METHYLATION BY DIASTEREOISOMERS OF METHYLCOBALAMIN

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1. Introduction

Methylcobalamin has an important function as a coenzyme in biological methylation systems, and it is also the form of vitamin B_{12} which is specifically transported by transcobalamin. Many important studies have been carried out on inorganic methylations by methylcobalamin of metal ions such as Pd(II), Tl(III), Cr(II), Cu(II), Pt(II), Pt(IV), Au(I), Au(III) [1–4] and especially with Hg(II) since the methyl-Hg(II) species formed are highly toxic and mutagenic agents [5–7]. This interesting problem raises the question of the biological activity of diastereoisomers of methylcobalamin in the processes mentioned. In our comparative studies with the α- and β-diastereoisomers of methylcobalamin as well as with dimethyl(Co,N^3_{bzm})-cobalamin we chose the methylation of mercury (II) ions as a model reaction.

2. Materials and methods

The Coα-(α-5,6-dimethylbenzimidazolyl)-Coβ-methylcobamide (β-MeB_{12} or β-methylcobalamin), α-methylcobalamin (α-MeB_{12}) and Co-methyl (3,5,6-trimethylbenzimidazolyl)-cobamide (diMeB_{12}) were synthesized from cyanocobalamin (Fluka) and dimethylsulphate [8]. Paper electrophoresis of corrinoids was performed in 0.5 M acetic acid. Kinetic studies were carried out under pseudo-first-order conditions (in Hg(II) species) according to DeSimone et al. [7] in 0.1 M acetate or citrate buffers at 30°C, pH range 3.7–5.6, sodium acetate–acetic acid and pH range 2.6–7.6, citric acid–Na_2HPO_4 (McIlvaine buffer). The kinetic runs were carried out spectrophotometrically using a modified Beckman DG apparatus, monitoring the formation of aquocobalamin at 349 nm or its decrease when the reverse reaction was investigated. In studies with α-MeB_{12} and diMeB_{12} the reaction rates were additionally determined by measuring the MeHg(II) formation using a Perkin Elmer No. 900 Gas Chromatograph equipped with a ^{63}Ni electron capture detector. The reactions were stopped by the addition of 1 equiv. conc. HCl and finally the MeHg chloride was extracted from the reaction mixture with benzene. The GC analyses were performed with a glass column (2m × 2mm) packed with 15% polydiethyleneglycol succinate on Chromosorb W AW HMDS, 60–80 mesh. The following conditions were used: injector and manifold at 180°C, column-isothermal hold at 140°C and the EC-detector at 200°C (DC-mode, Potential 2.6 V) and nitrogen as a carrier gas (60+25) ml per min. The kinetic data were analyzed by conventional methods.

3. Results and discussion

The α-MeB_{12}, β-MeB_{12} and diMeB_{12} are decomposed at different rates by Hg(II) species in both citrate and acetate buffers (fig.1). In these reactions MeHg(II) cation and aquocobalamin are formed stoichiometrically, as determined spectrophotometrically (B_{12}) and with gas chromatography (MeHg(II)Cl). The methylations of Hg(II) species by the α- and β-diastereoisomers of methylcobalamin and diMeB_{12} are first-order reactions in both Hg(II) and Co-methyl-corrinoid. The reaction order was determined in 0.1 M citrate buffer, at pH 3.0 and the following values were obtained: α-MeB_{12}, $n = 0.9$; β-MeB_{12}, $n = 1.07$; and diMeB_{12}, $n = 1.04$. The methylation process is pH dependent

Table 1
Rate constants for the methylation of Hg(II) in 0.1 M citrate buffer, pH 3.0 – the anion effect

Added salt	Rate constant ($sec^{-1}M^{-1}$) α-MeB_{12}	β-MeB_{12}	diMeB_{12}
None	7.7	$4.3 \times 10^{+2}$	4.7
KI	3.5×10^{-1}	$3.2 \times 10^{+1}$	3.4×10^{-3}
KBr	7.2×10^{-1}	$9.5 \times 10^{+1}$	5.9×10^{-2}
KCl	1.9×10^{-1}	$1.8 \times 10^{+2}$	1.0×10^{-4}
NaN_3	2.1	$2.0 \times 10^{+2}$	1.7×10^{-1}
NaCN	1.3	$3.0 \times 10^{+2}$	2.2×10^{-1}

The molar ratio of Hg(II) to salts was 1:1, [Co-methyl-corrinoid] = 3×10^{-5} M, [salt] = 3.33×10^{-4} M.

