Kinetics and Mechanism of the Interaction of *cis*-Diamminediaquaplatinum(II) with Organocobalamins

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The stoicheiometries and kinetics of the reactions of *cis*-diamminediaquaplatinum(II) with adenosylcobalamin and a series of alkylcobalamins have been examined. The reactions proceed with a 1.3 : 1 and a 1 : 1 stoicheiometry (Pt^{II} : cobalamin) for the interaction between *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ and adenosylcobalamin and the alkylcobalamins, respectively. In all cases the interactions generate the ' base-off ' form of the organocobalamins. Our kinetic studies indicate that the reactions are first order in organocobalamin and first order in *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ at low concentrations of Pt^{II}, but that they approach zero order as the Pt^{II} concentration is increased. The kinetic data are interpreted in terms of a fast equilibrium between the alkylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ followed by a rate-determining ligand exchange between N^3 of the 5,6-dimethylbenzimidazole ligand and a co-ordinated H_2O of the platinum complex. The mechanism of the ' base-on ' ⟶ ' base-off ' conversion for the adenosylcobalamin–diamminediaquaplatinum(II) complex is different from that for the alkylcobalamin–diamminediaquaplatinum(II) complexes. The interaction between adenosylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ dramatically reduces the light sensitivity of the carbon–cobalt bond. Prolonged exposure of the adenosylcobalamin–diamminediaquaplatinum(II) complex to visible light in the presence of air yields 5'-deoxy-5'-oxoadenosine as the only nucleoside product. No photolysis occurs in the absence of air. These observations suggest that even in the presence of only stoicheiometric amounts of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$, the platinum complex interacts with the 5'-deoxyadenosyl ligand of the coenzyme. Carbon-13 and ^{31}P n.m.r. spectroscopy studies using [5'-^{13}C]-adenosylcobalamin show that the adenosylcobalamin–diamminediaquaplatinum(II) complex consists of at least four ' base-off ' conformers. These ' base-off ' conformers are probably the consequence of a downward distortion of the corrin ring resulting from the interaction between the 5'-deoxyadenosyl ligand, the corrin ring, and the *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ ion.

In a previous report we have described the interaction between *cis*-diamminediaquaplatinum(II) nitrate and adenosylcobalamin and a series of alkylcobalamins.[1] We are interested in these cobalamin–platinum complexes because it has been reported that the administration of pharmacological doses of vitamin B_{12} and leucovorin significantly increased the life-span of leukemic rats treated with *cis*-diamminedichloroplatinum(II).[2] These observations suggest that vitamin B_{12} may be used to counteract the severe side effects of *cis*-diamminedichloroplatinum(II) chemotherapy.[3] Thus, the cobalamin–diamminediaquaplatinum(II) complexes may represent a new class of platinum-containing anti-tumor agents. Indeed, Myasishcheva *et al.*[4] and Soyna *et al.*[5] have reported that cobalamin–tetrachloropalladate(II) complexes are anti-tumor agents.

In this paper, we describe an extension of the kinetic and mechanistic studies on the interaction of *cis*-diamminediaquaplatinum(II) nitrate with adenosylcobalamin and a number of alkylcobalamins. This interaction results in a ' base-on ' ⟶ ' base-off ' conversion for all the organocobalamins examined. The adenosylcobalamin–diamminediaquaplatinum(II) complex is no longer light sensitive in the absence of oxygen, while irradiation of the complex with visible light in the presence of oxygen yields 5'-deoxy-5'-oxoadenosine as the only nucleoside product.

Experimental

Materials.—*cis*- and *trans*-Diamminedichloroplatinum(II) were obtained from D. F. Goldsmith Metal and Chem. Inc. and were used as received. Diamminediaquaplatinum(II) nitrate solutions were prepared as follows. $[Pt(NH_3)_2Cl_2]$ (0.30 g) and $AgNO_3$ (0.34 g) in H_2O (or D_2O) (10 cm^3) were stirred for 3 days at room temperature to produce a 0.10 mol dm^{-3} diamminediaquaplatinum(II) nitrate solution. The AgCl precipitate was removed by filtration and a small aliquot of the filtrate was tested for Ag^+ ions with 0.1 mol dm^{-3} HCl. Stock solutions were kept in the dark and were never stored more than 5 days. In all experiments the solutions were maintained at pH *ca.* 5; at this pH adenosylcobalamin and the alkylcobalamins are predominantly in the ' base-on ' form. In addition, at this pH *cis*-diamminediaquaplatinum(II) nitrate consists mainly of the monomeric dicationic form *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ ($pK_{a1} = 5.6$, $pK_{a2} = 7.2$).[6,7]

