The Interaction of Methylcobalamin with Tetracyanoplatinate(II), Tetrathiocyanoplatinate(II) and Tetrachloroplatinate(II)

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The interactions of methylcobalamin (CH_3-B_{12}) with $Pt(CN)_4^{2-}$, $PtCl_4^{2-}$, and $Pt(SCN)_4^{2-}$ in aqueous solution were studied by UV–visible and ^1H NMR spectroscopy. Together with earlier results on the mechanism of the Pt(IV)-dependent methyl-transfer reaction from CH_3-B_{12} to Pt(II), these studies suggest at least three Pt binding sites on CH_3-B_{12}. One site, which is occupied by all three complexes ($K_1 = 4 \times 10^3$ M^{-1} for $Pt(CN)_4^{2-}$ and 3×10^3 M^{-1} for $PtCl_4^{2-}$), is located on the Co–CH_3 side of the corrin macrocycle, and is involved in the methyl-transfer process in the presence of a Pt(IV) complex. An additional site for $Pt(SCN)_4^{2-}$ is the N-3 of the benzimidazole group, resulting in dissociation of this group from the cobalt. An additional site for $Pt(CN)_4^{2-}$ has a binding constant of 16 M^{-1} and ^1H NMR changes indicate perturbation but not dissociation of the benzimidazole group. Only the first interaction is discerned for $PtCl_4^{2-}$.

Introduction

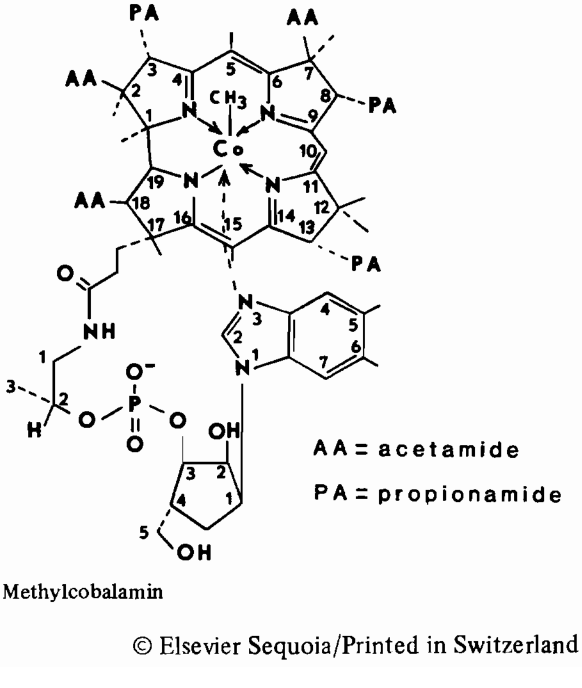
Ever since it was demonstrated [1] that demethylation of methylcobalamin (CH_3-B_{12}) by platinum complexes requires the presence of both Pt(IV) and Pt(II) complexes, this unusual reaction has been extensively investigated in several laboratories [2–4]. Our kinetic and product studies of the demethylation by Pt(IV) ($PtCl_6^{2-}$, $Pt(CN)_4Cl_2^{2-}$, or $Pt(CN)_5Cl^{2-}$) and Pt(II) ($PtCl_4^{2-}$ or $Pt(CN)_4^{2-}$) couples have established the following pathway [3b, c]:

$$CH_3\text{-}B_{12} + PtL_4^{2-} \overset{K_1}{\rightleftharpoons} CH_3\text{-}B_{12}\cdots PtL_4^{2-} \qquad (1)$$

$$CH_3\text{-}B_{12}\cdots PtL_4^{2-} + XPtL_4'Y^{2-} \overset{K_2}{\rightleftharpoons} [CH_3\text{-}B_{12}\cdots PtL_4\text{–}X\text{–}PtL_4']^{3-} + Y^- \qquad (2)$$

$$[CH_3\text{-}B_{12}\cdots PtL_4\text{–}X\text{–}PtL_4']^{3-} \xrightarrow[H_2O]{k} H_2O\text{-}B_{12}^+ + CH_3PtL_4X^{2-} + PtL_4'^{2-} \qquad (3)$$

The nature of the interaction between CH_3-B_{12} and the Pt(II) complex in eqn. 1 is of importance to this reaction and to B_{12} chemistry in general. In this report, we present the results of spectroscopic studies on the interaction of CH_3-B_{12} with $Pt(CN)_4^{2-}$, $Pt(SCN)_4^{2-}$, and $PtCl_4^{2-}$.



