Thermodynamic and Kinetic Data for the Base-On/Base-Off Equilibration of Alkylcobalamins

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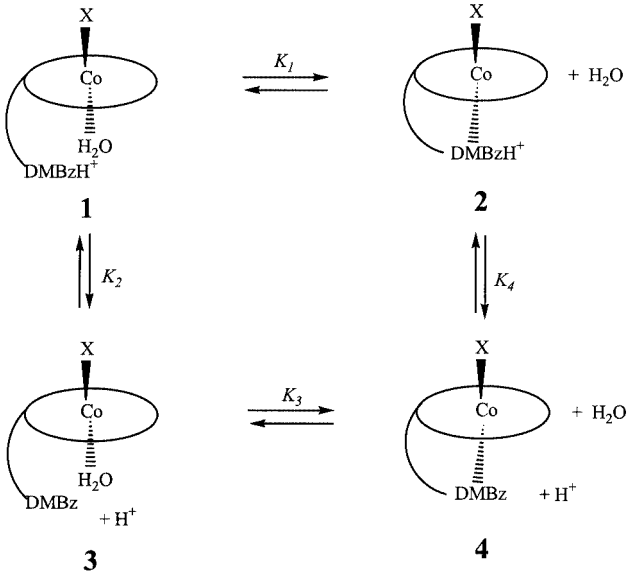
The base-on/base-off equilibration of a series of alkylcobalamins (XCbl) was studied as a function of the electronic properties of X. A square scheme was developed to incorporate all species that participate in this equilibration. The equilibrium between five- and six-coordinate species was studied for several alkylcobalamins (protonated base-off) and alkylcobinamides as a function of pressure by using UV/Vis spectroscopy. In addition, the kinetics of the acid-induced base-on/base-off equilibration of β-$NCCH_2$Cbl, β-CF_3Cbl and CNCbl were studied; the data obtained reveal evidence for an acid-catalysed reaction path. The results of this study and the observed correlations are discussed in reference to those reported in the past, and enable the formulation of an overall equilibration scheme.

Introduction

In general there is a need to investigate ligand substitution reactions *trans* to the axial alkyl ligand in coenzyme B_{12} and other β-alkylcobalamins, for comparison with the work performed on substitution reactions *trans* to a non-alkyl ligand. Methylcobalamin (CH_3Cbl) and coenzyme B_{12} (AdoCbl) undergo substitution of their axial benzimidazole ligand by a protein histidine residue during complexation to the CH_3Cbl-dependent methionine synthase,[1] the class I[2] AdoCbl-dependent mutases, methylmalonyl coenzyme A mutase, and glutamate mutase,[3] and the class III AdoCbl-dependent D-lysine-5,6-aminomutase.[4]

One of the most characteristic chemical properties of cobalamins is the base-on/base-off equilibrium in which the axially coordinated dimethylbenzimidazole (DMBz) nucleotide is displaced by H_2O and protonated. This reaction is accompanied by a large change in the electronic spectrum upon conversion of the (red) base-on to the (yellow) base-off form. The base-on/base-off equilibrium can best be described by the reactions outlined in Scheme 1.



Scheme 1

In this square scheme, **1** represents the protonated base-off cobalamin species, by far the major contributor at low pH, **2** the protonated base-on species, which must be a minor contributor at all pH values, **3** the deprotonated base-off species, which can be a significant contributor at neutral pH for some X, and **4** the deprotonated base-on species, the major species at neutral pH. The introduction of **2** is new and is based on the kinetic data for the base-on/base-off equilibration presented in this report. Coordination (ring closure) of the protonated base-off cobalamin species

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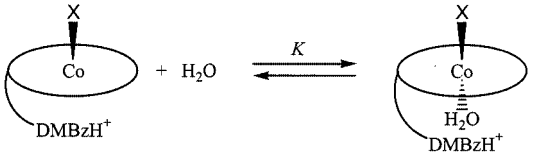
1 is expected to be unfavorable with respect to chelation of the deprotonated base-off species **3**, such that $K_1 << K_3$.

Although it is known that the base-on form (**4**) is a six-coordinate species, one of the remaining questions is whether the base-off forms (**1** and **3**) are six-coordinate aqua complexes or five-coordinate species without a water molecule occupying the sixth coordination site, or whether they exist in equilibrium between the five- and six-coordinate forms. Physicochemical measurements including electronic absorption, IR and NMR spectra of corrinoid Co^{III} complexes have suggested that the presence of an alkyl group as a strong donor ligand increases the fraction of the five-coordinate species.[5−8] The temperature dependence of the UV/Vis spectra of alkyl cobinamides (RCbi's, derivatives in which the axial nucleotide is chemically removed) suggests that the ratio of six-coordinate to five-coordinate species increases on decreasing the temperature.[5] Increasing temperature also favours the five-coordinate form when diethyl phosphite is the upper axial ligand, and in this case it was also shown qualitatively that increasing pressure favours the six-coordinate form.[9] No structural data on the five-coordinate forms have yet been reported and more direct evidence for the reversible coordination of water is clearly desirable.

