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Probing the nature of the Co(III) ion in cobalamins: deactivation of the metal towards ligand substitution in 10-nitrosoaquacobalamin, and the kinetics of the ligand substitution reactions of iodocobalamin †

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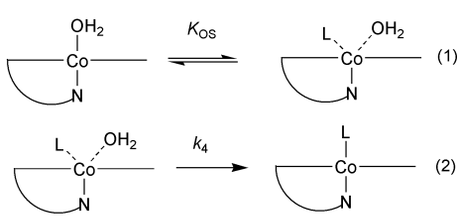
Aquacobalamin (vitamin B_{12a}) formally contains a diamagnetic Co(III) ion encapsulated in the small macrocyclic cavity of the corrin ring. Since the ligand substitution reactions, which proceed through a dissociative interchange mechanism, are fast, the usual inertness of d^6 Co(III) has been modified; this points to the ability of the corrin ring to perturb the properties of the metal ion. This phenomenon is explored further in the present work by replacing the H atom at C10 by an electron withdrawing NO group in 10-nitrosoaquacobalamin. This compound, 10-nitrosoaquacobalamin, is characterised by NMR and UV–vis spectroscopy, and by FAB-MS. The pK_a of coordinated H_2O is determined to be 10.71 ± 0.04 (25 °C, I = 2.2 mol dm^{-3}, $NaClO_4$), ΔH = 120 ± 11 kJ mol^{-1} and ΔS = 198 ± 38 J K^{-1} mol^{-1} (the pK_a of B_{12a} itself is 8.09). The strongly electron-withdrawing NO group deactivates the metal ion towards ligand substitution, and neither 1.2 mol dm^{-3} pyridine nor 0.7 mol dm^{-3} N_3^- result in displacement of coordinated H_2O; either the reactions are very slow (no observable change in 72 hours) or log K has been decreased by at least 1 and 4 orders of magnitude for coordination of these two ligands, respectively. Hence, the electronic structure of the corrin ring can directly influence the axial ligand binding properties of the metal ion. We demonstrate by studying the temperature-dependence of the kinetics of the substitution of I^- in iodocobalamin by imidazole, N_3^- and $S_2O_3^{2-}$ that, despite the considerable steric barrier of the corrin's peripheral substituents, the nucleophilic participation of the incoming ligand is retained even when changing H_2O to I^-, which increases the size of the departing ligand.

Introduction

It is now generally accepted that the ligand substitution reactions of vitamin B_{12a} (Fig. 1), where the axially-coordinated H_2O ligand of Co(III) is readily replaced by a ligand, L, from solution, proceed through a dissociative interchange mechanism (I_d, Scheme 1, eqns. 1 and 2, where N represents the axial



Scheme 1

base 5,6-dimethylbenzimidazole, and charges are omitted for convenience),[1–3] despite earlier suggestions[4–6] that the mechanism is strictly dissociative (D). The distinction between a limiting D mechanism and an interchange I_d mechanism for an octahedral complex rests on one being able to demonstrate directly or indirectly the existence of the five coordinate intermediate. When the observed pseudo first-order rate constant shows saturation with the concentration of incoming [L], such a distinction becomes possible (see Results and discussion).

The B_{12a} kinetics results are perhaps surprising for two reasons. Firstly, not only is the Co(III) ion encapsulated by the small corrin macrocycle (corrins, unlike porphyrins, have a direct linkage between the A and D ring which results in a smaller macrocyclic cavity) but also the substituents on the periphery of the corrin ring provide a formidable steric barrier to incoming L.[7] Yet an I_d mechanism necessarily requires nucleophilic participation by incoming L in the transition state. Secondly, the reactions are surprisingly fast for Co(III), which is the classic example of an inert transition metal ion. A comparison of representative second-order rate constants for substitution of H_2O by L in Co(III) complexes with four N-donor equatorial ligands shows that the approximate lability ratio of the metal ion towards substitution in corrin, porphyrin, cobaloxime and ammine systems is $10^9 : 10^6 : 10^4 : 1$.[8] This is clear evidence for a *cis* labilising effect of the corrin ring, *i.e.*, the electronic properties of the *cis* ligand have a direct and marked effect on the kinetics of the ligand binding properties in the axial position.

We address these two questions further in this paper. Firstly, we probed the effect of replacing H at the C10 position of the corrin with an electron-withdrawing nitroso group (Y = NO) on the ligand substitution properties of Co(III). Secondly, we increased the steric bulk on the departing ligand, X, by making X = iodide (Fig. 1) and studied the kinetics of its substitution by three representative ligands, N_3^-, $S_2O_3^{2-}$ and imidazole, to determine whether an increase in the bulk of X is sufficient to switch the mechanism from I_d to D.

† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR assignments for 10-NO-H_2OCbl$^+$; comparison of the ^{13}C resonances of H_2OCbl$^+$, 10-Cl-H_2OCbl$^+$ and 10-NO-H_2OCbl$^+$; comparison of the side chain amide N–H chemical shifts of 10-NO-H_2OCbl$^+$ to Cbl. See http://www.rsc.org/suppdata/dt/b2/b202158g/

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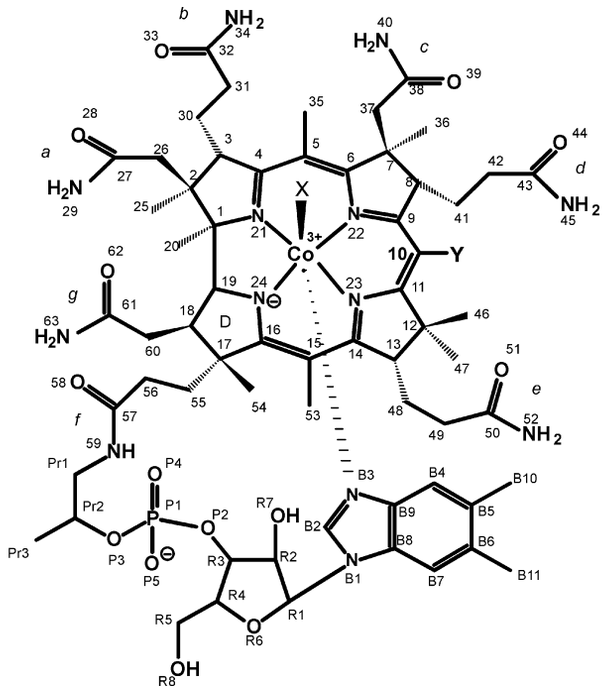


Fig. 1 The standard view of derivatives of vitamin B_{12}. In aquacobalamin, vitamin B_{12a}, X = H_2O and the substituent at C10, Y, is H. The base occupying the lower coordination site of Co(III) is 5,6-dimethylbenzimidazole (Bzm). The portion of the molecule from C7 to C13 is referred to as the eastern quadrant of the molecule.

Experimental

Hydroxocobalamin (>99% pure, HPLC) was obtained from Roussel. Imidazole (Merck), NaN_3 (Riedel-de-Haën), NaI and $Na_2S_2O_3$ (Saarchem) were of the highest purity available and used as received. Water was purified with a Millipore MilliQ system. Preliminary spectroscopic observations were conducted on a Cary 1E or Cary 3E spectrophotometer using 1 cm pathlength cuvettes. For studying the kinetics of the substitution of coordinated I^- in iodocobalamin (ICbl), sufficient NaI was added to solutions of B_{12a} (*ca.* 50 μmol dm^{-3}) to ensure >97% complex formation (log *K* for coordination of I^- by B_{12a} = 1.5[9]). Ionic strength was maintained at 2.2 mol dm^{-3} with $NaClO_4$ and solutions were buffered at pH 7.00 with MOPS (0.1 mol dm^{-3}). The kinetics were studied under pseudo first-order conditions at 560 nm using a Hi-Tech Scientific SF-51 stopped flow spectrometer. The experimentally determined rate constants, k_{obs}, were determined by fitting the absorbance *vs.* time traces to an equation of the form $A_1\exp(-k_{obs}t) + A_2$ using a non-linear least squares technique and a Newton–Raphson procedure. The temperature of the system was maintained (±0.1 °C) with a water-circulating bath.

The synthesis of 10-nitrosoaquacobalamin was a modification of the method Wagner used to synthesise 10-nitrosocyanocobalamin from cyanocobalamin.[10,11] In a typical synthesis, 0.109 g of hydroxocobalamin was dissolved in 40 cm^3 glacial acetic acid and stirred under nitrogen. Dry NaCl (0.6239 g) was added to a nitrogen-purged round bottom flask and to this was added nitrosylsulfuric acid (1.2587 g). The brown NOCl gas formed was carried in the N_2 stream and bubbled through the B_{12a} solution for 4 h. The reaction was followed by HPLC; a maximum of 40% conversion to 10-NO–H_2OCbl^+ was obtained. The solution was desalted on XAD, eluted with 10% CH_3CN and purified on HPLC using a semi-preparative C18 column.

NMR spectra were obtained on a Varian Unity Inova 500 MHz NMR spectrometer equipped with pulsed field gradients on samples in 95% H_2O–5% D_2O. Two dimensional homonuclear (TOCSY and ROESY) spectra with Watergate solvent suppression and heteronuclear (HMQC and HMBC) experiments were carried out as described previously.[12] ^1H and ^{13}C NMR assignments (Table S1 of the ESI), including those of the amide hydrogens, were made as described in detail elsewhere.[13]

10-NO–HOCbl was characterised by FAB-mass spectrometery. The molecular ion peak (MH^+) occurs at 1374.6, whilst $(MNa)^+$ occurs at 1396.6; this gives a molecular weight of 1373.6, whilst the expected molecular weight of 10-NO–OHCbl is 1373.8. Treatment of a solution of 10-NO–H_2OCbl^+ with excess cyanide in neutral pH and at pH >13 leads to the formation of 10-NO–$(CN)_2Cbl^-$ and 10-*iso*NO–$(CN)_2Cbl^-$, respectively, with the position of the main bands as reported[11] (Fig. 2).