The corrinoids were prepared from cyanocobalamin by published procedures: adenosyl-, methyl-, ethyl-, vinyl-, and carboxymethyl-cobalamin,[8] [^{13}C]methyl- and [5'-^{13}C]-adenosyl-cobalamin,[9] [^{13}C]methyl 3,5,6-trimethylbenzimidazolylcobamide,[10] methyl- and adenosyl-epicobalamin.[11] All other chemicals were reagent grade and were used as received.

Methods.—Pulse Fourier-transform ^{13}C (62.9 MHz), ^{31}P (101.3 MHz), and ^1H (250.1 MHz) n.m.r. spectra were obtained at 37 ± 0.3 °C using a Bruker 250 MHz spectrometer locked to the solvent D_2O. ^{13}C and ^{31}P spectra were obtained under conditions of simultaneous broad-band proton noise decoupling. Peak positions were determined by computer examination of the final Fourier-transformed spectra and the chemical shifts were measured with respect to external neat $SiMe_4$ or 85% H_3PO_4 standards. Absorption spectra were recorded with a Cary model 15 spectrometer. Stoicheiometries for the reaction of *cis*-$[Pt(NH_3)_2(OH_2)_2]$-$[NO_3]_2$ were determined by spectrophotometric titration with Pt^{II} : cobalamin ratios in the range 0.1—5 : 1. Reaction rates were measured with the Pt^{II} complex in 20—800-fold excess over cobalamin, so that the Pt^{II} concentration remained

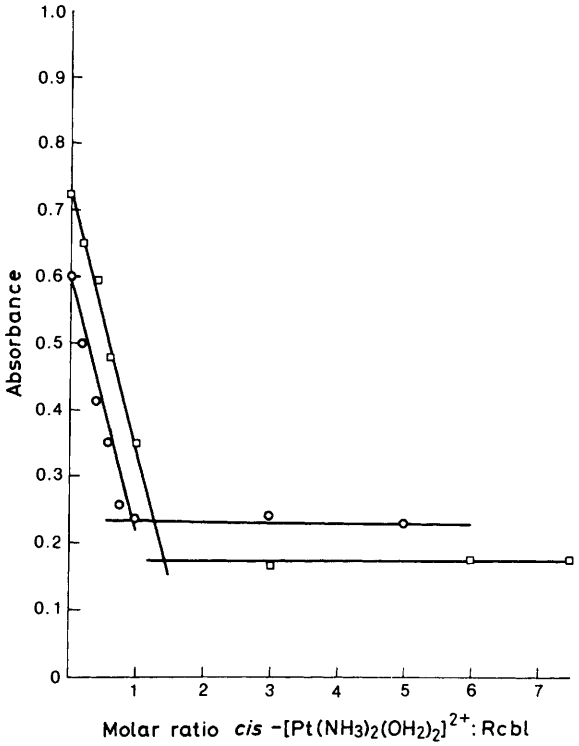


Figure 1. Stoicheiometry of the complexes of methylcobalamin (6.00×10^{-5} mol dm^{-3}) (○) and adenosylcobalamin (8.32×10^{-5} mol dm^{-3}) (□) with *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ (determined at 520 nm)

essentially constant during the course of the reaction. The α band of the ' base-on ' form (520 nm) or of the ' base-off ' form (455 nm) of the organocobalamins was chosen for the kinetic studies.

Results

As shown before,[1] the addition of *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ to solutions of methyl- or adenosyl-cobalamin causes a time-dependent decrease in the absorbance at 520 nm with a concomitant increase at 455 nm. This ' base-on ' to ' base-off ' conversion in the presence of the Pt^{II} complex is irreversible. The *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$–cobalamin complex can be extracted into phenol and back extracted into water by the addition of acetone and diethyl ether. Paper chromatography using the solvent systems described by Dolphin [12] does not lead to dissociation of these complexes. Furthermore, the cobalamin–$[Pt(NH_3)_2(OH_2)_2]^{2+}$ complexes are indefinitely stable in aqueous solution in the dark, the addition of NaCl or thiourea does not dissociate them.