Wirt and Chance[10] provided evidence for a five-coordinate/six-coordinate equilibrium in base-off CH_3Cbl and AdoCbl from the temperature dependence of the 1s-3d pre-edge transition in the X-ray absorption edge spectra of these complexes. In addition to the spectroscopic evidence, thermodynamic parameters for this equilibrium provide evidence that the base-off RCbl's exist as an equilibrium mixture of six-coordinate aqua and five-coordinate species, and that this equilibrium is sensitive to the nature of the organic ligand.[11]

Recently, the equilibrium between the five- and six-coordinate base-off forms of both methylcobalamin and vinylcobinamide was studied as a function of pressure, and it was found that this equilibrium is shifted towards the formation of the six-coordinate species upon increasing pressure.[12] This has led us to investigate the equilibrium between the five- and six-coordinate species [shown by way of example for the protonated base-off species **1** in Equation (1)] for many of the available alkylcobalamins (protonated base-off) and cobinamides, in order to investigate the influence of the group X on this equilibrium and to understand the mechanistic implications of its occurrence.



5 **1** (1)

In addition, a systematic study of the kinetics of the base-on/base-off equilibration for β-$NCCH_2$Cbl, β-CF_3Cbl and CNCbl was undertaken, and the data obtained reveal evidence for the acid-catalysed reaction path shown in Scheme 1. The reported results enable us to draw a consistent mechanistic picture for such ligand displacement reactions.

Results

According to Scheme 1, the base-on/base-off reaction must be viewed as the sum of two consecutive equilibria, either deprotonation of **1** followed by coordination (ring closure) of **3**, involving dissociation of a coordinated water molecule, or coordination (ring closure) of **1** followed by deprotonation of the base-on species **2**. It follows from the square scheme that $K_2K_3 = K_1K_4$. Values of $pK_{base\text{-}off}$ [Equation (2), where $K_{base\text{-}off}$ represents the apparent acid dissociation constant of a mixture of species **1** and **2**] have been reported for a large number of cobalamins, and range from about 4.0 (*n*-heptylCbl) to −2.4 in the case of H_2OCbl.[13,14] The pK_a of the detached axial nucleoside α-ribazole ($pK_a = 5.56$),[15] or the virtually identical value for the appropriate microscopic pK_a of the detached axial nucleotide, α-ribazole-3′-phosphate ($pK_a = 5.54$),[16] can be used for the value of pK_2.

$$K_{base\text{-}off} = \frac{K_2(1+K_3)}{(1+K_1)} \quad (2)$$

Based on the definition of $K_{base\text{-}off}$ [Equation (2)], it can be shown (see Supporting Information; see also footnote on the first page of this article) that f_{off}, the fraction of base-off species, is given by Equation (3).

$$f_{off} = \frac{([H^+]+K_2)}{([H^+]+K_{base\text{-}off})(1+K_1)} \quad (3)$$

Equations (2) and (3) simplify to Equations (4) and (5) on the basis that it is reasonable to expect K_1 to be very small, so that $1 + K_1 \approx 1$.

$$K_{base\text{-}off} = K_2(1+K_3) \quad (4)$$

$$f_{off} = \frac{([H^+]+K_2)}{([H^+]+K_{base\text{-}off})} \quad (5)$$

Equation (5) indicates that the fraction of base-off cobalamin depends on the pH, such that an increase in $[H^+]$ will increase the concentration of **1**, and therefore the fraction of base-off species in solution. However, under neutral experimental conditions (pH ≥ 7) or under conditions in which typical cyanation reactions were studied (pH = 11), Equation (5) simplifies to Equation (6). Equations (5) and (6) allow the calculation of the fraction of base-off form from $K_{base\text{-}off}$, K_2 and $[H^+]$ under all experimental

conditions, where Equation (6) is identical to that used before for pH ≥ 7.[13]

$$f_{off} = \frac{K_2}{K_{base\text{-}off}} \quad (6)$$

Effect of Pressure on the UV/Vis Spectra of XCbl and XCbi

There is good evidence that Cbi's (and protonated, base-off Cbl's) with strong donor ligands (e.g. vinyl, methyl, sulfite, dialkyl phosphites) exist as temperature-dependent mixtures of five- and six-coordinate aqua forms based on UV/Vis and EXAFS data.[5,10,17] Increasing temperature favours the five-coordinate form, and with diethyl phosphite as the β-axial ligand, it has also been shown qualitatively that increasing pressure favours the six-coordinate form.[9]