Methylcobalamin

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TABLE I. Chemical Shift Changes in the 270 MHz ^1H NMR Spectrum of Methylcobalamin Complexed with $Pt(CN)_4^{2-}$.

Resonance[a]	Δδ (Hz)[b]	Resonance[a]	Δδ (Hz)[b]
Co-CH_3	30.0	C19β-H	<−5
C1α-CH_3	13.2	B2-H	11.1
C2α-CH_3	20.0	B4-H	−2.2
C5-CH_3	−0.8	B5-CH_3	0.7#
C7α-CH_3	5.9	B6-CH_3	5.1#
C10-H	−0.7	B7-H	0
C12β-CH_3	5.0	R1-H	−3.0
C15-CH_3	0.7	R5′-H	0
C18β-CH_2	1.8*	R5″-H	0
C2β-CH_2	0.7*	Pr1″-H	0
C12α-CH_3	13.9	Pr3-CH_3	−3.2

[a] C = corrin ring, B = benzimidazole, R = ribose, Pr = propanolamine. Assignments followed by the same symbol may be reversed (see ref. 9). [b] Chemical shift difference of: 100% methylcobalamin–platinum complex ($[CH_3\text{-}B_{12}]_{tot}$ = 1.0 m*M*, $[Pt(CN)_4^{2-}]$ = 0.3 *M*) *vs.* 98% monomer ($[CH_3\text{-}B_{12}]_{tot}$ = 0.08 m*M*).

Experimental

Materials

K_2PtCl_4, $Na_2Pt(CN)_4$ and $Na_2Pt(SCN)_4$ were purchased from D. F. Goldsmith, Inc. and were recrystallized from aqueous solution. Methylcobalamin was synthesized according to a literature method [5]. The concentration of CH_3-B_{12} was determined from the absorption spectrum using the published molar absorptivity [6].

Preparation of the CH_3-B_{12}/$Pt(SCN)_4^{2-}$ Complex

A solution of $K_2Pt(SCN)_4$ (0.13 g, 0.26 mmol) in 5 ml 0.5 *M* NaCl was mixed with a solution of CH_3-B_{12} (0.10 g, 0.074 mmol) in 10 ml 0.5 *M* NaCl under dim light, and cooled to 5 °C. After about 12 hr in the dark, a salmon-red microcrystalline precipitate formed and the solution became decolorized. The solid was collected, washed with cold water, and air-dried. Elemental analysis revealed a Pt/Co ratio of 2.3 ± 0.1 (three separate isolations).

Methods

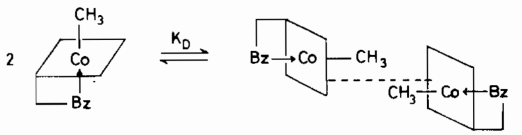
Spectrophotometric studies were carried out with a GCA/McPherson spectrophotometer. The ^1H NMR studies were carried out with a Brüker 270 MHz pulse Fourier-transform spectrometer at 26 °C. Chemical shifts were measured with respect to TSP or dioxane as internal references. An external TSP reference verified the constancy of the internal reference chemical shifts under our conditions. The EPR spectra were recorded on a Varian EPR spectrometer at 4 K.

Results and Discussion

In a previous study [3c] of the demethylation of CH_3-B_{12} by Pt(IV)/Pt(II) couples, we obtained convincing evidence for reversible complexation of CH_3-B_{12} with $Pt(CN)_4^{2-}$ and $PtCl_4^{2-}$ (eqn. 1). Equilibrium constants of 4 (±2) × 10^3 and 3 (±2) × 10^3 M^{-1}, respectively, were obtained from changes in the electronic spectrum (1.0 *M* NaCl, pH 7.2, 23 °C). Almost identical values were calculated from a kinetic study of the demethylation reaction. Based on mechanistic considerations, we proposed that this interaction takes place on the β-side (*i.e.*, CH_3-Co side) of the corrin ring.