The results of the spectrophotometric titrations of methylcobalamin and adenosylcobalamin with *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ are presented in Figure 1. Definite breaks are noted at a 1 : 1 stoicheiometry for the methylcobalamin–diamminediaquaplatinum(II) complex and at a 1.0 : 1.3 ratio for the corresponding adenosylcobalamin–platinum(II) complex. A 1 : 1 stoicheiometry was also observed for the interaction between ethylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$. These stoicheiometries were confirmed by calculating the concentration of the ' base-on ' and ' base-off ' forms at each experimental point. It should be noted that in these experiments only the ' base-on ' ⟶ ' base-off ' conversion can be detected. Thus, stoicheiometry refers only to that platinum(II) : organocobalamin ratio at which the ' base-on ' ⟶ ' base-off ' conversion is complete. These observations suggest that the interaction between the alkylcobalamins (Rcbl) and *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ can be described by equation (1).

Table. Kinetic parameters for the reaction of *cis*-diamminediaquaplatinum(II) with organocobalamins (Rcbl) at 37 °C

R	k/s^{-1}	K/dm^3 mol^{-1}	$-\log(K_1/$ dm^3 mol^{-1}) *
C_2H_5–	3.3×10^{-5}	3.2×10^2	0.80
CH_3–	2.9×10^{-5}	6.8×10	2.00
$CH_2{=}CH_2$–	4.0×10^{-5}	3.5×10	2.3
$^-O_2CCH_2$–	4.0×10^{-5}	3.3×10	2.5
epi-CH_3–	3.0×10^{-5}	1.1×10	2.5

* $-\log K_1 = 4.7 - pK_a$ (ref. 16, p. 139).

' base-on ' Rcbl + *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ ⟶ ' base-off ' Rcbl, *cis*-$[Pt(NH_3)(OH_2)_2]^{2+}$ (1)

The 1.0 : 1.3 stoicheiometry for the adenosylcobalamin–platinum(II) reaction suggests that the Pt^{II} complex interacts at more than one site of the adenosylcobalamin molecule.

Kinetics.—Kinetic measurements of the reactions of the organocobalamins with *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ were carried out at pH 5. At this pH the reaction rates are independent of small variations in pH and the uncomplexed cobalamins are in the ' base-on ' form. No supporting electrolytes were used and the increase in ionic strength due to the platinum(II) complex had little effect on the reaction rates. Acetate and phosphate buffers interfered with the complexation reaction, and the reactions do not proceed to completion at neutral pH [0.05 mol dm^{-3} tris(hydroxymethyl)aminomethane].

Plots of log $(A_\infty - A_t)$ *versus* time (t) gave straight lines for more than 85% of the reactions. The reactions are first order in organocobalamin according to equation (2).

$$-\mathrm{d}[\text{' base-on ' Rcbl}]/\mathrm{d}t = k_{obs.}[\text{' base-on ' Rcbl}]_{tot} \quad (2)$$

At relatively low levels of *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ the reactions are first order in *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$. As the Pt^{II} concentration is increased, the reactions become less than first order in *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$. The reaction of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with ethyl-, vinyl- and carboxymethyl-cobalamin and with methylepicobalamin all result in a ' base-on ' ⟶ ' base-off ' conversion. The kinetic behaviour of these reactions presented in Figure 2 can be expressed by equation (3). The kinetic parameters for the interaction

$$k_{obs.} = \frac{kK[Pt^{II}]}{1 + K[Pt^{II}]} \quad (3)$$

between *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ and the organocobalamins are summarized in the Table. Methylcobalamin interacts also with *trans*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ at pH 4 ($pK_{a1} = 4.3$, $pK_{a2} = 7.46$) and yields a ' base-off ' complex; however, the rate of this reaction is very slow and an accurate kinetic analysis was not feasible.

