Methylation of Platinum Complexes by Methylcobalamin

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Abstract: The biomethylation reaction between platinum and methylcobalamin (MeB_{12}) has been shown to involve both Pt(II) and Pt(IV) oxidation states. Kinetic, equilibrium, and 270-MHz ^1H NMR studies have been used to show that an "outer-sphere" complex is formed between charged Pt(II) salts and the corrin macrocycle, which catalytically labilizes the Co–C σ bond to electrophilic attack. This research provides us with the first example of "activation" of the Co–C σ bond of MeB_{12} through the interaction of a charged species with the corrin macrocycle. The significance of this discovery is discussed in terms of the reactions catalyzed by B_{12} enzymes.

Introduction

Chemical model systems have contributed significantly to our understanding of biological processes. Reactions between methylcobalamin and metal or metalloid ions have been used as a model system for the biomethylation of toxic elements.[1] In fact, mechanistic studies with methylcobalamin have been used to predict the physical conditions required for biomethylation to occur.[1-5] Such reactions are of considerable environmental significance since in general the methylated organometallic derivatives are more toxic than their inorganic precursors to higher organisms.

Agnes et al.[6] were the first to report that the methylation of platinum by methylcobalamin required the addition of platinum in both oxidation states (Pt(II) and Pt(IV)). A pathway for this reaction was formulated and called the "redox switch" reaction.[6] Later, Taylor and Hanna showed that under their conditions MePt was a product of this reaction, and they obtained some preliminary kinetic data to support the redox switch reaction mechanism.[7]

To date, we have reported on two general mechanisms for B_{12}-dependent methylation of metals or metalloids. The first reaction involves heterolytic cleavage of the Co–C σ bond of methylcobalamin with transfer of a carbanion methyl group to the more oxidized state of the metal (i.e., electrophilic attack on the Co–C σ bond), and the second reaction involves homolytic cleavage of the Co–C bond leading to the transfer of a methyl radical (i.e., free-radical attack on the Co–C σ bond).[1,3,5] The electrophilic mechanism occurs with inorganic compounds having a standard reduction potential (E^0) of +0.85 V or higher, while the free-radical mechanism occurs with inorganic compounds having E^0 of +0.50 V and lower.[8] Platinum is especially interesting because the E^0 for Pt(IV)/Pt(II) couple is +0.76 V and cannot be classified with either those elements that react electrophilically or those which react by a free-radical mechanism. Therefore, a study of the biomethylation of platinum is of considerable mechanistic interest since both Pt(IV) and Pt(II) oxidation states are required for methyl transfer to occur.

In this paper we present a detailed kinetic and mechanistic study of the methylation of platinum complexes by methylcobalamin. On the basis of these data a detailed exploration for the role of the two oxidation states of platinum is proposed. The unique feature of this mechanism is the formation of a complex between Pt(II) and methylcobalamin which labilizes the Co–C σ bond for subsequent methyl transfer to platinum. Studies of the nature of this complex could contribute to our understanding of how B_{12}-dependent enzymes interact with the cobalamin coenzymes.

Experimental Section

Materials. Platinum complexes were purchased from either Ventron or Goldsmith, Inc. The K_2PtCl_4 used for 270-MHz NMR studies was recrystallized from D_2O. All other chemicals were reagent grade and were used as received.

Methylcobalamin (MeB_{12}) was synthesized by the method described by Dolphin.[9] Concentrations of MeB_{12} in solution and identification of B_{12} reaction products were determined from their absorbance spectra and from published molar extinction coefficients.[9-11] Methylcobalamin *B*-pyrrole ring lactam (MeB_{12}-lactam) was synthesized by the method of Bonnett et al.[10]

Kinetic Measurements. Reaction rates were estimated with the absorbance increase at 351 nm (absorbance maximum for aquo-B_{12}) with a GCA/McPherson Instrument connected to a circulating thermostated cell. All reactions were performed at 25 °C in the dark, with both $Pt^{IV}Cl_6^{2-}$ and $Pt^{II}Cl_4^{2-}$ complexes in at least sevenfold excess with respect to MeB_{12}. Ionic strength was maintained at 1.0 M with LiCl throughout unless otherwise mentioned. The pH was controlled in the range 0.9 to ca. 7 with either HCl, acetate buffer, or natural pH.

Equilibrium Constant Measurements. All equilibrium constants were estimated spectrophotometrically with a GCA/McPherson spectrophotometer at 25 °C and 1.0 M ionic strength in Cl^- medium. Equilibrium constants for reactions which occur in the absence of Pt(II) complexes were measured according to eq 1[12]:

$$\frac{A_{304.5nm}}{[MeB_{12}]_{tot}} = \frac{K_2\epsilon_{base\text{-}on} + K_1[H^+]\epsilon_{base\text{-}off}}{K_2 + K_1[H^+]} \quad (1)$$

Equilibrium constants in the presence of $Pt^{II}Cl_4^{2-}$ were measured according to eq 2.[12]

$$\frac{A_{304.5nm}}{[MeB_{12}]_{tot}} = \frac{(K_2 + K_2K_3[Pt^{II}Cl_4^{2-}])\epsilon_{base\text{-}on} + K_1[H^+]\epsilon_{base\text{-}off}}{K_2 + K_2K_3[Pt^{II}Cl_4^{2-}] + K_1[H^+]} \quad (2)$$

270-MHz NMR Studies. NMR (270 MHz) was used to study the effect of $Pt^{II}Cl_4^{2-}$ on the methyl–cobalt σ bond. Reactions were set up using 0.50 mL of MeB_{12} (2×10^{-3} M) in D_2O. Spectra were recorded after each addition of 0.20 mL of $Pt^{II}Cl_4^{2-}$ (2.5×10^{-3} M in D_2O) at room temperature. No supporting electrolyte was added.