Organocobalamins (XCbl) with different X (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 (**4**) and two base-off forms (**1** and **3**), which can both be either five- or six-coordinate, as shown for **1** in Equation (1). UV/Vis spectra of cobalamins such as AdoCbl and ethylcobalamin in their protonated, base-off forms, exhibit reversible changes on heating the solution, changing the solvent or drying the solid (when a thin film was used to record the reflectance spectrum).[5,18] The spectrum of protonated base-off cobalamin at high temperature is similar to the spectra of cobinamides, suggesting that temperature affects the spectra of base-off cobalamin and the analogous cobinamides in the same way. The UV/Vis spectra of methylcobinamide, isopropylcobinamide and sulfitocobinamide at low temperature (−180 °C in ethanol or a mixture of ethanol, methanol and propan-2-ol) were assigned to the six-coordinate complexes. However, the spectra of these complexes at 20 °C were assigned to the five-coordinate complexes.[5] In the case of ethylcobalamin, the calculated[13] fraction of base-off cobalamin [ca. 4.7% from Table 1 for the two base-off forms **1** and **5** in Equation (1)] at equilibrium is in reasonable agreement with the values of 13 and ca. 15%, from NMR measurements and optical spectra, respectively.[5,18] The ratio of the species in Equation (1) is probably similar for ethylcobinamide, so that ethylcobalamin exists as a mixture of 85% **4**, 0% **3** and 15% **5**.[19] For AdoCbl, calculations suggest ca. 1.3% **3** and **5** in aqueous solution at room temperature, whereas the spectrum indicates the presence of ca. 90% **4** and ca. 10% **3** and **5**; by analogy with ethylcobalamin, one may assume 0% **3** and 10% **5**. We conclude that the base-off forms of AdoCbl and ethylcobalamin are mainly five-coordinate species at room temperature.

In addition to the spectroscopic evidence for the existence of a five-coordinate/six-coordinate equilibrium for corrinoid alkyl(aqua)cobalt complexes, and the sensitivity of this equilibrium to the inductive effect of the alkyl ligand, there are thermodynamic results that suggest the same equilibrium occurs in the base-off alkylcobalamins.[11] 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.[11] The UV/Vis spectrum of protonated base-off AdoCbl (3×10^{-5} M in 1 M $HClO_4$), exhibits a band at ca. 450 nm as shown in Figure 1. Increasing the pressure to 150 MPa resulted in a small increase in absorbance at the maximum with no isosbestic points being observed. Similar UV/Vis spectra were observed in the case of *n*PrCbl. On the basis that such five-coordinate species exhibit a band at ca. 440−460 nm, compared to a band at ca. 500−522 nm for six-coordinate cobalamins or cobinamides,[8,18] the observed spectral changes suggest that the protonated base-off forms of these complexes mainly exist as five-coordinate species in solution irrespective of pressure. The fractions of the six-coordinate species are apparently very small, i.e., no shift in the equilibrium towards six-coordinate species can be induced by pressure.

Table 1. Electrochemical, thermodynamic and activation parameters for base-on/base-off reactions for a series of cobalamins (XCbl's)

X	p*K* base-off [a]	K_3 [b]	Fraction base-off[c]	Δδ(^{31}P) [Hz][d]	ΔG_3 [kJ·mol^{-1}][e]	ΔH_3 [kJ·mol^{-1}][f]	ΔS_3 [J K^{-1} mol^{-1}][f]	$E_{1/2}$[g]
Et	4.16	20	4.7×10^{-2}			−37 ± 3	−100 ± 12	−1.54
*n*Pr	4.1	23.7	4.05×10^{-2}	−11.98	−8.23	−35 ± 3	−92 ± 12	−1.55
Ado	3.67	76.6	1.3×10^{-2}	−10.45	−10.74			−1.35
CH_3	2.89	467	2.1×10^{-3}	2.81	−15.21	−32 ± 2	−54 ± 4	−1.6
CH_2CF_3	2.6	923	1.1×10^{-3}	0.6	−16.89	−33 ± 3	−58 ± 8	
CF_2H	2.15	2600	3.84×10^{-4}	7.89	−19.48	−28 ± 2	−30 ± 6	
$NCCH_2$	1.81	5620	1.78×10^{-4}	8.42	−21.69			
CF_3	1.44	1.3×10^4	7.7×10^{-5}	24.71	−23.49	−29 ± 1	−20 ± 5	−1.27
CN	0.1	2.9×10^5	3.4×10^{-6}	35.68	−31.10	−38 ± 1	−21 ± 5	−1.00
H_2O	−2.13	4.9×10^7	2.0×10^{-8}	51.01	−43.81			−0.07

[a] See Equation (2) in the text. [b] See Equation (4) and Scheme 1 in the text. [c] Fraction base-off = $K_2/K_{base\text{-}off}$. [d] Ref.[21] [e] At 25 °C, ref.[21] [f] Refs.[11,13] [g] Refs.[34,35]

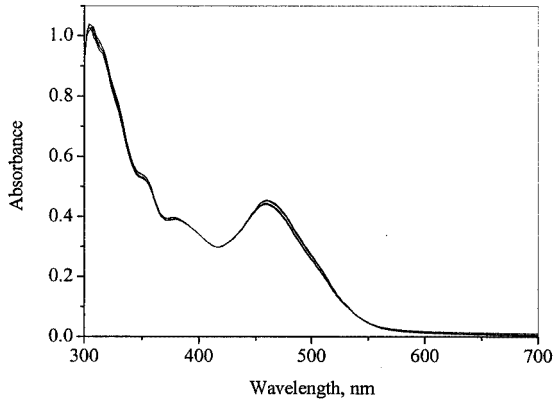


Figure 1. UV/Vis spectra of β-AdoCbl (base-off) (0.5 M $HClO_4$) recorded as a function of pressure (5, 50, 100 and 150 MPa)