A plot of $k_{obs.}^{-1}$ against $[Pt^{II}]^{-1}$ for the reaction between adenosylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ at various temperatures is presented in Figure 3. The kinetic behaviour of this reaction can also be described by equation (3). However, the photolysis experiments as well as the n.m.r. spectra of the adenosylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ complex (see below) clearly demonstrate that the interaction is more complex.

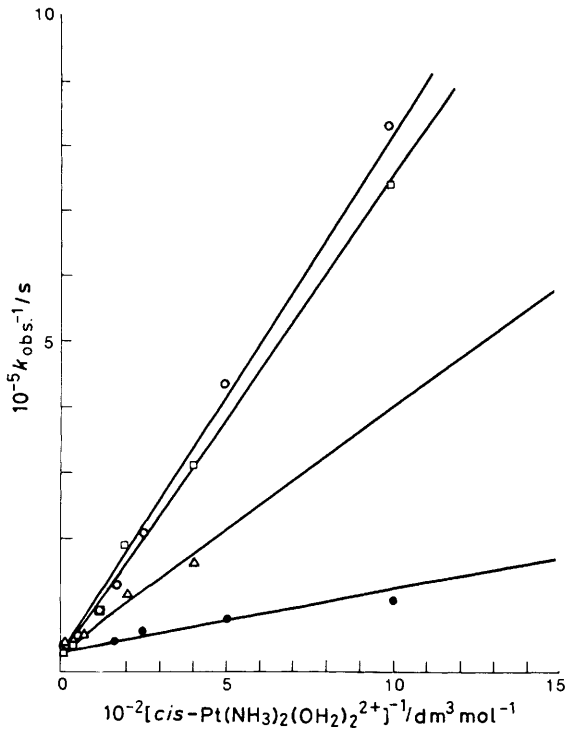


Figure 2. Plots of $k_{obs.}^{-1}$ *versus* $[cis\text{-}Pt(NH_3)_2(OH_2)_2^{2+}]^{-1}$ for the reactions of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with alkylcobalamins (37 °C): (○) carboxymethylcobalamin, (□) vinylcobalamin, (△) methylcobalamin, (●) ethylcobalamin; [Rcbl] = 5×10^{-5} mol dm^{-3}

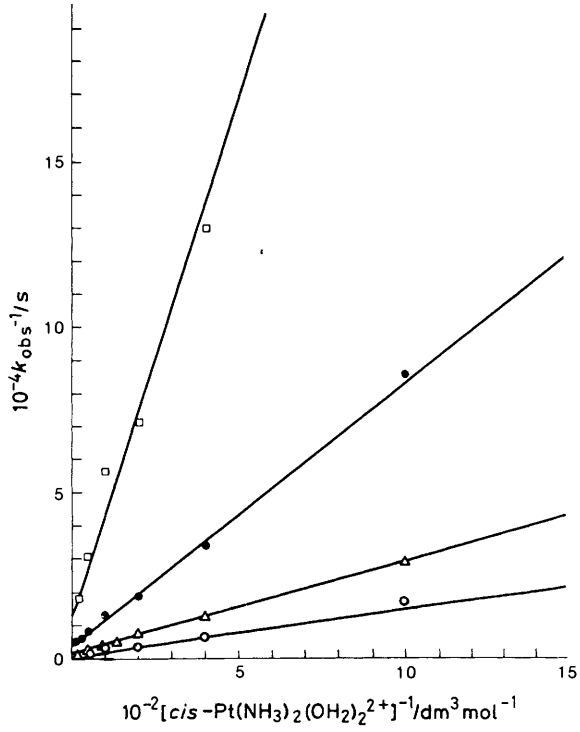


Figure 3. Plots of $k_{obs.}^{-1}$ *versus* $[cis\text{-}Pt(NH_3)_2(OH_2)_2^{2+}]^{-1}$ for the reactions of adenosylcobalamin (5×10^{-5} mol dm^{-3}) with *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ at 25 (□), 37 (●), 46 (△), and 54 °C (○)