Proton NMR Study of the Reactions of CH_3-B_{12} with $Pt(CN)_4^{2-}$ and $PtCl_4^{2-}$

Much of the ^1H NMR spectrum of CH_3-B_{12} has been assigned [8, 9]. The spectrum in aqueous solution is concentration-dependent and we have shown that this concentration dependence represents a monomer–dimer equilibrium [7] (eqn. 4). Most of the assignable resonances shift upfield with increasing concentration. The shift is largest for Co-C*H*$_3$. We have proposed that the dimer is a 'head-to-head' complex, which is held together by π–π attractive forces between parallel corrin rings. The upfield shifts are due to the ring current effect. The value of K_D calculated from the chemical shift of Co-C*H*$_3$ as a function of concentration is NaCl-dependent, and ranges from 195 ([NaCl] = 0 *M*) to 1 × 10^5 M^{-1} ([NaCl] = 5 *M*).



(4)

The addition of up to 5 equivalents of $Pt(CN)_4^{2-}$ or $PtCl_4^{2-}$ to aqueous CH_3-B_{12} (1.0 m*M*) does not cause any appreciable changes in the ^1H NMR spectrum of CH_3-B_{12}, despite the fact that almost complete complexation occurs under these conditions as judged from the equilibrium constants of eqn. 1. However, further addition of $Na_2Pt(CN)_4$ leads to shifts in the Co-C*H*$_3$ peak and other resonances (Table I). Experiments showing the dependence of the Co-C*H*$_3$ shift on $Pt(CN)_4^{2-}$ and NaCl concentrations are illustrated in Fig. 1A. The methyl peak is shifted progressively downfield with increasing $[Pt(CN)_4^{2-}]$ at a given total CH_3-B_{12} concentration. At a given platinum concentration, the methyl resonance is shifted upfield with increasing total

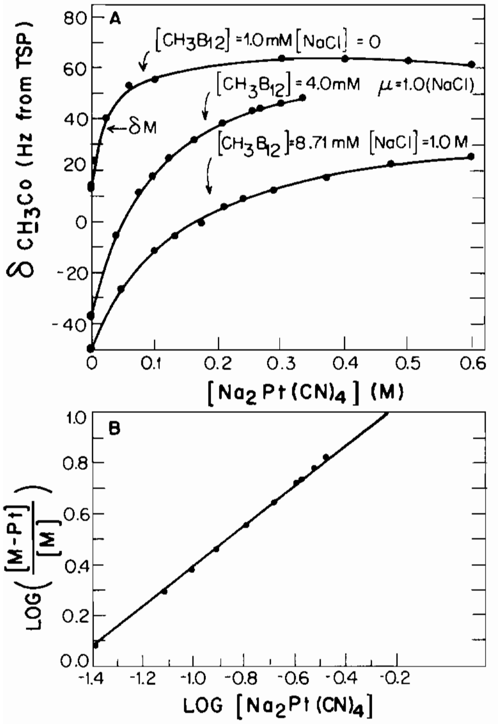


Fig. 1. (A) The effect of $Pt(CN)_4^{2-}$ on the chemical shift of Co-CH_3 of methylcobalamin; T = 26 °C, pH 7.2 (0.1 M phosphate, lower two curves) or natural pH (upper curve). (B) Plot according to eqn. 9 to determine K' of eqn. 5 for $\mu = 1.0\,M$ NaCl and $[CH_3\text{-}B_{12}] = 4.0$ mM.

$CH_3\text{-}B_{12}$ or NaCl concentration; *i.e.*, conditions which favor the dimer form of $CH_3\text{-}B_{12}$. These results suggest that complexation of monomeric $CH_3\text{-}B_{12}$ with platinum (eqn. 5) occurs in competition with dimer formation described in eqn. 4:

$$CH_3\text{-}B_{12} + Pt(II) \overset{K'}{\rightleftharpoons} CH_3\text{-}B_{12}\cdot[Pt(II)] \qquad (5)$$