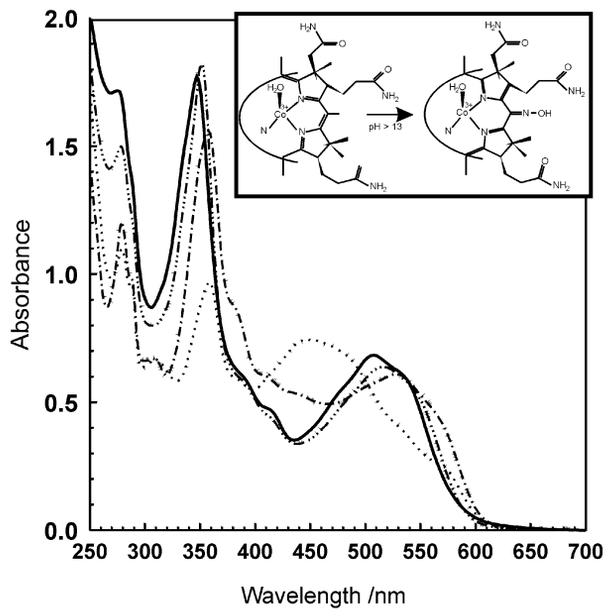


Fig. 2 UV–visible spectra of a 119 μM solution of 10-NO–H_2OCbl^+, pH 7, in aqueous solution (——). Treatment with 1 equivalent of CN^- leads to slow conversion to 10-NO–CNCbl (— · · · —). Addition of excess cyanide results in the formation of 10-NO–$(CN)_2Cbl^-$ (—·—); at pH > 13 (*insert*), this is converted to the isonitroso derivative (· · · · · ·).[11]

The pK_a of coordinated H_2O in 10-NO–H_2OCbl^+ was determined spectrophotometrically as described previously.[8]

Molecular orbital calculations on 10-NO–OHCbl were performed at the RHF–SCF level of theory using the ZINDO/1 model[14,15] with HYPERCHEM[16] as single-point calculations at the crystal structure geometry of 10-Cl–OHCbl,[8] with NO substituted for Cl at C10. The geometry of the substituent was set at that obtained from a molecular mechanics modelling of a nitroso group substituted on an sp^2 C backbone using Allinger's MM2 force field,[17] *viz.*, C–N = 1.387 Å, N–O = 1.174 Å, C–N–O = 125.8°.

Results and discussion

Synthesis and characterisation of 10-NO–H_2OCbl^+

It is clear that the axial coordination site and the corrin ring are in direct electronic communication because changes in either perturb the electronic spectrum of the cobalamins, which is dominated by π–π* transitions of the corrin. Thus, strong axial donor ligands cause the bands in the spectrum to shift to lower energy as the charge density on the metal ion, and hence on the corrin ring, increases.[18–20] The effect of substitution at C10 can be seen from the data in Table 1. An electron-withdrawing substituent such as NO or NO_2 (σ_m = 0.62; σ_p = 0.91; *R* = 0.42[21]) causes the bands to move to shorter wavelengths, whereas a resonance donating substituent such as Cl (σ_m = 0.37; σ_p = 0.23; *R* = −0.19[21]) causes them to move to longer wavelengths.

Table 1 The effect of substitution at C10 on the electronic spectrum of the cobalamins

C10 substituent[a]	Axial ligands	γ/nm	β/nm	α/nm	Ref.
H	H_2O, Bzm	351	527	554	31
	CN^-, Bzm	360.5	520	550	11
	CN^-, CN^-	367	540	580	10
Cl	H_2O, Bzm	364	546	576	8
	CN^-, Bzm	364	546	576	8
	CN^-, CN^-	369	561	602	8
NO	H_2O, Bzm	345	507	535	This work
	CN^-, Bzm	347	510	531	This work
	CN^-, CN^-	356	530	568	This work
NO_2	CN^-, CN^-	561	538	572	32

[a] See Fig. 1.

Table 2 Significant differences[a] in ^{13}C resonances (quoted in ppm) between H_2OCbl^+ and 10-NO–H_2OCbl^+, and between H_2OCbl^+ and 10-NO–H_2OCbl^+

Group[b]	H_2OCbl^+	10-NO–H_2OCbl^+	$\Delta\delta_1$[c]	10-Cl–H_2OCbl^+	$\Delta\delta_2$[d]
C10	97.58	132.77	35.19	106.84	9.26
C11	181.49	170.88	−10.61	177.75	−3.74
C9	177.24	171.28	−5.96		
C37	48.21	44.12	−4.09		
C8	60.02	56.77	−3.25		
C12	50.9	53.80	2.90	53.36	2.46
C15	106.85	109.65	2.80		
C46	34.84	32.20	−2.64	32.13	−2.71
C36	23.81	21.30	−2.51		
C5	110.63	113.06	2.43	112.15	1.52
C14	168.53	166.41	−2.12		
C13	56.42	58.05	1.63	57.92	1.50
C16	183.85	182.24	−1.61	182.51	−1.34
C19	77.73	76.25	−1.48		
C47	22.18	20.74	−1.44	24.63	2.45
C7	53.59	54.85	1.26		
C6	166.75	165.68	−1.07		
C26	45.95	44.90	−1.05		
C38	178.12	177.08	−1.04		

[a] Taken as $|\Delta\delta| \geq 1$ ppm. [b] See Fig. 1 for nomenclature. [c] $\Delta\delta_1 = \delta_{\text{10-NO–}H_2OCbl^+} - \delta_{H_2OCbl^+}$. [d] $\Delta\delta_2 == \delta_{\text{10-Cl–}H_2OCbl^+} - \delta_{H_2OCbl^+}$.