Results

Preliminary experiments showed that MeB_{12} was not demethylated by $Pt^{II}Cl_4^{2-}$ alone. A very slow reaction with MeB_{12} does proceed with $Pt^{IV}Cl_6^{2-}$ alone, but this reaction is so slow that it does not interfere with the kinetic experiments reported here. MeB_{12} (4×10^{-5} M) was completely demethylated by a mixture of $Pt^{II}Cl_4^{2-}$ (4×10^{-4} M) and $Pt^{IV}Cl_6^{2-}$ (4×10^{-4} M) within half an hour at room temperature (pH ca. 2, in 1.0 M LiCl). Both $Pt^{II}Cl_4^{2-}$ and $Pt^{IV}Cl_6^{2-}$ have been shown to be quite stable in neutral aqueous solution. Absorbance spectra of $Pt^{II}Cl_4^{2-}$ or $Pt^{IV}Cl_6^{2-}$ (0.01 M) in neutral aqueous solution do not change discernibly over a few hours. Moreover, when 0.002 M $Pt^{II}Cl_4^{2-}$ was added to a solution with pH range from 2 to 3 (in 1.0 M LiCl), there was no appreciable difference in pH before and after adding the $Pt^{II}Cl_4^{2-}$. Therefore, it is reasonable to consider $Pt^{IV}Cl_6^{2-}$ and $Pt^{II}Cl_4^{2-}$ as the only active platinum complexes involved in the demethylation of MeB_{12} under the experimental conditions throughout. Spectrophotometric measurements showed the

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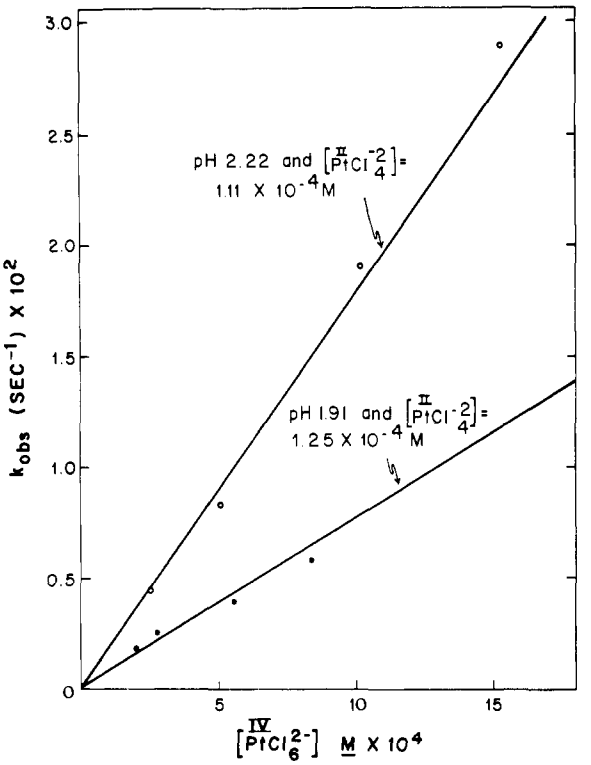


Figure 1. Plots of k_{obsd} vs. $[Pt^{IV}Cl_6^{2-}]$ for the reaction of methylcobalamin with a mixture of $Pt^{IV}Cl_6^{2-}$ and $Pt^{II}Cl_4^{2-}$.

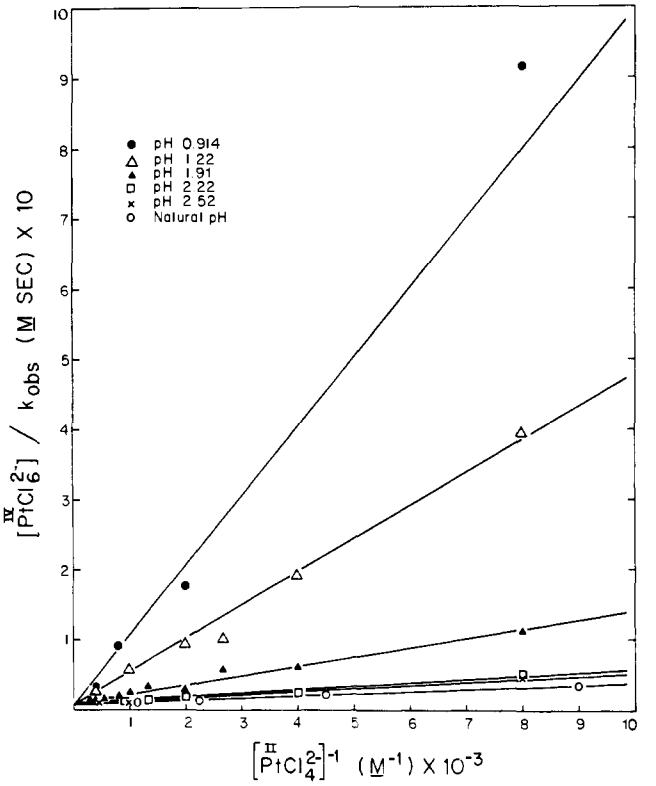


Figure 2. Plots of $[Pt^{IV}Cl_6^{2-}]/k_{obsd}$ vs. $[Pt^{II}Cl_4^{2-}]^{-1}$ for the reaction of methylcobalamin with a mixture of $Pt^{IV}Cl_6^{2-}$ and $Pt^{II}Cl_4^{2-}$. The solid lines were generated using eq 5 and the values for k and K' in Table I.