and strongly inhibited by monovalent inorganic anions (table 1). The inhibition differs within Co-methyl-corrinoids and the greatest effect was observed for chloride ion when the α-isomer or diMeB_{12} were studied. On the other hand, for the methylation of Hg(II) by α-MeB_{12} in water (no buffer) with $HgCl_2$, $HgBr_2$ and Hg(II)acetate, the following second-order rate constants were obtained: 3.2×10^{-1}, 3.3×10^{-2} and 1.2 $s^{-1}M^{-1}$, respectively. The complexation of 5,6-dimethylbenzimidazole in α-MeB_{12} by Hg(II) was not investigated kinetically. It was observed that methylation of Hg(II) salts occurs in absolute alcohol (table 2). Two different displacements on β-MeB_{12} by Hg(II) salts were investigated independently in terms of the Co–N bond and Co–C bond. The Hg(II)-acetate participates in both displacements and displaces the 5,6-dimethylbenzimidazole rapidly and CH_3^- very slowly. On the other hand, $HgCl_2$ and $HgBr_2$ do not displace 5,6-dimethylbenzimidazole but only CH_3^-. This was investigated by monitoring the spectra between 300 nm and 600 nm. The rate of demethylation of β-MeB_{12} in absolute ethanol is at least 10^4

Table 2
The observed rate constants for the methylation of Hg(II) salts by β-MeB_{12} in absolute ethanol

Salt	k_{obs} (sec^{-1})	Co-ordination of Co(III)
$Hg(OAc)_2$	9.11×10^{-5}	(base-off)
$HgCl_2$	6.3×10^{-4}	(base-on)
$HgBr_2$	2.17×10^{-4}	(base-on)

[salt] = 2.5×10^{-2} M, [β-MeB_{12}] = 3×10^{-5} M

times slower (in comparison to aqueous solution) and the reaction proceeds six times slower with yellow-(base-off) β-MeB_{12} (with Hg(II)acetate) than with red-(base-on) β-MeB_{12} (with $HgCl_2$). No reverse reaction (methylation of cobalamin) was observed with methyl-Hg(II)chloride in 0.1 M citrate buffer, pH 2.6, even after several days. When methyl-Hg(II)chloride was replaced by dimethylmercury, methylation of aquocobalamin occurs and β-MeB_{12} was formed. The reaction is pH dependent; a decrease in pH increases the reaction rate. The reaction with dimethyl-Hg(II) was investigated spectrophotometrically (table 3) and on a preparative scale. Hydroxocobalamin (3×10^{-5} M) and diMeHg(II) (3×10^{-3} M) were reacted in 0.1 M citrate buffer, pH 2.6 in darkness overnight at room temperature and desalted on an Amberlite XAD-2 column. Finally, the reaction mixture (+ 5 μl of 1% HCN) was separated by paper electrophoresis. Two components were detected: β-MeB_{12} as a main product and cyanocobalamin the identity of which was confirmed by spectrophotometry and photolysis. Kinetic studies showed that the quasi-reverse reaction is first-order in both diMeHg(II) and cobalamin when carried out in 0.1 M citrate buffer, pH 2.6.

DeSimone et al. [7] suggested that demethylation of uncomplexed (base-on)/methylcobalamin by Hg(II) is the predominant route for the methyl transfer step. Recently, Scovell [1] showed that a close similarity exists between methyl transfers by Hg(II) and Pd(II). In these systems the initial reaction is a displacement of 5,6-dimethylbenzimidazole by electrophiles and the attainment of the equilibrium (base-on) ≠ = ≠ (base-off). In our kinetic studies with the α- and β-diastereoisomers of MeB_{12} and with diMeB_{12} we observed

Table 3
Rate constants for the methylation of cobalamin by dimethyl-mercury in 0.1 citrate buffer, pH 2.6.

Added salt	Rate constant (sec^{-1} M^{-1})
None	4.4×10^{-1}
KI	4.0×10^{-1}
KBr	3.5×10^{-1}
KCl	3.9×10^{-1}
NaN_3	2.0×10^{-1}
NaCN	4.3×10^{-1}