Assuming eqns. 4 and 5 coexist in rapid equilibrium, K' can be calculated from the dependence of the observed chemical shift, δ_{obs}, on [Pt(II)] in the following manner. The relationship of δ_{obs} to the concentrations of three B_{12} species is given by (M = monomer; D = dimer; M-Pt = monomer-platinum complex):

$$[Co]\cdot\delta_{obs} = \delta_{M\text{-}Pt}[M\text{-}Pt] + \delta_M[M] + 2\delta_D[D] \qquad (6)$$

where:

$$[Co] = [M\text{-}Pt] + [M] + 2[D] \qquad (7)$$

According to the equilibrium in eqn. 4:

$$[D] = K_D[M]^2 \qquad (8)$$

Simultaneous solution of equations 6, 7 and 8 yield [M] and [M-Pt] as a function of δ_{obs}, [Co], K_D, δ_M, δ_D, and $\delta_{M\text{-}Pt}$. The values of [M] and [M-Pt] can be substituted into the logarithmic form of the equilibrium expression for eqn. 5, leading to eqn. 9.

$$\log\frac{[M\text{-}Pt]}{[M]} = \log[Pt] + \log K' \qquad (9)$$

Linear least-squares treatment of a number of measurements at different [Pt] while maintaining [Pt] > 10[Co], should yield log K' as the intercept of eqn. 9. The experimental conditions for determining K' were chosen to be relevant to the kinetic and mechanistic studies of the Pt(II)/Pt(IV) demethylation reaction; *i.e.*, $\mu = 1.0\,M$ (NaCl), pH 7.2 (0.1 M phosphate) T = 26 °C [3c]. The values of δ_M (0.248 ppm), δ_D (–0.306 ppm), and K_D (1030 M^{-1}) were determined previously [7]. The value of $\delta_{M\text{-}Pt}$ is estimated from the saturation effect of high [$Pt(CN)_4^{2-}$] on the chemical shift (Fig. 1A, upper curve). Using these conditions and parameters an excellent straight line fit results (Fig. 1B), yielding K' = 16 M^{-1}. The calculated slope of 0.80 indicates the involvement of one Pt(II) per $CH_3\text{-}B_{12}$ in eqn. 5.

The K' calculated in this manner is *ca.* 10^2-fold smaller than K_1 obtained kinetically and spectrophotometrically (eqn. 1) [3c]. This indicates that $Pt(CN)_4^{2-}$ binds to $CH_3\text{-}B_{12}$ at two sites – one represented by eqn. 1, and one by eqn. 5. Therefore, in eqn. 5, '$CH_3\text{-}B_{12}$' must actually represent the complex $CH_3\text{-}B_{12}\cdots PtL_4^{2-}$ of eqn. 1. The binding of the first platinum is obscured in the experiments of this report by the fact that $K_1 \gg K'$ and [Pt] $\gg$ total [$CH_3\text{-}B_{12}$].

The addition of $Pt(CN)_4^{2-}$ is accompanied by shifts of many of the assignable peaks in the ^1H NMR spectrum of $CH_3\text{-}B_{12}$ in addition to that of Co-CH_3 (Table I). Other resonances that are shifted significantly are: C2α-CH_3, C12α-CH_3, C1α-CH_3, B2-H, and C7α-CH_3. Based on this we propose that $Pt(CN)_4^{2-}$ causes a shift in the position of the benzimidazole group. This results in a change in the electronic configuration of the Co–C bond and a change in the ring current effects on the methyl groups facing the benzimidazole ring (*i.e.*, on the α-side of the corrin ring). This site cannot be the benzimidazole 3-nitrogen because this would lead to dissociation of the benzimidazole from the cobalt. The shift of the cobalt methyl is in the opposite direction in this complex compared to the 'base-off' form of $CH_3\text{-}B_{12}$ in which the benzimidazole is removed by protonation [9]. Also, the UV–visible spectrum does not indicate the gross changes that occur on conversion to the 'base-off' form.

The chemical-shift changes of $CH_3\text{-}B_{12}$ protons after addition of $PtCl_4^{2-}$ have also been examined.

