer varies with the alkyl ligand. However, for some compounds with the highest value of $-\Delta S$ (and the lowest $-\Delta G$), the loss of entropy upon coordination of the pendent axial nucleotide is largely uncompensated for by the entropic effect of the loss of an axial water ligand; i.e., these compounds are largely five coordinate.[62]

Kräutler et al. [63,64] have compared the crystal structure of AdoCbl with the structure of Cob(II)alamin, which is the intermediate that arises from homolytic cleavage of the β-axial bond. They found that the two structures are very similar, indicating that it is not just a distortion of the corrin moiety but rather a strong stabilization of the radicals by the environment that leads to the homolysis products. The structure of Cob(II)alamin showed that the cobalt ion is shifted 0.12 Å out of the plane of the corrin nitrogens toward the Bzm ligand. More recently, Jensen et al.[65] ascribed the difference between MeCbl and AdoCbl to a different electronic nature of the two Co−C bonds. According to B3LYP DFT calculations, the highest occupied molecular orbital (HOMO) energy is higher in AdoCbl than in MeCbl, favoring homolytic cleavage. This is due to the 5′-deoxyadenosyl group, which interacts with the corrin ring and induces more electron density on the cobalt center.

The above arguments indicate that AdoCbl and ethylcobalamin are mainly five coordinate in the base-off form. However, in the case of CF_3Cbl, CNCbl, and H_2OCbl, the percentage of the base-off forms is very small and they mainly exist as six-coordinate species. The structures of the other cobalamins (R = Me, CH_2Br, CH_2CF_3, vinyl, etc.) are intermediate and each exists as an equilibrium between the five- and six-coordinate species. Thus, in the case of AdoCbl, the complex with the highest fraction of five-coordinate base-off, it is not surprising that cyanation follows an associative reaction mechanism since the five-coordinate fraction is high enough to enable an associative reaction path. A decrease in the labilization effect of R in going from adenosyl to CH_2CF_3 and CF_3 causes a drastic decrease in the fraction of the base-off species resulting in a decrease in the fraction of the five-coordinate species. Thus, a mechanistic changeover first to an I_d mechanism for R = CH_2CF_3 and then to a limiting D mechanism for R = CF_3 is at hand. We conclude that these arguments, based on our kinetic observations and supported by earlier spectroscopic observations, now present a consistent picture to account for the surprising associative mechanism reported before for the cyanation of the coenzyme.[28]

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Supporting Information Available: Three figures presenting the changes in absorbance upon addition of CN^- to MeCbl (Figure S-1) and $BrCH_2$Cbl (Figure S-2), and the UV−vis spectra of β-CF_3Cbl, CF_3(CN)Cbl, and $(CN)_2$Cbl (Figure S-3). This material is available free of charge via the Internet at http://pubs.acs.org.

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