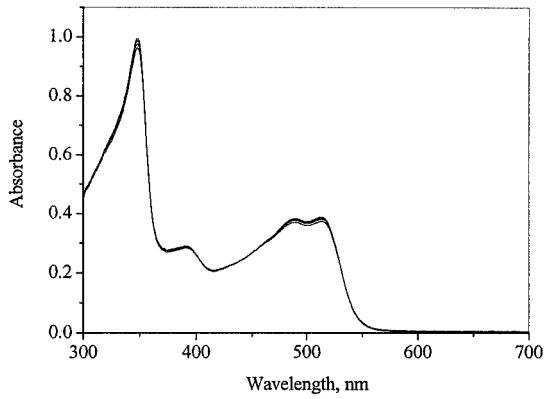


Figure 2. UV/Vis spectra of β-CF_3Cbl (base-off) (0.5 M $HClO_4$) recorded as a function of pressure (5, 50, 100 and 150 MPa).

The UV/Vis spectra of β-CF_3Cbi, β-CF_3Cbl (protonated base-off), H_2O(CN)Cbi (exists as a mixture of α- and β-isomers), and $(H_2O)_2$Cbi exhibit bands at 500−520 and 520−540 nm. Increasing the pressure to 150 MPa again resulted in a very small increase in absorbance with no isosbestic points observed [as shown in Figure 2 for β-CF_3Cbl (base-off)], suggesting that these complexes mainly exist as six-coordinate species irrespective of pressure. Thus, the fraction of the five-coordinate species in these compounds is very small. Interestingly, β-CF_3CH_2Cbl (protonated base-off), β-$NCCH_2$Cbl (protonated base-off) and the cobinamides of these complexes exhibit the main band at 454 and 478 nm, respectively.[20] The absorbance at these maxima increases slightly and is accompanied by a slight shift in the band position on increasing pressure. More importantly, however, a significant increase in absorbance at ca. 480−540 nm was observed upon increasing the pressure step-wise from 5 to 150 MPa. These changes in the UV/Vis spectra are accompanied by isosbestic points at 304, 365, 450 and 595 nm as shown in Figure 3 for β-$NCCH_2$Cbl (protonated base-off), suggesting that an equilibrium between the five- and six-coordinate species occurs [see also Supporting Information, Figure S-1 for the UV/Vis spectra of β-CF_3CH_2Cbl (protonated base-off) as a function of pressure]. Similar changes to those obtained upon increasing the pressure were obtained upon decreasing the temperature. This equilibration involves the coordination of a water molecule to the five-coordinate species to form the six-coordinate aqua complex, and indicates that these complexes exist as a mixture of five- and six-coordinate species in solution.

We recently studied this equilibrium for both MeCbl (protonated base-off) and vinylcobinamide,[12] and found that increasing pressure displaces the observed equilibrium [Equation (1)] from the five-coordinate towards the six-coordinate form for both complexes. A significant increase in the absorbance at ca. 490 nm was observed, accompanied by good isosbestic points. Values of the equilibrium constant {K = [six-coordinate]/[five-coordinate] as defined in Equation (1)} as a function of pressure were calculated

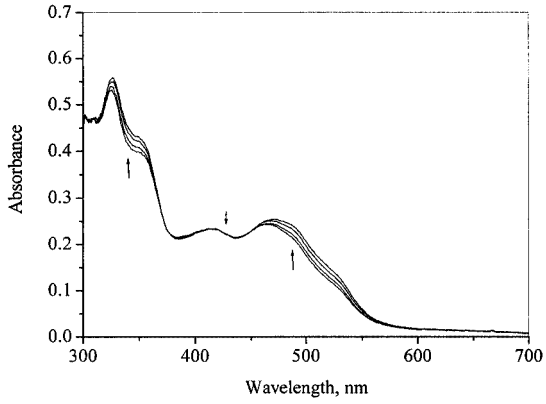


Figure 3. UV/Vis spectra of β-$NCCH_2$Cbl (base-off) (0.5 M $HClO_4$) recorded as a function of pressure (5, 50, 100, and 150 MPa, accompanied by an increase in absorbance around 480−540 nm)

from the increase in absorbance at 490 nm using the relative optical density for the fully formed six-coordinate and five-coordinate species derived from the temperature-dependence studies.[5] The reaction volume, ΔV, was found to be -12.4 ± 1.0 and -12.5 ± 1.2 cm^3 mol^{-1} for the conversion of the five- to the six-coordinate species in vinylcobinamide and MeCbl (base-off), respectively.[12] Their identical values underline the close parallel between Cbi's and protonated Cbl's, and their agreement with the theoretical value of -13.1 cm^3 mol^{-1} expected for the coordination of a water molecule to a five-coordinate species confirm that conversion of the five- to the six-coordinate form involves the coordination of one water molecule to the five-coordinate form.[12]

Based on the UV/Vis spectra recorded under pressure, we conclude that the protonated base-off species of the first two compounds (AdoCbl and *n*PrCbl) of the series of alkylcobalamins (see Table 1 for a list of these XCbl's) investigated during the course of this work are mainly five-coordinate species irrespective of pressure. For some of the XCbl's (X = Me, CF_3CH_2, $NCCH_2$ and vinyl) we suggest that the protonated base-off forms exist in solution as an equilibrium between five- and six-coordinate species. However, for

the last three complexes in the XCbl series (X = CF_3, CN and H_2O), the base-off forms of these compounds are mainly six-coordinate aqua species irrespective of pressure. It seems clear that the X group in the upper, β-axial position has a significant influence on this equilibrium, and by increasing the electron-withdrawing ability of the alkyl group the equilibrium is shifted towards the six-coordinate complexes.