Despite many attempts, we have been unable so far to obtain diffraction-quality crystals of 10-NO–$H_2OCbl^+X^-$ ($X^- = NO_3^-$, Cl^-, ClO_4^-). Crystals that have formed are invariably small and of poor quality. We have therefore had to rely on NMR spectroscopy to make tentative deductions concerning the structure of 10-NO–H_2OCbl^+ in solution.

The effect of substitution of H by NO at C10 on the ^{13}C NMR resonances of the corrin is shown in Table 2 and Fig. 3. (A full comparison of the ^{13}C resonances of H_2OCbl^+,[22] 10-NO–H_2OCbl^+ and 10-Cl–H_2OCbl^+,[8] is given in the ESI.) As expected, the greatest differences occur in the eastern half of the molecule (Fig. 1), with the C10 resonance shifting downfield by 35.2 ppm, and tapering off as one moves further away. Table 2 shows that the shifts in the ^{13}C resonances are significantly greater for substitution by NO than by Cl.[8] Thus, the NO group at the C10 position has a major effect on the electronic properties of the corrin ring; indeed, the electronic effect of NO is so large that even the resonances of relatively remote C atoms such as C3, C19 and C54 are affected (Fig. 3).

We considered the possibility that the shift at C19 might be significantly affected by the corrin fold (the dihedral angle between the mean planes through N21, C4, C5, C6, C9, N22 and C10, and through N24, C16, C15, C14, N23, C11 and C10, respectively[23]), which might be different in 10-NO–H_2OCbl^+ and in H_2OCbl^+. There is only a weak ($r^2 = 0.55$, $n = 10$) correlation between the fold angle and the C19 chemical shift; this would predict a fold angle of 8.5° for 10-NO–H_2OCbl^+ (*cf.* 18.7° in H_2OCbl^+).

In sufficiently acidic solution, the coordinated 5,6-dimethylbenzimidazole base (Bzm) of the cobalamins is protonated and displaced from the lower (α) coordination site (Scheme 2, eqn. 3). We could not determine $pK_{\text{base-off}}$ directly because 10-NO–H_2OCbl^+ is unstable towards denitrosation in strongly acidic solution; we therefore examined the possibility of correlations between Bzm ^{13}C chemical shifts and values of $pK_{\text{base-off}}$ for the on–off reaction. We obtained good correlations for the resonances of B4 ($r^2 = 0.98$), B7 ($r^2 = 0.98$), B6 ($r^2 = 0.98$), B5 ($r^2 = 0.97$) and B9 ($r^2 = 0.97$, $n = 7$ for all), and these gave estimates of −1.06, −1.52, −1.57, −1.58 and −1.21 (average −1.4 ± 0.2) for the $pK_{\text{base-off}}$ of 10-NO–H_2OCbl^+ (compared to

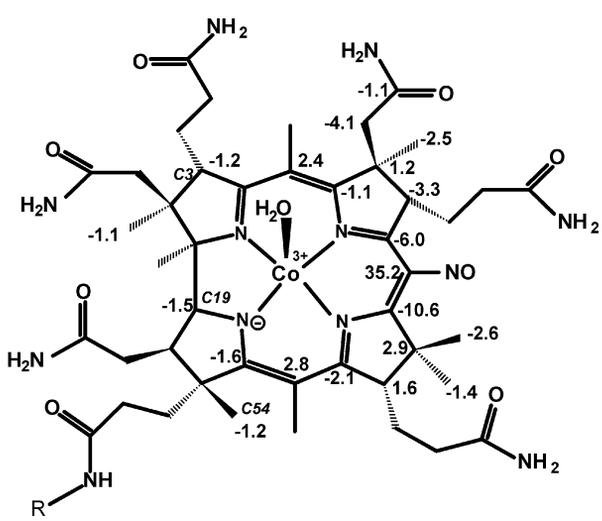
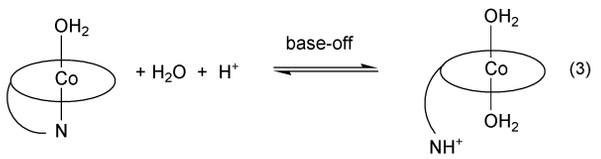


Fig. 3 Significant differences between the ^{13}C NMR resonances of 10-NO–H_2OCbl^+ and H_2OCbl^+ ($\delta_{\text{10-NO–}H_2OCbl^+} - \delta_{H_2OCbl^+}$).