consumption of 1 mol of $Pt^{IV}Cl_6^{2-}$ per mol of MeB_{12}, with $Pt^{II}Cl_4^{2-}$ required in only catalytic quantities. Aquocobalamin (aquo-B_{12}) and methylplatinum were shown to be the products of the reaction as demonstrated previously by Taylor and Hanna.[7] The overall stoichiometry for the demethylation of MeB_{12} by platinum complexes is expressed in eq 3.

$$MeB_{12} + Pt^{IV}Cl_6^{2-} + H_2O \xrightarrow[(Pt^{II}Cl_4^{2-})]{} \text{aquo-}B_{12} + MePt^{IV}Cl_5^{2-} + Cl^- \quad (3)$$

It has been previously shown by Taylor and Hanna that the appearance of isosbestic points at 490–492, 367, and 335 nm is consistent with conversion of MeB_{12} to aquo-B_{12} with no other discernible corrinoid intermediates accumulating in the reaction course. There was no observable change in the rates or products when the reactions were performed under anaerobic conditions. Oxygen was carefully excluded by bubbling argon through solutions of MeB_{12} and $Pt^{II}Cl_4^{2-}$ (1.0 M LiCl and 1.22 X 10^{-3} M HCl) and then similarly deoxygenated solutions of $Pt^{IV}Cl_6^{2-}$ were added in the dark through a syringe. Under these conditions the corrinoid product was exclusively aquo-B_{12}; no B_{12r} (cob(II)alamin) could be detected.

Kinetic and Equilibrium Studies. The kinetics for the demethylation of MeB_{12} were studied in the presence of at least a sevenfold excess of $Pt^{II}Cl_4^{2-}$ and $Pt^{IV}Cl_6^{2-}$. Under these conditions, MeB_{12} was quantitatively converted to aquo-B_{12}. The reactions were found to obey the rate law:

$$\frac{d[\text{aquo-}B_{12}]}{dt} = k_{obsd}[MeB_{12}]_{tot} \quad (4)$$

giving good linear plots of ln $(A_\infty - A_t)$ vs. time for at least 85% of the reaction. Reproducibility has been checked to be within 7%. Pseudo-first-order rate constants are plotted vs. $[Pt^{IV}Cl_6^{2-}]$ in Figure 1. The linearity and zero intercept are consistent with a first-order dependence in $Pt^{IV}Cl_6^{2-}$. The data for the kinetic dependence of the reaction on $Pt^{II}Cl_4^{2-}$ and pH are plotted in Figure 2 and were found to fit the empirical rate expression shown in eq 5:

$$k_{obsd} = \frac{kK'[Pt^{II}Cl_4^{2-}][Pt^{IV}Cl_6^{2-}]}{1 + K'[Pt^{II}Cl_4^{2-}]} \quad (5)$$

When $K'[Pt^{II}Cl_4^{2-}] \ll 1$, then $k_{obsd} \simeq kK'[Pt^{II}Cl_4^{2-}]\cdot[Pt^{IV}Cl_6^{2-}]$ and the reaction will show a first-order dependence on $Pt^{II}Cl_4^{2-}$. Experimentally, this was found to occur at relatively low pH and low $[Pt^{II}Cl_4^{2-}]$, as shown in Figure 2. Equation 5 predicts that as $K'[Pt^{II}Cl_4^{2-}]$ approaches 1 the rate will show a less than first-order dependence on $Pt^{II}Cl_4^{2-}$. Eventually when $K'[Pt^{II}Cl_4^{2-}] \gg 1$ the reactions become zero order in $Pt^{II}Cl_4^{2-}$, a phenomenon that was observed at relatively high pH and high $[Pt^{II}Cl_4^{2-}]$.

Plots of $[Pt^{IV}Cl_6^{2-}]/k_{obsd}$ vs. $1/[Pt^{II}Cl_4^{2-}]$ shown in Figure 2 at different pHs give a series of straight lines with different slopes and a similar intercept. Linear least-squares analysis of these plots gave the values of k (reciprocal of intercept) and K' (slope = $1/kK'$) listed in Table I.

A plausible reaction pathway that is consistent with the data shown in Figures 1 and 2 is outlined in Scheme I. The reaction pathway in Scheme I leads to the rate law

$$\text{rate} = \frac{d[\text{aquo-}B_{12}]}{dt} = \frac{k\cdot K_2\cdot K_3[Pt^{II}Cl_4^{2-}][Pt^{IV}Cl_6^{2-}][MeB_{12}]_{tot}}{K_2 + K_1K_2 + K_1[H^+] + K_2K_3[Pt^{II}Cl_4^{2-}]} \quad (9)$$

This rate law implies a first-order dependence on MeB_{12} and $Pt^{IV}Cl_6^{2-}$ under all reaction conditions, as well as a first-order dependence on $Pt^{II}Cl_4^{2-}$ at relatively low $[Pt^{II}Cl_4^{2-}]$ and low pH. However, reactions become less than first order as the $[Pt^{II}Cl_4^{2-}]$ increases and/or as $[H^+]$ decreases until eventually reactions are zero order in $Pt^{II}Cl_4^{2-}$. At relatively high $[H^+]$ and low $[Pt^{II}Cl_4^{2-}]$, reactions show inverse first-order dependency in H^+, approaching zero order as $[H^+]$ decreases.