*Photolysis of the Organocobalamin–*cis*-Diamminediaquaplatinum*(II) *Adducts.*—The rates of photolysis of the alkylcobalamin–*cis*-diamminediaquaplatinum(II) adducts in the presence of air are virtually identical to those of the corresponding alkylcobalamins at a similar concentration in 0.1 mol dm^{-3} $HClO_4$ (' base-off '). Photolysis of the methylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ complex (3.3 mmol dm^{-3}) in the presence of air yields formaldehyde, methanol, ethane, methane {identified by ^{13}C n.m.r. spectroscopy using a [^{13}C]methylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ complex} and a corrinoid with absorption maxima at 519, 495, and 350 nm. These spectral properties are very similar to those of aquacobalamin in strong acid,[13] suggesting that the corrinoid photolysis product is aquacobalamin in its ' base-off ' form. In accord with this conclusion the ^1H n.m.r. spectrum of this photolysis product shows resonances for the protons at positions B4, B2, and B7 of the 5,6-dimethylbenzimidazole (dbzm) moiety at 8.92, 7.93, and 7.18 p.p.m. respectively. These resonance positions are characteristic for cobalamins in the ' base-off ' form.[14]

In contrast, the rate of photolysis of the adenosylcobalamin–*cis*-diamminediaquaplatinum(II) adduct (prepared in a 1 : 1 or 1 : 10 ratio) is much smaller than that of adenosylcobalamin in the ' base-off ' form. Exposure of a solution (1.8 mmol dm^{-3}) of [5′-^{13}C]adenosylcobalamin–*cis*-diamminediaquaplatinum(II) complex (1 : 1) in the presence of air to a 150-W incandescent lamp at 25 cm for 15 h yields 5′-deoxy-5′-oxo-[5′-^{13}C]adenosine as the only labelled product. No [5′-^{13}C]-5′-deoxy-8,5′-cycloadenosine could be detected by ^{13}C n.m.r. spectroscopy. The same preparation is *not* photolabile under strictly anaerobic conditions. The nature of the corrinoid photolysis product depends on the ratio of adenosylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ used in the preparation of the adducts. Photolysis of a 1 : 1 adduct of

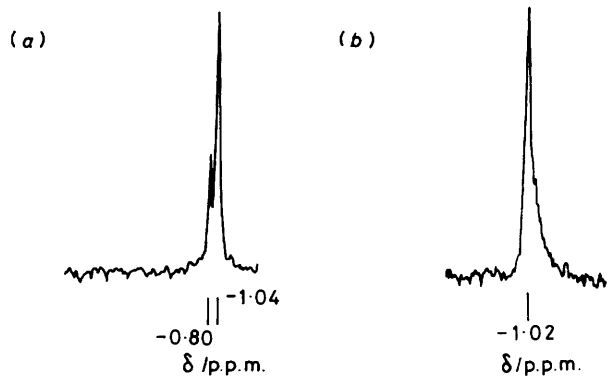


Figure 4. Proton noise decoupled ^{13}C n.m.r. spectra of (*a*) [^{13}C]-methylcobalamin (3 mmol dm^{-3}) and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ (10 mmol dm^{-3}); (*b*) [^{13}C]methylcobalamin (3 mmol dm^{-3}) and *trans*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ (10 mmol dm^{-3})

adenosylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ yields a corrinoid product with a visible spectrum very similar to that of aquacobalamin (maxima at 525 and 502 nm). In contrast, photolysis of an adduct formed with a ten-fold excess of *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ yields a corrinoid product with a visible spectrum similar to that of aquacobalamin in strong acid (' base-off ') (maxima at 520 and 493 nm).[13]

*Characterization of the Cobalamin–*cis*-Diamminediaquaplatinum*(II) *Adducts by N.M.R. Spectroscopy.*—The interaction between the organocobalamins and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ can be readily followed by ^{13}C n.m.r. spectroscopy if cobalamins specifically enriched with ^{13}C in the ligand in the β position are used. Figure 4(*a*) shows the ^{13}C n.m.r. spectrum of the [^{13}C]methylcobalamin–*cis*-diamminediaquaplatinum(II) adduct. The peak at −1.04 p.p.m. corresponds