Kinetics of the Base-On/Base-Off Equilibration

β-CF_3Cbl, β-$NCCH_2$Cbl and CNCbl were selected for this study, since the p$K_{base\text{-}off}$ values of these complexes are 1.81, 1.44 and 0.1, respectively.[15,21,22] These p$K_{base\text{-}off}$ values are in the range which allow a titration with acid to be carried out in order to dechelate the DMBz in the α-position. At the same time, the DMBz dissociation rate of these compounds can be monitored with stopped-flow techniques, since, from the electron-inductive effect of these X, the rate of DMBz dissociation is expected to be much slower for these complexes than for those with strongly donating X. The DMBz dissociation rates of other XCbl's such as MeCbl and CF_3CH_2Cbl are much too fast to be monitored by the stopped-flow technique.[23,24]

The kinetic results for the stopped-flow "pH-jump" reaction of 3×10^{-5} M β-$NCCH_2$Cbl (pH = 5, unbuffered solution) with different concentrations of $HClO_4$ (0.01−0.3 M) at I = 1 M ($NaClO_4$) and 5 °C are shown in Figure 4. The values of $k_{obsd.}$ first decrease with increasing acid concentration in the lower concentration range (0.005−0.05 M), and then increase linearly with increasing acid concentration at higher concentrations (0.05−0.3 M). A combination of these concentration dependencies results in the curved dependence observed in Figure 4. The first part of the plot at low acid concentration is incomplete since the reaction becomes too fast to be monitored by stopped-flow and the change in absorbance at 530 or 478 nm becomes too small. The second part of the plot is expected to level off (base-off, k_{-3}), but shows a significant increase in $k_{obsd.}$ with increasing acid concentration. This linear increase in $k_{obsd.}$ is ascribed to an acid-catalysed dechelation reaction, the slope of which represents the rate constant for the acid-catalysed reaction (k^H) and the intercept of which represents the rate constant for the spontaneous dechelation of DMBz (k_{-3}). We conclude that dechelation of DMBz to give β-$NCCH_2$Cbl (base-off) is catalysed by acid. Reenstra and Jencks previously reported that the reaction between CNCbl and HCN is also catalysed by acid, but no kinetic data were given.[25] In terms of the overall base-on/base-off equilibration presented in Scheme 1, the rate law for the acidification reaction of β-$NCCH_2$Cbl is given in Equation (7), in which k_{-3} and k_3 represent the rate constants for the spontaneous dechelation of **4** and chelation of **3**, respectively, $K_2/(K_2 + [H^+])$ represents the fraction of the base-off species in the form of **3** that can undergo chelation, and k^H represents the acid-catalysed reaction path, i.e. the product of the protonation constant for **4** and the dechelation rate constant k_{-1} of **2**, such that $k^H = k_{-1}/K_4$.

$$k_{obs} = k_{-3} + \frac{k_3 K_2}{K_2 + [H^+]} + k^H [H^+] \quad (7)$$

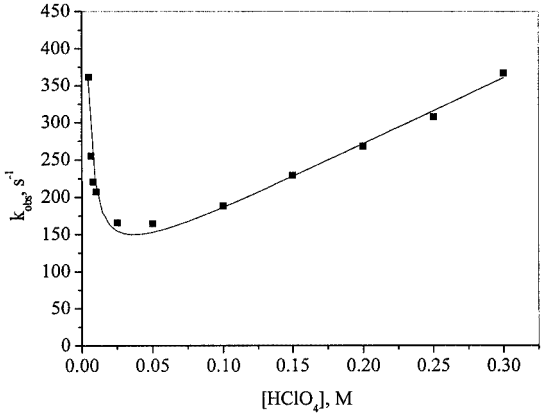


Figure 4. $k_{obsd.}$ versus [$HClO_4$] for the reaction between β-$NCCH_2$Cbl and $HClO_4$ at 5 °C and I = 1 M ($NaClO_4$); the solid line is a fit to Equation (7) in the text.