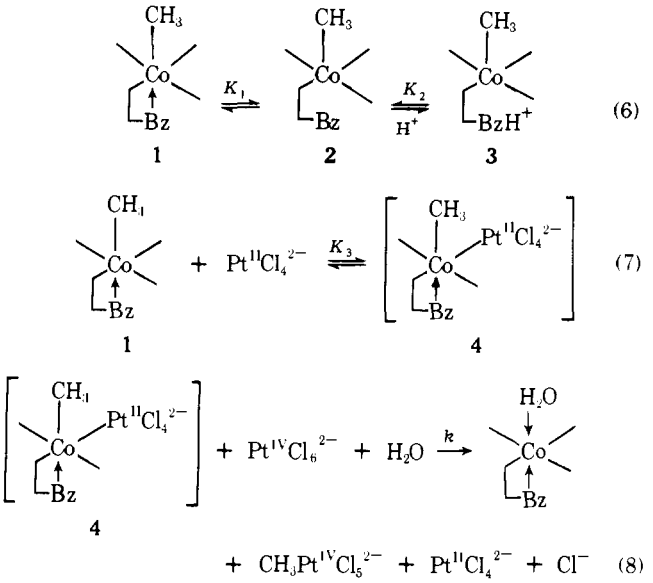
The pK_2 can be considered as approximately equal to 4.7,

Table I. Analysis of Kinetic Data for the Demethylation of MeB_{12} by a Mixture of $Pt^{IV}Cl_6^{2-}$ and $Pt^{II}Cl_4^{2-}$ at Various pHs[a]

pH	exptl $k \cdot K'/10^4$ [b]	$k/10$	$K'/10^2$
0.914	1.0		
1.22	2.0	16	1.3
1.91	7.4	9.0	8.5
2.22	16	14	14
2.52		9.0	26
≃7		11.2	32

[a] Temperature 25 °C; μ = 1.0 M (LiCl + HCl). [b] Here $k \cdot K'$ are experimental values obtained at relatively low pH and low $Pt^{II}Cl_4^{2-}$ concentration (i.e., in regions which show first-order behavior in $Pt^{II}Cl_4^{2-}$; see eq 5).

Scheme I[a]



[a] K_1 = chain opening equilibrium constant. K_2 = acid dissociation constant for benzimidazole (Bz). K_3 = association constant for complex.

which is the pK_a for free 5,6-dimethylbenzimidazole nucleotide in aqueous solution. The literature value for pK_1 is 2.0[9,11] at low ionic strength and therefore the rate law presented in eq 9 can be simplified to the following

$$\frac{d[\text{aquo-B}_{12}]}{dt} = \frac{k \cdot K_2 \cdot K_3[Pt^{II}Cl_4^{2-}][Pt^{IV}Cl_6^{2-}][MeB_{12}]_{tot}}{K_2 + K_1[H^+] + K_2K_3[Pt^{II}Cl_4^{2-}]} \quad (10)$$

Using the kinetic data of Table I and a value of $K_2 = 2.0 \times 10^{-5}$ M (based on pK_a = 4.7 for free 5,6-dimethylbenzimidazole in aqueous solution) then $k = (1.2 \pm 0.26) \times 10^2$ M^{-1} s^{-1}, $K_3 = (3.4 \pm 0.20) \times 10^3$ M^{-1}, and "apparent pK_1" = 2.2 at μ = 1.0 M (HCl + LiCl), 25 °C.

The presence of $Pt^{II}Cl_4^{2-}$ in a solution of MeB_{12} does not change the spectrum of MeB_{12} at pH 1 or lower (base-off MeB_{12}), or the spectrum of MeB_{12} at pH around 7 (base-on MeB_{12}). However, the presence of $Pt^{II}Cl_4^{2-}$ in a solution of MeB_{12} in the intermediate pH range significantly changes the equilibrium position between base-off and base-on MeB_{12}. The influence of $Pt^{II}Cl_4^{2-}$ on the percentage of base-on MeB_{12} in solution at various pHs is shown in Table II.

The equilibrium constant, K_1, at 25 °C can be estimated for MeB_{12} in both the absence (eq 1) and the presence of $Pt^{II}Cl_4^{2-}$ (eq 2) by examining absorbance changes at 304.5 nm. Measurements were made at low ionic strength in the absence of

Table II. Percentage of Base-on MeB_{12} in Solution as a Function of pH and $[Pt^{II}Cl_4^{2-}]$[a]

pH	$[Pt^{II}Cl_4^{2-}]$, M	% base-on MeB_{12}
0	0	≃0
0	4.2×10^{-3}	≃0
1.91	0	6.6
1.91	4.4×10^{-4}	12
2.61	0	29
2.61	4.4×10^{-4}	37
2.61	2.22×10^{-3}	53
2.91	0	37
2.91	4.4×10^{-4}	44
≃7	0	≃100

[a] $[MeB_{12}]_{tot} = 3.28 \times 10^{-5}$ M, μ = 1.0 M (LiCl + HCl), 25 °C.