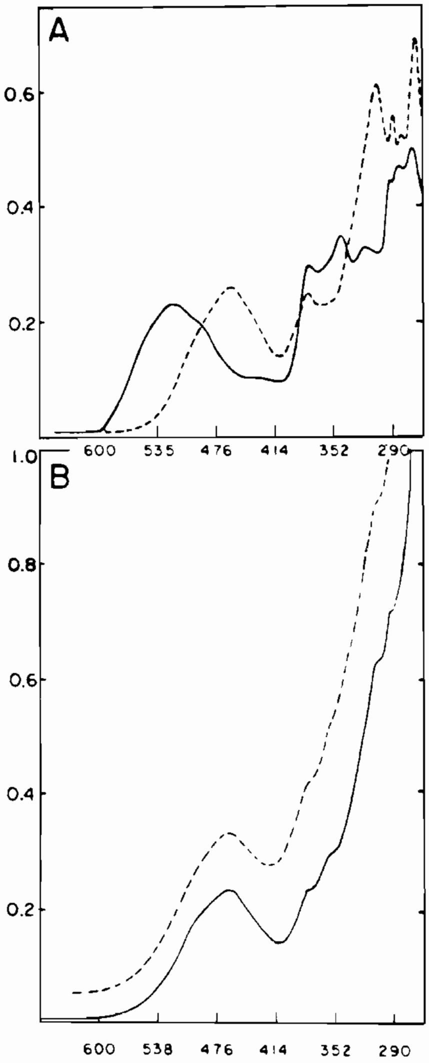


Fig. 2. UV-visible spectra of B_{12} complexes: (A) Base-on CH_3-B_{12}, pH 7.2 (——); Base-off CH_3-B_{12}, pH 1.0 (------). (B) CH_3-B_{12}/$Pt(SCN)_4^{2-}$ complex: pH 7.2 (——); pH 1.0 (------). $[CH_3\text{-}B_{12}] = 2.5 \times 10^{-5}$ *M*.

Even at high concentrations of CH_3-B_{12} and $PtCl_4^{2-}$ the changes are very small. The largest $\Delta\delta$ is only 13 Hz (0.05 ppm) and only four are greater than 6 Hz. The $\Delta\delta$ for Co-CH_3 is insignificant. The resonances showing the greatest shifts (B7-H, R1-H, C15-H, and C10-H) are not the same as for the $Pt(CN)_4^{2-}$ case. Thus the location or extent of binding to a second site remains undetermined for $PtCl_4^{2-}$.

$Pt(SCN)_4^{2-}$ Complexation with CH_3-B_{12}

Co-crystallization of CH_3-B_{12} with excess $Pt(SCN)_4^{2-}$ yields a complex with a Pt/Co ratio of 2.3 ± 0.1. A dilute solution of this complex is yellow and its visible spectrum at both neutral and acidic pH (Fig. 2) is very similar to CH_3-B_{12} at acidic pH (*i.e.*, protonated base-off CH_3-B_{12}). This indicates that one of the Pt groups is complexed to the 3-nitrogen of the benzimidazole group, resulting in detachment of the benzimidazole group from the cobalt atom. Similar reactions have been reported for alkylcobalamins with *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ [10] and with $PdCl_4^{2-}$ [11].

The ^1H NMR spectrum of the $Pt(SCN)_4^{2-}$/CH_3-B_{12} complex in aqueous solution is considerably broadened, owing to the presence of paramagnetic species. The EPR spectrum taken under air shows a resonance at g = 2.35 which is similar to the EPR spectrum of B_{12r}, although B_{12r} is normally oxidized rapidly by molecular oxygen to H_2O-B_{12}^+. In addition, a signal appears at g_z = 2.06, g_x = 2.01, g_y = 1.97. At the present time we are unable to identify the species represented by this signal.

The $Pt(SCN)_4^{2-}$/CH_3-B_{12} complex behaves as if it were a compound with an intact Co–C bond; exposure of an aqueous solution to light results in demethylation to yield H_2O-B_{12}^+. Furthermore, the complex behaves much like CH_3-B_{12} complexes of $Pt(CN)_4^{2-}$ or $PtCl_4^{2-}$, in that treatment with a Pt(IV) complex such as $PtCl_6^{2-}$ results in quantitative demethylation to H_2O-B_{12}^+ within a few hours in 0.1 *M* NaCl.

In conclusion, all three platinum(II) complexes bind to CH_3-B_{12}. The first binding site for $Pt(CN)_4^{2-}$ and $PtCl_4^{2-}$ is on the Co-CH_3 side of the corrin ring. Due to the similar reactivity in the demethylation reaction, $Pt(SCN)_4^{2-}$ may bind to this site as well. A second binding site for $Pt(SCN)_4^{2-}$ is the 3-nitrogen of the benzimidazole side chain. A second binding site for $Pt(CN)_4^{2-}$ causes a perturbation in the position of the benzimidazole ligand.

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