A non-linear least-squares fit of the data shown in Figure 4 to the rate law in Equation (7), with pK_2 = pK_{Bz} = 5.56,[15] results in $k_3 = (4.4 \pm 0.3) \times 10^5$ s^{-1}, $k_{-3} = 83 \pm 13$ s^{-1} and $k^H = 913 \pm 71$ M^{-1} s^{-1}. It follows that K_3 (= k_3/k_{-3}) equals 5300, which is in very good agreement with the value of 5620 previously reported from thermodynamic considerations.[13] Also, the value of k_{-3} is in good agreement with the acid-independent limiting rate constant obtained when the nucleophile concentration dependence was studied for the reaction of β-$NCCH_2$Cbl and CN^-.[26]

Similar results to those obtained for β-$NCCH_2$Cbl, were obtained for the reaction of β-CF_3Cbl with $HClO_4$. The data for the reaction of 3×10^{-5} M β-CF_3Cbl with $HClO_4$ (0.01−1 M) are shown in Figure 5. The data again show a decrease in $k_{obsd.}$, followed by an increase in $k_{obsd.}$ at higher acid concentrations. The non-linear least-squares fit of the data to the rate-law in Equation (7) resulted in $k_3 = (7.80 \pm 0.07) \times 10^4$ s^{-1}, $k_{-3} = 7.7 \pm 0.2$ s^{-1} and $k^H = 31.8 \pm 0.4$ M^{-1} s^{-1}. It follows that $K_3 = 1.01 \times 10^4$, which is in good agreement with the reported value of 1.3×10^4.[13] Also k_{-3} is in good agreement with the acid-independent limiting rate constant (8.2 s^{-1}) previously determined from the concentration dependence of the reaction of CN^- with β-CF_3Cbl.[27]

Similar results to those obtained for the acidification of β-$NCCH_2$Cbl and β-CF_3Cbl, were obtained for the reaction of CNCbl with $HClO_4$. The data for the reaction of 3×10^{-5} M CNCbl with $HClO_4$ (0.01−1.0 M) at 25 °C again show a decrease in $k_{obsd.}$ followed by a slight increase in $k_{obsd.}$ at higher acid concentrations (see Figure S-2 in the Supporting Information). The increase in $k_{obsd.}$ in this case was not as prominent as in the previous two cases because the value of p$K_{base\text{-}off}$ in this case is only 0.1,[22] which means that more acidic conditions are required to give signific-

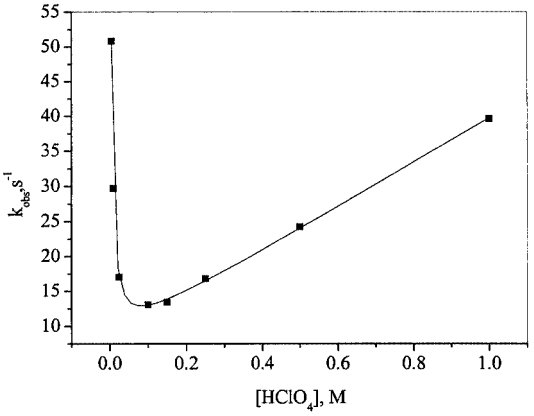


Figure 5. $k_{obsd.}$ versus [$HClO_4$] for the reaction between β-CF_3Cbl and $HClO_4$ at 10 °C and I = 1 M ($NaClO_4$); the solid line is a fit to Equation (7) in the text

ant proportions of the protonated base-off cobalamin. The non-linear least-squares fit of the data resulted in k_3 = (9.2 ± 0.6) × 10^3 s^{-1}, k_{-3} = 0.19 ± 0.04 s^{-1} and k^H = 0.28 ± 0.06 M^{-1} s^{-1}, from which it follows that K_3 = 4.85 × 10^4, which is significantly smaller than the value determined previously (2.9 × 10^5).[13] The value of k_{-3} is also higher than the limiting rate constant (0.042 s^{-1}) determined from the concentration dependence of the reaction of CN^- with CNCbl.[25,26] These differences are in part caused by the lower value of $pK_{base-off}$ and the consequently much higher concentration of $HClO_4$ required to obtain the base-off form.

By way of comparison, the values of k_{-3} for the spontaneous ring opening of β-$NCCH_2$Cbl, β-CF_3Cbl, and CNCbl to give the corresponding unprotonated base-off cobalamins were found to be 83, 7.7 and 0.19 s^{-1}, respectively. The rate constant k_3 for ring closure of the unprotonated base-off forms of these cobalamins was found to be 4.4 × 10^5, 7.8 × 10^4 and 9.2 × 10^3 s^{-1}, respectively, and the acid catalysed rate constant k^H for the ring opening of the unprotonated base-on forms was found to be 913, 31.8 and 0.28 M^{-1} s^{-1}, respectively. These rate constants all follow the same trend for the three complexes, which can be ascribed to the influence of the axial group (X) in the upper, β-position (see further discussion related to Table 1). Thus, our systematic study of the acid dependence of the base-on/base-off reactions has revealed rate constants for the individual reaction steps that present a consistent picture of the overall mechanism and are in agreement with the earlier reported overall equilibrium data for this process, which plays a crucial role in the ligand substitution reactions at the DMBz site of the complexes.

Discussion

It seems clear that the alkyl group (X) in the *trans* position, which controls the equilibrium between the base-on and base-off cobalamin species shown in Scheme 1, as well as the ratio between the five- and six-coordinate species shown in Equation (1), must play a crucial role in controlling the ligand displacement mechanism of these complexes.