Scheme 2

−2.13 for H_2OCbl^+ itself). This $\Delta pK_{base\text{-}off} = 0.7$ increase on substituting H for NO at C10 is in agreement with the effect observed with 10-Cl–XCbl's ($\Delta pK_{base\text{-}off} = 1.8$ for X = CN and 0.4 for X = Me[8]) and suggests that, as with 10-Cl–CNCbl and 10-Cl–MeCbl compared to CNCbl and MeCbl, respectively, the Co–N(Bzm) bond in 10-NO–H_2OCbl^+ is longer than in H_2OCbl^+ (1.925 Å[24]). If this analysis is correct, this is a surprising result as one might have expected that a strongly electron-withdrawing group at C10 would decrease, not increase, the axial bond lengths.

We have previously shown[8] that the ^1H chemical shifts of the side chain amides of 10-Cl–CNCbl and 10-Cl–H_2OCbl^+ are very similar, with the exception of the *c* side chain amide, where the ^1H resonance is shifted by 0.54 ppm downfield. This is diagnostic of an H-bond from the donor coordinated H_2O to the acceptor carbonyl oxygen of the *c* side chain,[25–28] confirming that the H-bond observed in the solid state,[8] and the analogous H-bond observed in the crystal structures of H_2OCbl^+[24] and of aquacyanocobyric acid,[29] is retained in solution. We have found that there is no significant difference between the ^1H amide resonances of 10-NO–H_2OCbl^+ and the average ^1H amide resonances of representative cobalamins (Table S3). (The difference in the *c-anti*-^1H resonance is a mere 0.20 ppm.) There therefore appears to be no H-bonding between the *c* side chain and coordinated H_2O in 10-NO–H_2OCbl^+. Why the H-bond should occur in H_2OCbl^+, aquacyanocobyric acid and 10-Cl–H_2OCbl^+ but not in 10-NO–H_2OCbl^+ is unclear.

We found that for the ionisation of coordinated H_2O in H_2OCbl^+, $\Delta H = 36.0 \pm 1.9$ kJ mol^{-1} and $\Delta S = -34 \pm 6$ J K^{-1} mol^{-1} so that pK_a (25 °C) = 8.09 (I = 0.5 M, $NaNO_3$).[8] For 10-Cl–H_2OCbl^+, $\Delta H = 32.9 \pm 1.5$ kJ mol^{-1} and $\Delta S = -36 \pm 6$ J K^{-1} mol^{-1} so that pK_a (25 °C) = 7.65 (I = 0.5 M, $NaNO_3$).[8] We find for 10-NO–H_2OCbl^+ (Fig. 4) $\Delta H = 120 \pm 11$ kJ mol^{-1} and $\Delta S = 198 \pm 38$ J K^{-1} mol^{-1} so that pK_a (25 °C) = 10.71 ± 0.04 (I = 2.2 M, $NaClO_4$).

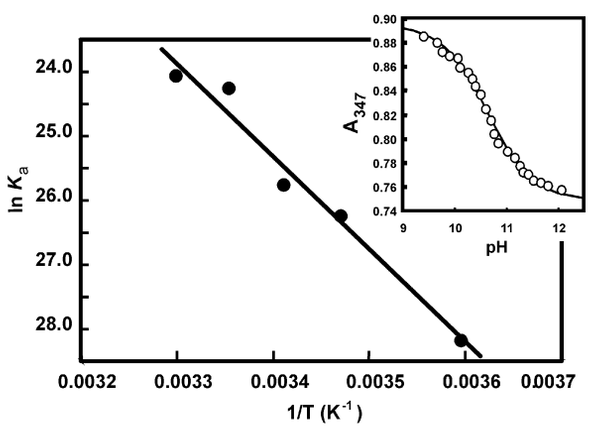


Fig. 4 The temperature dependence of the pK_a of the ionisation of coordinated H_2O in 10-NO–H_2OCbl^+. *Insert*: Typical dependence of the γ band absorbance of 10-NO–H_2OCbl^+ on pH. The solid line is the best non-linear least squares fit to the experimental data.

The very significant increase in the pK_a of coordinated H_2O is in line with our observations for 10-Cl–H_2OCbl^+. As we have been unable to obtain diffraction-quality crystals of 10-NO–$HOCbl^+$ we performed molecular orbital calculations on the crystal structure of 10-Cl–H_2OCbl^+, with the Cl at C10 replaced by a NO group and coordinated H_2O replaced by OH^- The partial charge found on the oxygen atom in coordinated OH^- in 10-NO–HOCbl by applying the ZINDO/1 model was −0.566; this compares to a value of −0.456 in 10-Cl–HOCbl and −0.503 in HOCbl.[8] Thus, as the electron-withdrawing properties of the C10 substituent increases, the charge density on coordinated OH^- increases (the metal–hydroxide bond becomes more ionic), and the pK_a monotonically increases ($r^2 = 0.91$, $n = 3$) with increasing negative charge. This is further evidence that *cis* effects occur in the cobalamins and that a property of the axial ligand can be perturbed by modifying the electronic structure of the equatorial ligand.