Table III. Kinetic Parameters for the Demethylation of MeB_{12} by a Mixture of $Pt^{IV}Cl_6^{2-}$ and $Pt^{II}Cl_4^{2-}$ at μ = 2.0 M[a]

pH	$k/10$[b]	$K'/10^4$ [b]
1.22	1.25	0.46
1.91	0.95	1.7

[a] μ = 2.0 M (LiCl + HCl), 25 °C. [b] Defined in eq 5.

platinum and the estimated pK_1 of 2.0 was found to agree with the literature values.[9,11] The same method at high ionic strength (1.0 M LiCl, 25 °C) gave a pK_1 of 1.64. However, in the presence of $Pt^{II}Cl_4^{2-}$ (2.22×10^{-3} M) in 1.0 M LiCl an apparent pK_1 of 2.24 was determined using eq 2 and $K_3 = 3.4 \times 10^3$ M^{-1} obtained from kinetic measurements. This value is in agreement with the apparent pK_1 of 2.2 determined kinetically using eq 10. It should be noted that the value of apparent pK_1 measured here is dependent on the $Pt^{II}Cl_4^{2-}$ concentration present in solution. There was no significant change of pK_1 value from free MeB_{12} when $[Pt^{II}Cl_4^{2-}]$ in solution is 4.0×10^{-4} M or lower.

Kinetic measurements of the reaction pathway presented in Scheme I were found to be extremely sensitive to ionic strength. Values for k and K' at 2.0 M ionic strength maintained with LiCl are presented in Table III. By comparing Tables I and III, it is apparent that as the ionic strength in the reaction mixtures is doubled from 1.0 to 2.0 M, k decreases by an order of magnitude while K' increases by 25-fold. Similar large effects on reaction rates were observed upon changing the anion in reaction mixtures. For example, the addition of acetate (10^{-2} M) slows down the reaction rate by a factor of two, and reaction rates in $NaClO_4$–$HClO_4$ solution were much slower than those with chloride. In contrast to the common anion in solution, kinetic measurements are not affected by the nature of common cation in solution. For example, the same rates were obtained when NaCl or KCl was substituted for LiCl to control the ionic strength.

Other charged complexes of Pt^{II} including $Pt^{II}(CN)_4^{2-}$, $Pt^{II}(SCN)_4^{2-}$, and $Pt^{II}(NH_3)_4^{2+}$ could be substituted for $Pt^{II}Cl_4^{2-}$ in the presence of $Pt^{IV}Cl_6^{2-}$ with comparable reaction rates. However, a mixture of the neutral *cis*-$Pt^{II}(NH_3)_2Cl_2$ complex and $Pt^{IV}Cl_6^{2-}$ demethylates MeB_{12} very slowly. This result suggests that the charge on the Pt^{II} complex is important in the formation of the complex with MeB_{12}.

The demethylation of the *B*-pyrrole ring γ-lactam derivative[10] of MeB_{12} by a mixture of $Pt^{II}Cl_4^{2-}$ and $Pt^{IV}Cl_6^{2-}$ was examined under identical conditions to those used for MeB_{12}. A rate expression similar to eq 10 was observed for the lactam and a kinetic analysis gave $k_{lac} = 3.1 \times 10$ M^{-1} s^{-1}, apparent

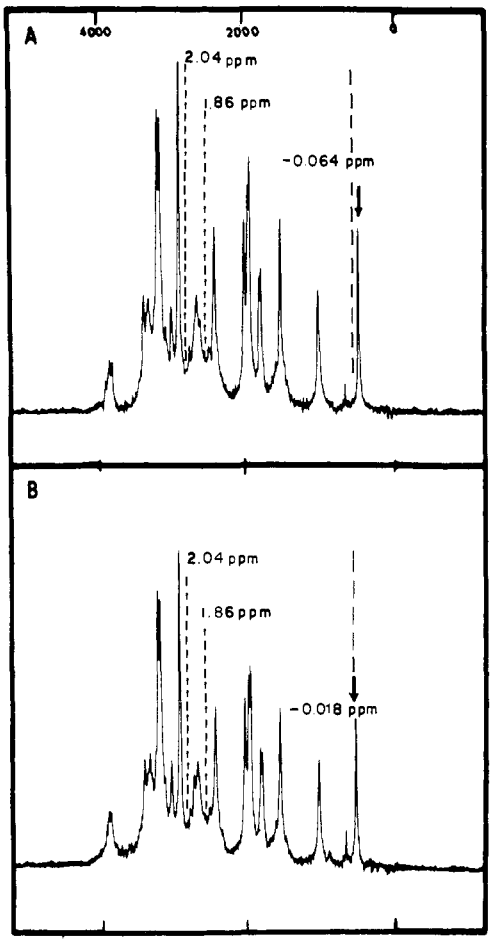


Figure 3. The 270-MHz ^1H NMR spectra of methylcobalamin in D_2O, pH ≃7. (A) Free methylcobalamin; (B) methylcobalamin with equal moles of $Pt^{II}Cl_4^{2-}$.

$pK_1 = 2.3$, $K_{3,lac} = 3.7 \times 10^3\ M^{-1}$ at $\mu = 1.0$ M Cl^- and 25 °C. The primary effect of lactam derivatization was a fourfold decrease in the rate constant, k, for the demethylation of the MeB_{12} derivative.

^1H NMR Studies. Independent evidence for the formation of a complex between MeB_{12} and $Pt^{II}Cl_4^{2-}$ was obtained from 270-MHz NMR. The ^1H NMR spectra for MeB_{12} and the MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex are presented in Figure 3 (here only the region from $\delta = -0.55$ to 3.1 is shown). Our NMR studies show that the 5,6-dimethylbenzimidazole moiety remains coordinated to the cobalt atom in the presence of $Pt^{II}Cl_4^{2-}$ because there is no observable chemical shift for the aromatic protons at δ 5.85, 6.20, 6.90, and 7.11, respectively. However, the proton resonance of the methyl group σ bonded to the cobalt atom shifts downfield significantly. This chemical shift is proportional to the concentration of $Pt^{II}Cl_4^{2-}$ as seen in Figure 4. The spectra of MeB_{12} and MeB_{12}–$Pt^{II}Cl_4^{2-}$ complexes are also different in the region σ 1.86 to 2.04.