The complex-formation constants for the substitution of the α-axial water molecule in a series of alkyl cobinamides by nitrogenous ligands 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.[5,18,28] This weakening of the bond between cobalt and all other ligands (X) 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, water can readily be removed to produce a five-coordinate complex when L = PhNC, or a dimer with a metal−metal bond when L = CN^- or MeNC, and the formation constant is indeed very low.[29]

The stretching frequency ν_{CN} (for cyanide *trans* to X), when X = H_2O, was found to be 2133 cm^{-1}. However, this value was found to be 2082 cm^{-1}, when X = Et, which is close to the stretching frequency for free CN^- (2079 cm^{-1}),[18,29c] i.e., the coordinated cyanide ion becomes more ionic in character. It is clear from this argument that the positive charge on Co decreases from X = H_2O to X = Et. It was also found that the Co−N and Co−C bond lengths in RCbl's increase in the order H_2O < CN < CF_3 < $NCCH_2$ < CF_2H < CF_3CH_2 < CH_3 < Ado.[30−32] This suggests that AdoCbl is a labile complex for ligand substitution reactions. Furthermore, in the case of AdoCbl steric effects may also play an important role in controlling its substitution behaviour.[33]

Table 1 shows that there is a significant increase in $E_{1/2}$ from −1.54 V (X = Et) to −0.07 V (X = H_2O), which reflects the effect of the X group on $E_{1/2}$.[34,35] These data suggest that there is a relationship between the electrochemical behavior and electron density on the Co centre in XCbl's. However, electrochemical studies on XCbl [X = CH_3, CH_3CH_2, $CH_2CH_2CH_3$, $CH_2CH(CH_3)_2$ and $CH_2C(CH_3)_3$], suggest that the bulkiness of X may have a significant influence on the $E_{1/2}$ values.[35] The decrease in $E_{1/2}$ may reflect changes in the Co−X distance which modulates the energy of the lowest σ-type MO (LUMO) of these complexes. The energy of the σ-LUMO (the orbital in which the electron added to RCo must enter) increases when the Co−X distance decreases.[35]

Kräutler et al.[36,37] have compared the crystal structure of AdoCbl with that of cob(II)alamin, which is the intermediate that arises from homolytic cleavage of the β-axial bond. It was found that the two structures are indeed very similar, indicating that it is not just a distortion of the corrin moiety that leads to the homolysis products, but rather strong stabilisation of the radicals by the environment. The structure of cob(II)alamin showed that the cobalt ion is shifted 0.12 Å out of the plane of the corrin nitrogen atoms towards the DMBz ligand. More recently, Jensen et al.[38] ascribed the difference between MeCbl and AdoCbl to the

different electronic nature of the two Co−C bonds. According to B3LYP DFT calculations, the 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 centre.

As mentioned previously, 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^{2-} or an organic ligand, where there is a possibility of a five-coordinate complex.[5] The ratio of the base-on form (six-coordinate species) to the base-off forms (five- and six-coordinate aqua species) is related to the value of K_3 for the formation of the base-on species from the base-off species. Thus, when the $pK_{base\text{-}off}$ value of the protonated base-off complex is high and close to the value for free DMBz (viz. 5.54),[15,16] the fraction of five-coordinate species in this equilibrium will be significant. It was found that the change in the spectrum on drying or heating cobalamin (a qualitative measure of the ease with which DMBz is displaced) does depend on the nature of the axial ligand in the order expected from the $pK_{base\text{-}off}$ values: CN ($pK_{base\text{-}off}$ = 0.1) ≈ ethynyl (0.7) < CF_3 (1.44) < vinyl (2.4) < methyl (2.89) < Ado (3.67) < ethyl (4.14).[14,39]

The values of K_3, the fraction of base-off species, the values of $pK_{base\text{-}off}$ and the thermodynamic parameters associated with the base-off/base-on equilibrium of XCbl are summarized in Table 1. This table shows the effect of the alkyl group (X) located in the *trans* position and the *trans* effect order for the different XCbl complexes. The value of K_3 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 XCbl decreases significantly from 0.047 in the case of CH_3CH_2Cbl to 2×10^{-8} for H_2OCbl.

The differences between the ^{31}P NMR chemical shifts for the base-on and base-off forms of XCbl, $\Delta\delta(^{31}P)$, are also included in Table 1. These values show a regular variation with the apparent free energy of coordination of the pendant axial DMBz ligand.[21] These effects are interpreted in terms of variations in the axial Co−N bond length leading to progressive changes in the base-on phosphodiester conformation throughout the series of cobalamins. Interestingly, $\delta(^{31}P)$ for base-on Cbl's is linearly related to the axial Co−N bond length (slope = −2.26 ± 0.08 ppm/Å),[32] but $\delta(^{31}P)$ for the base-off Cbl's is the same for all XCbl. When the axial ligand is bound moderately weakly in a base-on cobalamin such as MeCbl (K_3 = 467) or CF_3CH_2Cbl (K_3 = 923), the RO−P−OR bond angle is nearly identical for the base-on and base-off species and hence a negligible value of $\Delta\delta(^{31}P)$ results. However, for those cobalamins such as AdoCbl (K_3 = 76.6) or EtCbl (K_3 = 20) in which the axial base is bound more weakly, the axial Co−N bond becomes longer (2.24 Å in AdoCbl), the RO−P−OR bond angle decreases and the ^{31}P resonance is shifted upfield, producing a negative $\Delta\delta(^{31}P)$. However, in the case of CNCbl, where the axial ligand is bound significantly more tightly (K_3 = 2.9×10^5), the Co−N bond length is short (1.97 Å), the RO−P−OR bond angle increases, and the ^{31}P resonance is shifted downfield and produces a positive $\Delta\delta(^{31}P)$.[21]