Ligand binding properties of 10-NO–H_2OCbl^+

Addition of 1.2 mol dm^{-3} pyridine and 0.7 mol dm^{-3} N_3^- to a solution of 10-NO–H_2OCbl^+ (*ca.* 120 μmol dm^{-3} pH 7.0) caused no observable changes in the spectrum even after 72 hours of observation. In our experience, 20% complex formation results in ready observation of the associated spectral changes. Hence, either log K values for coordination of pyridine and N_3^- are <0.2 and 0.8, respectively (unless fortuitously all three compounds have exactly the same UV–visible spectrum, which seems highly unlikely), or the kinetics of ligand substitution are extraordinarily slow. Log K values for coordination of pyridine and N_3^- by B_{12a} itself are 1.16 and 4.3, respectively,[30] and for substitution of H_2O in 10-Cl–H_2OCbl^+ the values are 1.02 and 4.6, respectively.[30] Thus, substitution of H by resonance-donating Cl has virtually no effect on the magnitude of log K, but, assuming the effect is thermodynamic and not kinetic, the electron-withdrawing NO group has markedly deactivated the metal ion towards axial substitution of coordinated H_2O, with log K (in the case of azide) decreasing by at least four orders of magnitude. Whether thermodynamic or kinetic, this result demonstrates that the ligand binding properties of Co(III) may be controlled by modifying the electronic properties of the corrin ring. To date we have been unable to obtain reliable kinetic data for substitution of H_2O in 10-NO–H_2OCbl^+; the matter is under active investigation and the results will be reported on elsewhere.

The kinetics of the substitution of iodide in iodocobalamin

The D and I_d mechanisms of the ligand substitution reactions at octahedral transition metal complexes may be distinguished by plots of the observed pseudo-first order rate constants, k_{obs}, against the concentration of the incoming ligand.[2] Provided a sufficiently high concentration of the ligand, L, can be used, k_{obs} will reach a saturating value, k_{sat}. In the case of a D mechanism, this value corresponds to the rate of the unimolecular release of the departing ligand (H_2O in the case of B_{12a}) from the metal complex, whilst for an I_d mechanism it corresponds to the rate constant for interchange between the entering and the departing ligand (k_4 in eqn. 2). Hence, for the former, k_{sat} must be *strictly* independent of the identity of the entering ligand, but dependent in the latter case, since the transition state in the latter mechanism entails nucleophilic participation by the entering ligand. To demonstrate that the ligand substitution reaction at the metal centre proceeds through an I_d mechanism, it is sufficient to find ligands that show saturation behaviour (not necessarily a trivial task because[3] saturation may well occur at experimentally unattainable concentrations of L), and to demonstrate that the k_{sat} values are different. It is usually found[1,2] that where plots of k_{obs} against [L] show curvature, the saturating limit is at an inconveniently high [L] value. Hence k_{sat} is usually obtained from a plot of k_{obs}^{-1} against $[L]^{-1}$, the intercept of which corresponds to k_{sat}^{-1}.[2] For an I_d mechanism, the equilibrium constant, K_{OS}, for the formation of the outer sphere complex can be obtained from the ratio of the intercept and the slope of such a plot.[2]

We studied the kinetics of the substitution of iodide in ICbl by imidazole, azide and thiosulfate as a function of

temperature. In order to maintain the ionic strength at a constant and reasonable level (we used 2.2 mol dm^{-3}; the effect of ionic strength on these reactions has never been comprehensively reported, but is expected to be significant), we were limited to thiosulfate concentrations < 0.35 mol dm^{-3}. The primary kinetic data are given in Table S4.

Plots of k_{obs} against $[S_2O_3]^{2-}$ did not show significant deviation from linearity (Fig. 5A), and if k_{obs} saturates, it does so