Discussion

The base-off to base-on equilibrium for free MeB_{12} is expressed in eq 6, Scheme I. The equilibrium thermodynamics for this system can be determined by two parameters: (1) the chain opening constant (K_1) and (2) the acid dissociation constant of 5,6-dimethylbenzimidazole (K_2).[13] Assuming that pK_2 is equal to the pK_a for free 5,6-dimethylbenzimidazole nucleotide, then K_1 can be estimated spectrophotometrically using eq 1. The pK_1 was found to be sensitive to ionic strength with a value of 1.64 in 1.0 M LiCl at 25 °C. The pK_1 is also very sensitive to the nature of common anion in solution, with a value of 0.83 in 1.0 M $NaClO_4$ at 25 °C.

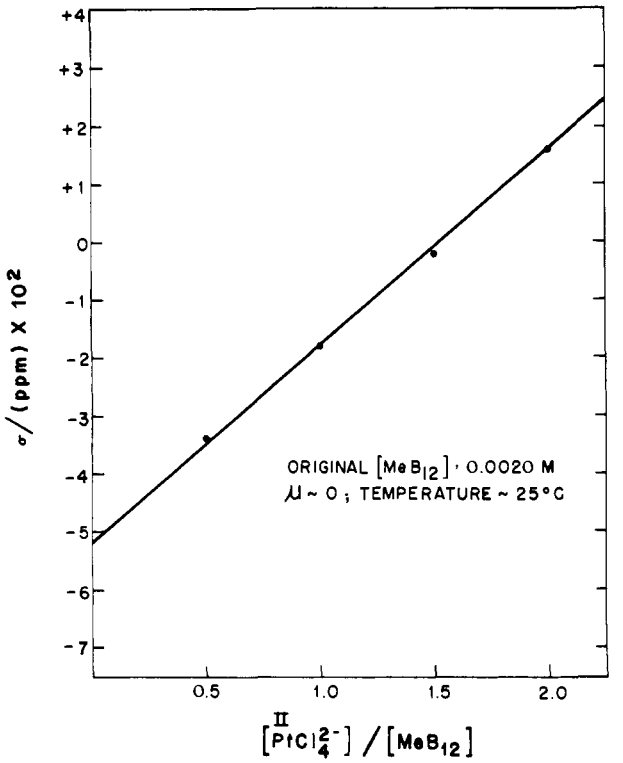
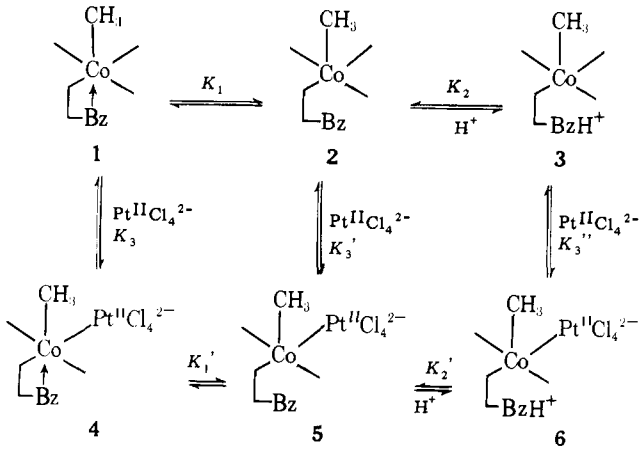


Figure 4. Plots of the chemical shift for the methyl group resonance at the Co atoms vs. $[Pt^{II}Cl_4^{2-}]/[MeB_{12}]$. Original $[MeB_{12}] = 0.0020$ M; $\mu \simeq 0$; temperature ≃25 °C.

In 1.0 M Cl^- and pH ≤1, MeB_{12} can be considered all base off and at pH ≥4, it can be considered all base on. In the intermediate pH range, MeB_{12} exists as a mixture of base-on and base-off forms. The influence of $Pt^{II}Cl_4^{2-}$ on the percentage of base-on MeB_{12} in solution at various pHs, which is shown in Table II, provides strong evidence that in the presence of the complexing ion $Pt^{II}Cl_4^{2-}$, additional equilibria occur to give more base-on MeB_{12}. For example, the presence of 2.2×10^{-3} M $Pt^{II}Cl_4^{2-}$ at pH 2.61 increases the amount of base-on MeB_{12} from 29 to 53%. Since base-on and base-off MeB_{12} have a marked difference in their absorbance spectra, this increase of base-on MeB_{12} is very unlikely due to experimental error, and since the presence of 2.2×10^{-3} M $Pt^{II}Cl_4^{2-}$ does not change the pH in solution (at least for the time scale and experimental conditions for the measurements), this observation provides strong nonkinetic evidence for Scheme I. It should be emphasized that $Pt^{II}Cl_4^{2-}$ has no observable effect on the absorbance spectra of MeB_{12}, either the base-on or base-off form. This was demonstrated by adding $Pt^{II}Cl_4^{2-}$ to a MeB_{12} solution at either a pH of 0 or 7.