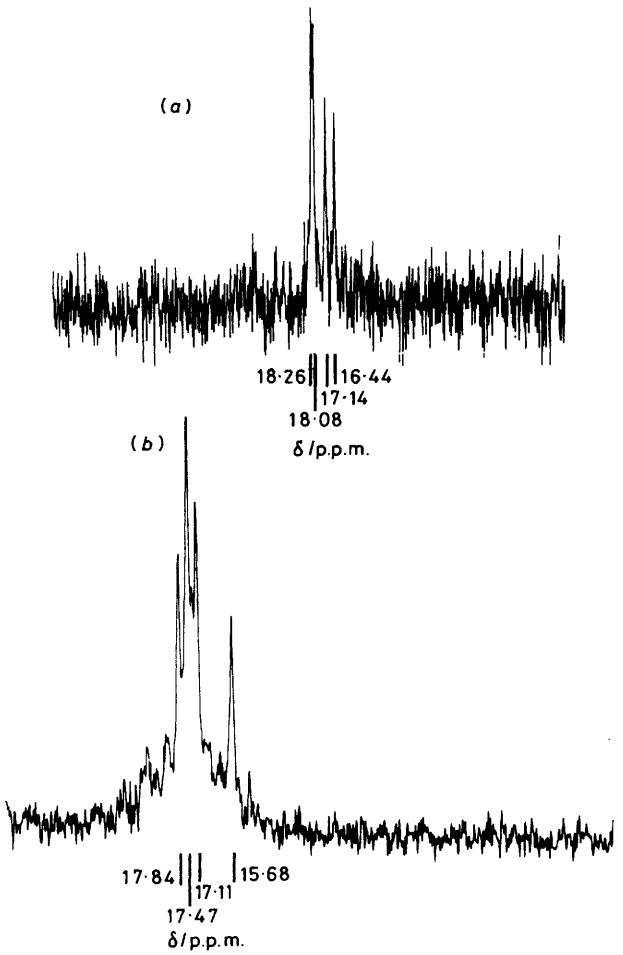


Figure 5. Proton noise decoupled ^{13}C n.m.r. spectra of [^{13}C]-adenosylcobalamin (2 mmol dm^{-3}) and *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ (*a*) 1 : 1, (*b*) 1 : 6

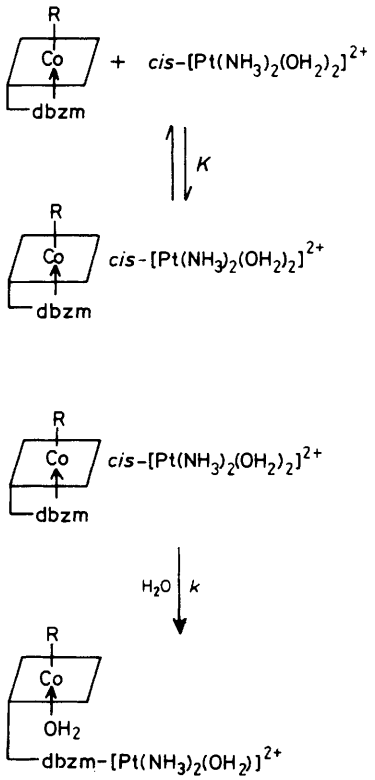
to the Co–methyl group of methylcobalamin in which N^3 of the 5,6-dimethylbenzimidazole moiety is detached from the cobalt atom and is co-ordinated to platinum.[15,*] The minor resonance at −0.80 p.p.m. may correspond the Co–methyl group of a complex in which the platinum atom forms a bridge between N^3 of the lower ligand and the carbonyl oxygen of the *e*-propionamide side chain. The addition of thiourea to the methylcobalamin–*cis*-diamminediaquaplatinum(II) adduct does not affect the ^{13}C n.m.r. spectrum. This minor resonance is not seen in the ^{13}C n.m.r. spectrum of the [^{13}C]methylcobalamin–*trans*-diamminediaquaplatinum(II) adduct [Figure 4(*b*)]. The ^{13}C n.m.r. spectrum of the [5′-^{13}C]adenosylcobalamin–*cis*-diamminediaquaplatinum(II) adduct (1 : 1) is more complex [Figure 5(*a*)]. At least four species with signals at 18.26, 18.08,, 17.14, and 16.44 p.p.m. are present in this preparation. This spectral region is characteristic of the chemical shift of [5′-^{13}C]adenosylcobalamin in the ' base-off ' form,[15] indicating that the 5,6-dimethylbenzimidazole ligand is no longer co-ordinated to cobalt. The ^{13}C n.m.r. spectrum is not simplified when the *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$: adenosylcobalamin ratio is increased. However, at higher ratios the chemical shifts as well as the relative intensities of the peaks are changed [Figure 5(*b*)].