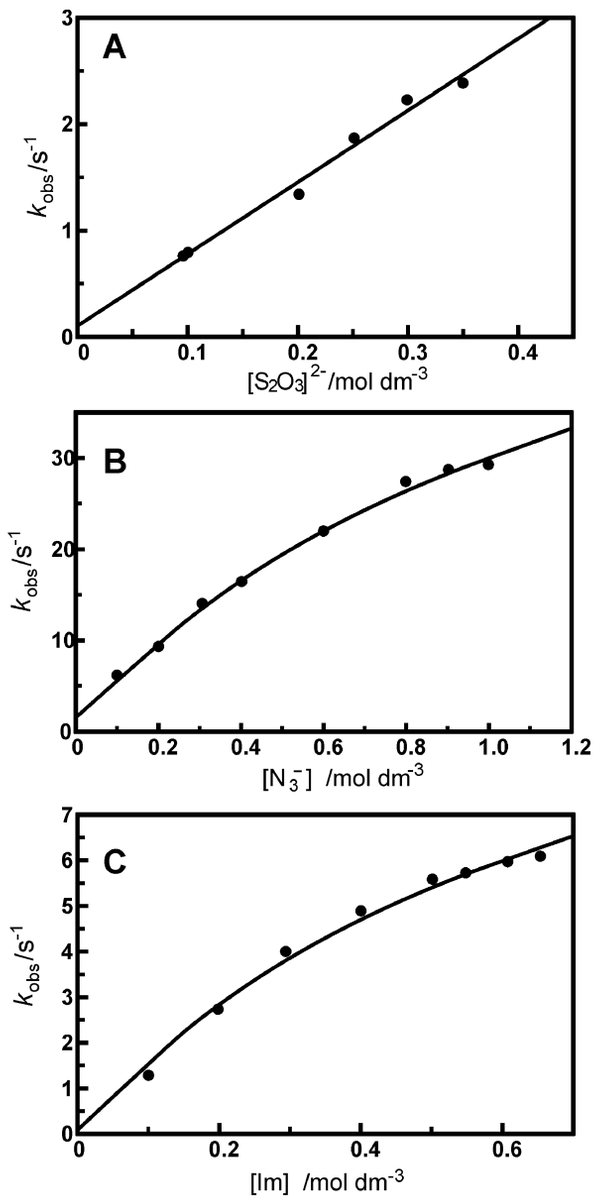


Fig. 5 Plot of the observed pseudo-first order rate constant as a function of ligand concentration for replacement of iodide in iodocobalamin by (A) $S_2O_3^{2-}$, (B) N_3^- and (C) imidazole.

above the concentrations used. By contrast, the analogous plots for N_3^- and imidazole show clear evidence of curvature (Fig. 5B and 5C), and all attempts to fit a linear function to either the N_3^- or imidazole data resulted in systematic deviations of the residuals from the best fit straight line. At [imidazole] > 0.6 mol dm^{-3}, k_{obs} becomes independent of ligand concentration, but in a manner that does not fit any reasonable kinetics model. We suspect that the activity of the ligand decreases sharply above this concentration in a solution with an ionic strength of 2.2 mol dm^{-3} because of self-association. As there is no obvious way of measuring the ligand activity in order to correct the ligand concentration values, the studies were confined to concentrations < 0.6 mol dm^{-3}.

A comparison of Fig. 5B and 5C shows that k_{sat} for N_3^- and imidazole is very different. We therefore can conclude immediately that the mechanism cannot be a strictly D mechanism, and that k_{sat} corresponds to the interchange rate constant, k_4 in eqn. 2, for iodide and the incoming ligand. Values of k_4 were obtained by the double reciprocal plot described above; Fig. 6A

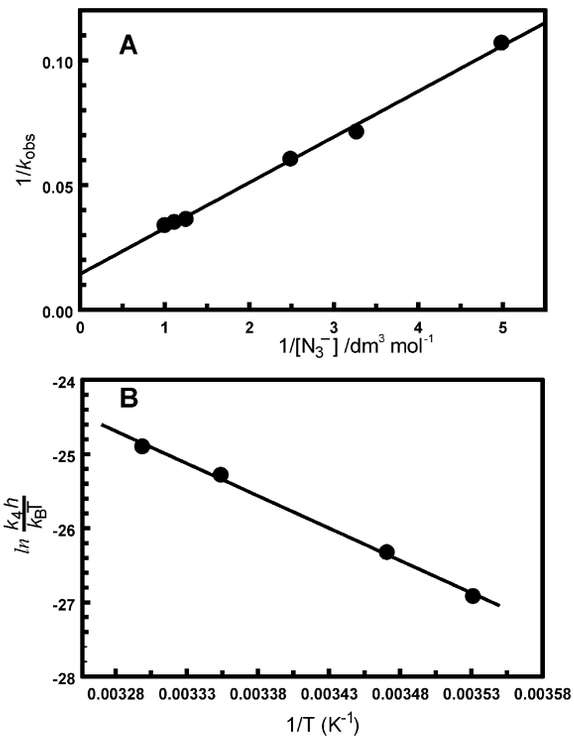


Fig. 6 **A**: A plot of $1/k_{obs}$ against $1/[N_3^-]$ at 25.0 °C, extrapolates to $1/k_{sat}$, the reciprocal of the saturating rate constant. Since k_{sat} values for the three ligands studied are different (at 25 °C, 70 ± 7 s^{-1} for N_3^-; 11.5 ± 0.5 s^{-1} for imidazole; and approximately 61 s^{-1} for $S_2O_3^{2-}$), the mechanism of substitution of iodide in ICbl by these ligands must be an I_d mechanism. The ratio of the intercept to the slope gives the equilibrium constant, K_{OS}, for formation of the outer sphere complex in an I_d mechanism. **B**: The temperature dependence of k_{sat} from which $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values are determined.