The molar ratio of diMeHg(II) to salt was 1:1, [salt] = 3.33×10^{-4} M

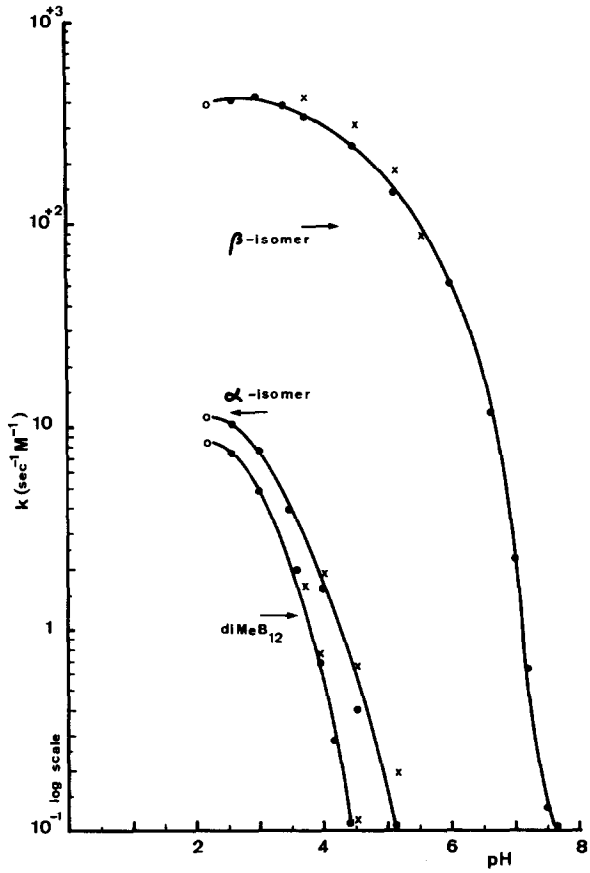

Fig.1. The pH-dependence of the methylation of Hg(II) by Co-methyl-corrinoids in acetate and citrate buffers. (×) in 0.1 M acetate buffer, (•) in 0.1 M citrate buffer, (○) in 0.1 M citric acid.

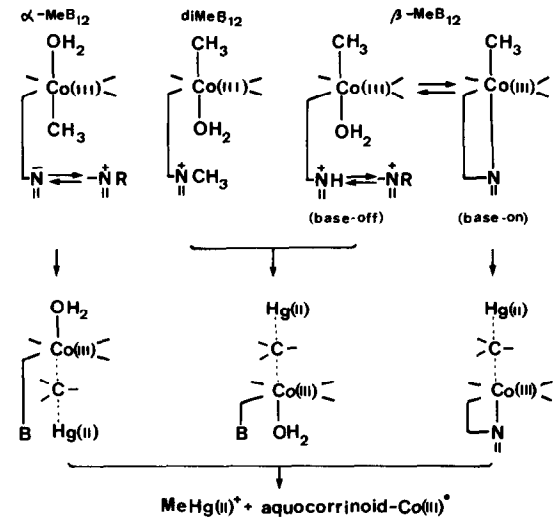

Fig.2. S_E2 displacement of the α- and β-co-ordinated methyl-carbanions by Hg(II). R = H or Hg(II)X, B = base, (*)-aquocobalamin from the α- and β-MeB_{12}, aquo(methyl-N^3_{bzm})-cobalamin from $diMeB_{12}$. The following equilibria exist: (base-on) ⇌ (base-off), $\equiv N^+Hg(II)X \rightleftharpoons -N= \rightleftharpoons \equiv N^+H$, $H_2O\text{-}Co\text{-}CH_3 \rightleftharpoons HO\text{-}Co\text{-}CH_3$ and $\text{-}Co\text{-}CH_3 \rightleftharpoons Co\text{-}CH_3$.

strong pH dependence for the methylation of Hg(II). The rates of methylation increase for both the (base-on) and (base-off) substrate with a decrease in pH. An increase in $[H^+]$ shifts the equilibrium, converting a significant concentration of reactive (base-on) β-MeB_{12} to the less reactive protonated (base-off) β-MeB_{12}. Therefore, the rate of the methylation should decrease with a decrease in pH. The observed pH dependence (fig.1) suggests that decreasing pH influences not only the equilibrium (base-on) ⇌ (base-off) but also the formation of more active Hg(II)-electrophiles: $Hg^{+2} > HgX^{+1} > HgX_2 > HgX_3^{-1} > HgX_4^{-2}$, since their geometry and electronegativity is changed. This suggestion receives support from the observed anion inhibition as Hg(II) also forms anionic complexes with halides and cyanide, $Hg(II) + 4X \rightleftharpoons Hg(II)X_4^{-2}$ with formation constants in the range of 10^{41} to 10^{16}. As the pH falls to around 3 the activity passes through a maximum and then decreases as expected from the known pK_a (2.5) of the equilibrium (base-on) ⇌ (base-off). Support for the view that the (base-off) substrate is demethylated slower than the (base-on) substrate is found in the observed 10^2 times lower rate constant for $diMeB_{12}$ as well as the methylations of Hg(II) salts in ethanol. The recently observed inversion on carbon in a similar transmethylation with alkyl-cobaloxime [9], kinetic data and similarity of the transfer of co-ordinated CH_3^- into Hg(II) species (no change in the oxidation state of reactants) support an earlier suggestion [7] that the methylation proceeds through an S_E2 reaction, resulting probably in an inversion of configuration (fig.2). Since dimethylmercury can be formed during the methylation as the result of a second displacement of CH_3^- by $MeHg(II)^+$ [6,7] the reaction is quasi-reversible because of the formation of β-MeB_{12}.

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