The interaction between the organocobalamins and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ was also monitored by ^{31}P n.m.r. spectroscopy. For methylcobalamin the ^{31}P resonance is not a sensitive probe to monitor the ' base-on ' ⇌ ' base-off ' conversion, indeed lowering the pH of a solution of methylcobalamin from 7 to 2 does not cause a change in the ^{31}P chemical shift (41.6 Hz). Thus it is not surprising that the ^{31}P n.m.r. spectrum of the 1 : 1 methylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adduct shows a single resonance at 41.6 Hz. In contrast, the ^{31}P n.m.r. spectra of the 1 : 1 adenosylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adduct show the presence of at least four species with signals at −8.06, −12.70, −25.39, and −48.34 Hz.

The ^1H n.m.r. spectra of the alkylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adducts show signals at approximately 7, 8, and 9 p.p.m. for the protons at B7, B2, and B4 respectively, indicating that in these adducts the cobalamins are in the ' base-off ' form.[14] In contrast, the ^1H n.m.r. spectrum of the adenosylcobalamin–*cis*-diamminediaquaplatinum(II) complex reflects the presence of several species. While the ^1H n.m.r. spectrum of free adenosylcobalamin shows eight resonances in the downfield region (>5 p.p.m.) corresponding to the protons at A6, A2, B7, B2, R1, B4, C10, and R′1 (for numbering system see ref. 14) the spectrum of the 1 : 1 adenosylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adduct reveals at least 40 resonances in this region. Even in the presence of a five-fold excess of *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ the spectrum of the adenosylcobalamin adduct shows at least 23 proton signals downfield from 5 p.p.m.

Discussion

The interaction between the alkylcobalamins and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ can be described by the mechanism outlined in Scheme 1.

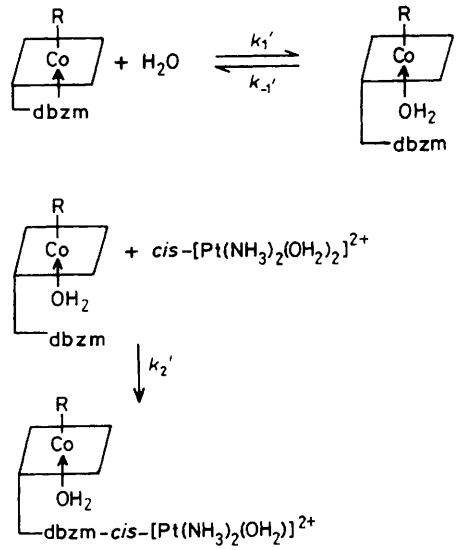


Scheme 1.

* Because of a different bulk magnetic susceptibility correction at the higher field, these shifts are 1 p.p.m. upfield from those obtained at 25.2 MHz.

The mechanism involves a fast pre-equilibrium between an alkylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ to form a complex in which the cobalamin is in the 'base-on' form, followed by a rate-limiting reaction involving ligand exchange of N^3 of the 5,6-dimethylbenzimidazole ligand with a water molecule ligated to the platinum(II).

Other mechanisms involving the reversible dissociation of the 5,6-dimethylbenzimidazole ligand from the cobalt atom followed by a reaction of the 'base-off' form with *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ can be eliminated.



Scheme 2.

In Scheme 2, if k_1' (and k_{-1}') $\gg k_2'$, such a mechanism dictates that the reaction be first order in *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$, in disagreement with our observations. On the other hand, if $k_2' \gg k_1'$, then the rate-limiting step should very much depend on the alkyl group in the β position.[16] However, the kinetic parameters presented in the Table demonstrate that within experimental error the reaction rates for the slow step are identical for all alkylcobalamins tested. Furthermore, Brown *et al.*[17] have shown that for methylcobalamin the rate of dissociation of the 5,6-dimethylbenzimidazole is fast [$k_{off} = (1.30 \pm 0.11) \times 10^4$ s^{-1} at 5 °C and pH 5.5].