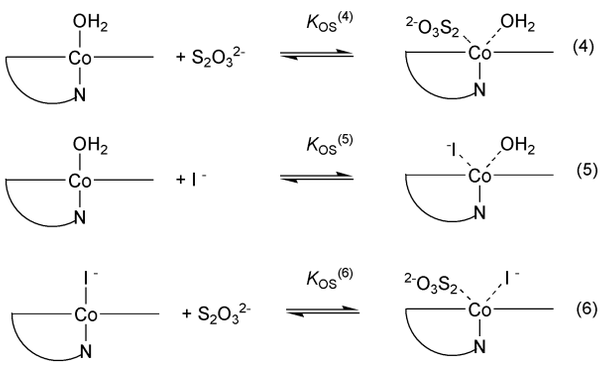
shows a representative plot. The values of k_4 and K_{OS} are listed in Table 3. In the case of thiosulfate, only the second-order rate constant, k_{II}, for substitution of I^- by $S_2O_3^{2-}$ could be determined from the slope of graphs such as shown in Fig. 5A. The activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were determined from the slope and intercept, respectively, of a plot of ln (k_4h/k_BT) against T^{-1}, where h and k_B are the Planck and Boltzmann constants, respectively (Fig. 6B). The values of these parameters (and, specifically, the values for substitution of I^- by imidazole and N_3^-, respectively) confirm that the saturating rate constants in the two cases are very different (Table 3). This is borne out by the significantly different values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, which, for a D mechanism, should be identical within experimental error. Hence, the mechanism is best described as an interchange mechanism.

In the case of thiosulfate, $k_{II} = k_4K_{OS}$. The composite rate constant can be deconvoluted provided the value of K_{OS} is known. Values of K_{OS} for eqns. 4 and 5 (see Scheme 3) are known:[3] $K_{OS}^{(4)} = 0.019$ and $K_{OS}^{(5)} = 0.212$. Hence $K_{OS}^{(6)} = K_{OS}^{(4)}/K_{OS}^{(5)} = 0.089$. From the temperature dependence of $K_{OS}^{(4)}$ and $K_{OS}^{(5)}$,[3] we can calculate that $\Delta H_{K_{os}^{(6)}} = -14$ kJ mol^{-1} and $\Delta S_{K_{os}^{(6)}} = -67$ J K^{-1} mol^{-1}. Hence, $\Delta H_{k4}^{\neq}$ $(S_2O_3^{2-}) = \Delta H_{kII}^{\neq} - \Delta H_{Kos}^{\neq(6)}$, = 78 kJ mol^{-1}, and, by similar reasoning, $\Delta S_{k4}^{\neq} = 51$ J K^{-1} mol^{-1}. Therefore we estimate $k_4 = 61$ s^{-1} for the interchange between $S_2O_3^{2-}$ and I^-. To check these results, we assumed that thiosulfate shows saturation behaviour but above the experimental concentrations, and therefore treated the thiosulfate data in the

Table 3 The interchange rate constant, k_4, and the outer sphere formation constant, K_{OS}, for the replacement of iodide in iodocobalamin with imidazole and azide, and the second order rate constant for the reaction with thiosulfate[a]

Ligand	T/°C	k_4/s^{-1}	k_{II}/dm^3 mol^{-1} s^{-1}	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	K_{OS}/dm^3 mol^{-1}
Imidazole	10	3.5(4)				0.80(9)
	15	5.1(9)				1.1(3)
	20	9.8(9)				0.9(1)
	25	11.5(5)		57(9)	−32(25)	1.8(9)
N_3^-	10	13(1)				0.99(8)
	15	24(3)				0.86(2)
	25	70(7)				0.8(1)
	30	102(8)		73(3)	33(11)	0.88(5)
$S_2O_3^{2-}$	10		1.67(7)			
	15		2.7(2)			
	20		4.5(1)			
	25		6.8(4)	64(1)[b]	−16(5)[b]	
	25	61[d]		78[c]	51[c]	

[a] The number in parenthesis is the standard error of the last significant figure. [b] Values for k_{II}; see text. [c] Estimated values for k_4, see text. [d] Calculated from the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, see text.



Scheme 3

same way as the azide and imidazole data in order to obtain k_4 and K_{OS} values. We obtained $\Delta H^{\ddagger} = 81 \pm 13$ kJ mol^{-1}, $\Delta S^{\ddagger} = 49 \pm 25$ J K^{-1} mol^{-1} and $K_{OS}^{(6)} = 0.5 \pm 0.3$, in good agreement with the values obtained by deconvolution of the k_{II} values.

The values of the activation parameters confirm that the mechanism cannot be a D mechanism which in all cases should be identical within experimental error. Hence, the mechanism is best described as an interchange mechanism. Moreover, since the values of k_4 for the three ligands studied differ by less than an order of magnitude, it is reasonable to conclude that the reactions are under dissociative activation, and that the mechanism is an I_d mechanism.

Conclusion

The Co(III) ion in the cobalamins is in direct electronic communication with the corrin such that perturbation of the electronic structure of the corrin can significantly affect the properties of the metal ion; we showed that replacement of H at C10 with an electron-withdrawing NO group increases the pK_a for coordinated H_2O as the Co–O bond becomes more ionic, and significantly decreases the affinity of the metal for axial ligands. Despite its small size, and its encapsulation in the sterically crowded environment of the corrin, substitution of the axial ligand proceeds through an interchange mechanism with nucleophilic participation of the incoming ligand in the transition state.

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