Using eq 2 together with $K_3 = 3.4 \times 10^3\ M^{-1}$, which was obtained from kinetic measurements, an apparent pK_1 of 2.24 was determined. This value is in good agreement with the apparent pK_1 obtained from kinetics using eq 10. It should be noted that the apparent pK_1 is an observed base-off to base-on equilibrium constant for a combination of free MeB_{12} and MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex whose ratio depends on the $Pt^{II}Cl_4^{2-}$ concentration in solution. Therefore, the value of 2.24 is at best a lower limit approximation of pK_1'—the base-off to base-on equilibrium constant for the MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex. These results demonstrate that $Pt^{II}Cl_4^{2-}$ affects the electron density at the cobalt atom of MeB_{12}. The increase in the observed pK_1 from 1.64 to 2.24 shows that 5,6-dimethylbenzimidazole has a stronger basicity toward the cobalt atom in MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex than in free MeB_{12}, indicating that the electron density along the cobalt–carbon bond in MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex is less than that for free MeB_{12}. A

Scheme II



plausible equilibrium system for the base-on and base-off species of MeB_{12} which exists in the presence of $Pt^{II}Cl_4^{2-}$ is expressed in Scheme II.

Although the kinetic and equilibrium quotient data do not indicate whether complex **4** or **5** of Scheme II is the active species for subsequent methyl group transfer in the presence of $Pt^{IV}Cl_6^{2-}$, two lines of evidence suggest that complex **4** (i.e., base-on MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex) is more likely the active species. In the first place, kinetic studies at low pH (pH <1) have shown that complex **6** is inactive in methyl transfer. It seems unlikely that deprotonation of the benzimidazole moiety to form complex **5** would greatly effect the reactivity. Secondly, Taylor and Hanna[7] have observed that methylcobinamide, the MeB_{12} analogue that lacks the dimethylbenzimidazole base, reacts very slowly with $Pt^{II}Cl_4^{2-}$ and $Pt^{IV}Cl_6^{2-}$.

Independent evidence for the formation of a MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex was obtained with 270-MHz ^1H NMR. Hydrolysis of $Pt^{II}Cl_4^{2-}$ during the NMR experiments was shown to be negligible because the 270-MHz ^1H studies were conducted over a time frame of 15 min with the aid of Fourier transform. We have shown that $Pt^{II}Cl_4^{2-}$ is stable in aqueous solution for several hours even in the absence of chloride ion. The Co–CH_3 resonance of MeB_{12} is shifted downfield in the presence of $Pt^{II}Cl_4^{2-}$ (Figure 3) with the magnitude of the shift being proportional to the $Pt^{II}C_4^{2-}/MeB_{12}$ ratio (Figure 4).

Using lanthanide shift reagents, Hensens et al.[14] have assigned 70% of the ^1H NMR of MeB_{12} in solution. Most of the benzimidazole nucleotide and the groups which project below the plane of the corrin ring have been assigned. It is apparent from our NMR studies that the 5,6-dimethylbenzimidazole base remains coordinated to the cobalt atom in the MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex, because there is no observable chemical shift of the aromatic protons. There is no interaction between $Pt^{II}Cl_4^{2-}$ and any group which projects below the plane. It appears that complexation most likely occurs with groups which project above the plane of the corrin ring (i.e., acetamide and propionamide side chains). Our studies on the *B*-pyrrole ring lactam analogue of MeB_{12} show that complexation with the acetamide side chain on the *B*-pyrrole ring does not occur because the equilibrium constant for complex formation is almost identical with that observed for MeB_{12}. The acetamide side chains on the *A*- and *D*-pyrrole rings would appear to be the most likely sites for complex formation with $Pt^{II}Cl_4^{2-}$.

It is of interest to compare the properties of the MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex with the properties of the fluoroalkylcobalamins.[15] The introduction of electronegative fluorine atoms into the alkyl group results in an upfield shift for the protons of the σ-bonded methyl group and a decrease in the pK_1 of the benzimidazole nucleotide. The fluoroalkyl-B_{12} analogues were shown to be competitive inhibitors for methyl

Scheme III

$$Pt^{II}L_4 + Y \overset{K}{\rightleftharpoons} Pt^{II}L_4\text{-}Y$$

$$X\text{-}Pt^{IV}L_4^*\text{-}Z + Pt^{II}L_4\text{-}Y \rightleftharpoons X\text{-}Pt^{IV}L_4^*\text{-}Z\text{-}Pt^{II}L_4\text{-}Y$$

$$X\text{-}Pt^{IV}L_4^*\text{-}Z\text{-}Pt^{II}L_4\text{-}Y \rightleftharpoons X\text{-}Pt^{II}L_4^*\text{-}Z\text{-}Pt^{IV}\text{-}L_4\text{-}Y$$

$$X\text{-}Pt^{II}L_4^*\text{-}Z\text{-}Pt^{IV}L_4\text{-}Y \dashrightarrow X + Pt^{II}L_4^* + Z\text{-}Pt^{IV}L_4\text{-}Y$$

$$\text{rate} = kK[Pt^{II}L_4][X\text{-}Pt^{IV}L_4^*\text{-}Z][Y]$$

transfer from MeB_{12} in biological methane formation. Complexation of MeB_{12} with $Pt^{II}Cl_4^{2-}$, on the other hand, results in a downfield shift for the σ-bonded methyl group and an increase in pK_1. It should also be pointed out that a similar downfield shift of the 5′-methylene protons on coenzyme B_{12} is observed when this coenzyme binds to B_{12} apoenzymes.[16] Parallel studies using ^{13}C NMR have recently been performed on coenzyme B_{12}–platinum interactions.[17]