In accord with the first mechanism involving a fast pre-equilibrium between the alkylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ followed by a slow ligand exchange reaction, the equilibrium constants K are correlated linearly with the 'base-on' ⇌ 'base-off' equilibrium constants (K_1). Furthermore, as indicated above, the values of k are independent of the nature of the alkyl ligand. For carboxymethylcobalamin the value for K is higher than would be predicted from the pK_1 values for the 'base-on' ⇌ 'base-off' interconversion. This aberration may be due to the carboxylate group which offers a second potential binding site for *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$.

Our ^1H and ^{13}C n.m.r. data show that in the final alkylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ complexes the Pt atom binds to N^3 of the 5,6-dimethylbenzimidazole nucleotide in a monodentate mode. A similar interaction between methylcobalamin and lithium tetrachloropalladate(II) has been proposed by Yurkevich *et al.*[18]

The interaction between adenosylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ is distinct from the interaction described above for the alkylcobalamins. Adenosylcobalamin has two potential binding sites on the 5′-deoxyadenosyl ligand (N^1 and N^7)[19] and one potential binding site on the 5,6-dimethylbenzimidazole moiety (N^3). All the evidence points to an initial binding at N^7 and/or N^1 of the 5′-deoxyadenosyl ligand in the β position, which leads to the dissociation of the lower nucleotide ligand from the cobalt atom. The most compelling evidence for this interaction is provided by the photolysis of the 1 : 1 adenosylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adduct. It has been well documented that the first step in the photolysis of adenosylcobalamin is the homolytic cleavage of the carbon–cobalt bond to cob(II)alamin and a 5′-deoxyadenosyl radical. The nature of the photolysis products is determined by secondary reactions of cob(II)alamin and the nucleosidyl radical. Thus, in the absence of oxygen, cob(II)alamin is quite stable but the 5′-deoxyadenosyl radical cyclizes to 5′-deoxy-8,5′-cycloadenosine. In the presence of oxygen cob(II)alamin is oxidized to aquacobalamin and the nucleosidyl radical yields both 5′-deoxy-5′-oxoadenosine and 5′-deoxy-8,5′-cycloadenosine.[20] In contrast, the adenosylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adduct is *not* photolabile in the absence of oxygen, while in the presence of oxygen the rate of photolysis is much smaller than that of adenosylcobalamin in 0.1 mol dm^{-3} $HClO_4$ ('base-off' form). Furthermore, photolysis of the adenosylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adduct in the presence of oxygen yields only 5′-deoxy-5′-oxoadenosine, *no* 5′-deoxy-8,5′-cycloadenosine could be detected by ^{13}C n.m.r. These results clearly demonstrate that even when a stoicheiometric amount of *cis*-$Pt[(NH_3)_2(OH_2)_2]^{2+}$ is present the Pt atom interacts only with the 5′-deoxyadenosyl ligand and that this interaction locks the nucleoside in one or more positions that disallow the cyclization reaction between the $C^{5'}$ carbon of the ribose moiety and C^8 of the purine ring.

The ^1H, ^{13}C, and ^{31}P spectra of the adenosylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adduct all point to the presence of at least four different species. In all these species the adenosylcobalamin is in the 'base-off' form. Adenosylcobalamin has five potential binding sites above the corrin ring, N^7 and N^1 of the 5′-deoxyadenosyl ligand and the carbonyl oxygens of the three acetamide side chains (*a*, *c*, and *g*) which project up from the ring. We propose that the species observed in the adenosylcobalamin–*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ adduct represent complexes in which the Pt atom is bound to N^7 or N^1 of the nucleoside ligand in a monodentate manner and complexes in which the Pt atom bridges N^7 (N^1) of the nucleoside ligand and a carbonyl oxygen of one of the acetamide side chains. We further propose that the binding of the Pt atom to these ligands in the β position forces the corrin ring into 'downward' distorted configurations which cause the dissociation of the lower ligand. Grate and Schrauzer[21] have suggested that secondary alkylcobalamins are capable of existence because the corrin ring can adopt such 'downward' distorted configurations in response to the bulky secondary alkyl group. They have also suggested that these distortions can become severe enough to cleave the coordinate bond between the lower ligand and the cobalt atom.

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