Table 1 also shows that the enthalpy of formation of the base-on species, ΔH_3, (the average value is −33 ± 6 kJ·mol^{-1})[11,13] is independent of the nature of the upper axial group (X), despite the variation in K_3. Thus, all the variation in K_3 and $pK_{base\text{-}off}$ must be due to variation in the entropy change, ΔS_3, upon coordination of the axial nucleotide; ΔS_3 varies from −100 ± 12 (X = Et) to −20 ± 5 (X = CF_3) and −21 ± 5 J K^{-1} mol^{-1} (X = CN). This suggests that for the compounds with the strongest electron-donating group (X = Et and Pr) and the most negative ΔS_3 values, the entropy loss associated with the restriction of the pendant axial nucleotide during formation of the base-on species is largely uncompensated by any gain in entropy due to loss of an axial water ligand. This in turn suggests that the base-off species of these complexes are largely pentacoordinated under these conditions. The equilibrium in Equation (1) is displaced towards the formation of the six-coordinate species by increasing electron-withdrawing X groups; thus, for X = CF_3 and CN, the loss of entropy associated with the axial DMBz coordination is compensated for by an increase in entropy due to axial water loss.[11] These results are in agreement with our findings for the equilibrium between five- and six-coordinate species obtained from the UV/Vis spectra recorded under pressure.

We conclude from the above discussion, and based on the trends observed in Table 1, that the base-off forms of some XCbl's such as AdoCbl and ethylcobalamin are mainly five-coordinate. 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 structure of the base-off forms of the other cobalamins (X = Me, CH_2Br, CH_2CF_3, vinyl, etc.) are intermediate and exist as an equilibrium mixture of the five- and six-coordinate species. A decrease in the labilisation effect of X on going from adenosyl to CH_2CF_3 and CF_3 causes a drastic decrease in the fraction of the base-off species and also in the fraction of the five-coordinate species. These effects can play a significant role in the ligand substitution behaviour of the investigated complexes.

Our systematic study of the acid dependence of the base-on/base-off reactions has revealed rate constants for the individual reaction steps that present a consistent picture for the overall mechanism and are in agreement with the earlier reported overall equilibrium data for this process. In addition, the reported kinetic data reveal evidence for an acid-catalysed pathway for the dechelation of DMBz.

Experimental Section

Materials: All the chemicals used were p.A. grade and used as received without further purification. $HClO_4$ and $NaClO_4$ were purchased from Merck. Ultrapure water was used in the kinetic and thermodynamic measurements. The preparations and measurements were carried out in diffuse light since all the alkylcobalamins are known to be very light-sensitive.[18,40] Cyanocobalamin and Ad-

oCbl were supplied by Sigma and H_2Ocbl acetate by Roussel. The other alkylCbl's were prepared as described in the literature[20,41] by treating 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), freshly activated with 1.0 M HCl, was added, and the reduction was allowed to proceed for 30 min. Alkyl halide (ca. 1 mmol; R = *n*Pr, CF_3CH_2, CF_3 and $NCCH_2$ for the preparation of *n*PrCbl, CF_3CH_2Cbl, CF_3Cbl and $NCCH_2$Cbl, respectively) was introduced, and the reaction was allowed to proceed for 30 min. Alkylcobinamides were prepared by treating (cobinamide)Co^{II}, generated by zinc reduction of Factor B[42,43] [a mixture of the two diastereomers of (aqua)(cyano)cobinamide], with the appropriate alkyl halides. The reaction mixtures were desalted by chromatography on Amberlite XAD-2[15] and the β-isomers were separated by HPLC.[20,44,45]

Instrumentation and Measurements: The pH of the solutions was measured using a Mettler Delta 350 pH meter with a combined glass electrode, calibrated with standard buffer solutions at pH = 4.0, 7.0 and 10.0. UV/Vis spectra were recorded with Shimadzu UV-2101 and Cary 1 spectrophotometers. UV/Vis spectra under high pressure were recorded with the Shimadzu spectrophotometer utilizing a home-made high-pressure unit.[46] 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 aqueous ammonium phosphate buffer (pH = 3.0) and acetonitrile as described previously.[20,44,45] Kinetic measurements were carried out with an Applied Photophysics SX 18MV stopped-flow instrument coupled to an online data acquisition system. At least eight kinetic runs were recorded under all conditions, and the reported rate constants represent the mean values. All kinetic measurements (pH jump experiments) were carried out under pseudo-first-order conditions, i.e. the acid concentration was in at least tenfold excess over the cobalamin concentration. The UV/Vis spectrophotometers and stopped-flow instruments were thermostatted to the desired temperature ±0.1 °C.

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