The consistency of the kinetic data with eq 10 and the agreement of the apparent pK_1 measurements from both kinetic and equilibrium quotient studies, the observation of the base-on and base-off equilibrium position shift due to the presence of $Pt^{II}Cl_4^{2-}$, together with 270-MHz ^1H NMR studies on the MeB_{12}—$Pt^{II}Cl_4^{2-}$ complex, provide strong support for the mechanism proposed in Scheme I. The present studies do not, however, reveal how $Pt^{IV}Cl_6^{2-}$ interacts with the MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex to give the final products. A consideration of platinum and cobalamin chemistry has suggested two most likely pathways. The first pathway is a two-electron "redox switch" between Pt(II) and Pt(IV) first suggested by Agnes et al.[6] and outlined below:

$$CH_3\text{-}B_{12}\text{-}Pt^{II}Cl_4^{2-} + Pt^{*IV}Cl_6^{2-} + H_2O \overset{k}{\rightarrow} \text{aquo-}B_{12} + CH_3Pt^{IV}Cl_5^{2-} + Pt^{*II}Cl_4^{2-} + Cl^- \quad (11)$$

This pathway bears a strong resemblance to the mechanism for Pt(II)-catalyzed substitutions of Pt(IV) complexes recently reviewed by Mason[18] and depicted in Scheme III. The methylation of platinum by MeB_{12} involves an equilibrium between MeB_{12} and $Pt^{II}Cl_4^{2-}$, while the substitution reaction of Scheme III involves a complex between the displacing ligand Y and $Pt^{II}L_4$. However, the cobalamin reaction is much more complex because MeB_{12} can appear in three different forms: (1) base on, (2) base off and (3) protonated base off, and this complexity is reflected in the rate law, eq 10. Equation 10 approaches the rate law for Scheme III at relatively low pH and low $Pt^{II}Cl_4^{2-}$ concentrations, where the rate is first order in Pt(II) as well as first-order in Pt(IV) and MeB_{12}.

A second mechanism which would be consistent with the observed kinetics is direct electrophilic attack by $Pt^{IV}Cl_6^{2-}$ on the Co–C σ bond of MeB_{12} as described in eq 12:

$$CH_3\text{-}B_{12}\text{-}Pt^{II}Cl_4^{2-} + Pt^{*IV}Cl_6^{2-} + H_2O \overset{k}{\rightarrow} \text{aquo-}B_{12} + CH_3Pt^{*IV}Cl_5^{2-} + Pt^{II}Cl_4^{2-} + Cl^- \quad (12)$$

The function of the MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex in this mechanism would be to labilize the Co–C bond to electrophilic attack by $Pt^{IV}Cl_6^{2-}$. An electrophilic mechanism has been proposed to account for the methylation of Hg(II)[19] and Pd(II)[20] by MeB_{12}.

When the ionic strength in reaction mixtures was changed from 1.0 to 2.0 M LiCl, then k decreases by an order of magnitude, while K' increases 25-fold. This increase in K' with increasing Cl^- concentration is consistent with our NMR data which were obtained at low ionic strength. Our NMR study shows that only a small amount of complex is formed over the concentration range 1 to 4×10^{-3} M $Pt^{II}Cl_4^{2-}$ and 2×10^{-3} M MeB_{12}.

The unique feature of the mechanism for the methylation

of platinum by MeB_{12} is the obligatory formation of a MeB_{12}–$Pt^{II}Cl_4^{2-}$ complex. Although the structure of this complex is not yet understood in detail, it seems likely that the propionamide and acetamide side chains of the A and D rings of the corrin macrocycle are likely sites for complexation. It seems reasonable to propose that these side chains are involved in the interaction of the cobalamins with B_{12}-dependent proteins. Recently, Abeles et al.[21] showed that a red to yellow isomerization occurs in the B_{12} enzyme, diol dehydrase, when a *B*-pyrrole ring monoester derivative of B_{12} coenzyme is substituted for 5′-deoxyadenosylcobalamin. This monoester coenzyme analogue shows a substrate-dependent red to yellow transition, and the yellow form of the coenzyme analogue is about 5% as active as 5′-deoxyadenosylcobalamin itself. An explanation for these results might be found in the suggestion of Brown and Wood in 1972[22] that the interaction of B_{12} with proteins could lead to corrin ring isomerizations which may represent an important feature in understanding substrate-directed labilization of the Co–C bond in the B_{12} enzymes. This suggestion was based on the observation that 2′,2′-isopropylidene-5′-deoxy-β-(D)-ribosylcobinamide could exist as two stable corrin ring isomers. The isomers possessed distinctly different NMR spectra and photolability to visible light.[22] In 1975, first Hogenkamp et al.[23] and then Cockle et al.[24] used 270-MHz ^1H NMR to demonstrate that a red-yellow shift similar to that observed by Brown and Wood[22] could be explained by corrin ring isomerizations of the cobalamins.

There is no doubt that isomerization of the corrin ring system would lead to a change in the electronic configuration of the macrocycle, which in turn could labilize or stabilize the Co–C σ bond. Temperature changes have been shown to lead to an isomerization of the corrin ring, although this isomerization does not affect the stability of the Co–C σ bond.[24] Our studies of the methylation of platinum complexes by MeB_{12} provide the first example of Co–C bond labilization by complexation of the corrin macrocycle with a coordination compound. This labilization of Co–C bond is very sensitive to the overall structure of corrin macrocycle, as demonstrated with the methylcobalamin *B*-pyrrole ring lactam which is only 25% as active as methylcobalamin. A continuation of these investigations could lead to a better understanding of the chemistry of the cobalamins and provide insight into the importance of the corrin side chains and ring isomerizations to the functioning of B_{12}-dependent proteins.

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