

Mechanism of Co-methylation of Co Corrinoids in the Presence of Thiols

John M. Pratt,* Mohamed S. A. Hamza and Gabriel J. Buist

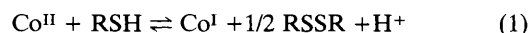
Department of Chemistry, University of Surrey, Guildford, UK GU2 5XH

The Co atom in Co^{II} cobinamide is methylated by MeI in the presence of thiols (RSH) such as dithiothreitol in aqueous solution at 25 °C by a pH-independent pathway involving the reaction of MeI with the [RSH⁰-Co^{II}] complex.

The B₁₂-dependent methyl transfer enzymes, which involve the alternate formation of Co^I and Me-Co corrinoids,¹⁻³ are less well studied than the isomerases and there are serious gaps in our knowledge of potentially relevant reactions *in vitro*, e.g. there is no model for Me transfer from an N atom to Co. The alkylation of Co^I corrinoids by MeI and other alkylating agents was discovered by several groups in 1962-1963 [see ref. 4(a)] and subsequent kinetic studies established Co^I as one of the most reactive nucleophiles known ($k_2 = 3.4 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$).⁵ Methylation of aquo-Cbl† by MeI in the presence of thiols (RSH) or H₂S was reported in 1963-1964^{6,7} but few experimental details were given and no comparison made between Cbls and Cbis. Mechanisms proposed include the reaction of MeI with RS⁻-Co^{III},⁷ RS⁻-Co^I,⁶ and Co^I,⁵ but no kinetic studies have been reported in the intervening 30 years. Because of the likely early origins of B₁₂,^{8,9} and the greater role of S-containing compounds in the primitive environment,¹⁰ we have re-investigated the methylation of Co^{III} corrinoids by MeI (soluble in water up to *ca.* 0.2 mol dm⁻³)¹¹⁻¹³ in the presence of various RSH. Co^{II}-Cbl and Co^{II}-Cbi were usually prepared by reducing aqueous solutions of the Co^{III} aquo-Cbl (B_{12a}, BDH) and diaquo-Cbi¹⁴ under N₂ with sodium formate.¹⁵ Kinetic studies were carried out by UV-VIS spectrophotometry at 25 °C in aqueous solution.

Initial experiments at pH 6 showed the following order of reactivity towards MeI with the formation of Me-Cbl or Me-Cbi: Co^{II}-Cbi + RSH (DTT, ME and TG all comparable) \gg Co^{II}-Cbl +/- RSH (*i.e.* no significant effect of RSH) \gg Co^{II}-Cbi alone (no detectable reaction). Further studies with Co^{II}-Cbi and DTT alone (pK 9.1 for the -SH group)¹⁶

revealed only small changes to the Co^{II} spectrum (see below) over the range of pH 2-8 but formation of Co^I at pH \geq 9, as expected from eqn. (1). The addition of MeI caused a simple conversion of Co^{II} to Me-Cbi‡ over the range of pH 4-8 but other changes in spectrum occurred at pH \leq 3. Me-Cbi and Co^{II}-Cbi are probably both five-coordinate.³



The kinetics of the reaction were followed from the rise in absorbance at 375 nm (*i.e.* near the γ -band of Me-Cbi),^{4b} using $6 \times 10^{-5} \text{ mol dm}^{-3}$ Co, $8 \times 10^{-3} \text{ mol dm}^{-3}$ DTT and 0.025 mol dm⁻³ MeI at pH 6.0 as standard conditions and varying each parameter in turn. All runs followed first-order kinetics for at least two half-lives. The values of the pseudo-first-order rate constants k_{obs} were essentially independent of pH 3-8 and of [Co] but limiting values were obtained with both DTT (see Fig. 1) and MeI (constant over $1-6 \times 10^{-2} \text{ mol dm}^{-3}$ MeI); this suggests a (Co-RSH·MeI) adduct as the key intermediate. Use of $\leq 1 \times 10^{-3} \text{ mol dm}^{-3}$ DTT or $< 10^{-2} \text{ mol dm}^{-3}$ MeI caused the loss of isosbestic points and/or gave final spectra similar to those at pH \leq 3, thereby precluding the determination of valid rate constants. Treating these products with CN⁻ in the presence of light and O₂ produced a pronounced band at 485 nm (as well as those belonging to dicyano-Cbi) characteristic of a 'stable yellow corrinoid',^{4c,17} *i.e.* shows the occurrence of side-reactions which are suppressed by high concentrations of MeI or DTT. Ignoring the results at low [DTT], the plot of k_{obs} vs. [DTT] gives a hyperbolic curve reaching a limiting value of the first order rate constant $k_1 = 1.8 \times 10^{-2} \text{ s}^{-1}$ at high [DTT] with 50% of the maximum rate observed at $4 \times 10^{-3} \text{ mol dm}^{-3}$ DTT (see Fig. 1); this corresponds to an apparent equilibrium constant $K = [\text{Co}^{\text{II}}\text{-DTT}]/[\text{Co}^{\text{II}}][\text{DTT}] = 2.5 \times 10^2 \text{ dm}^3 \text{ mol}^{-1}$. Adding increasing amounts of DTT to the Co^{II}-Cbi in the absence of MeI causes

† Abbreviations: Cbl, cobalamin, a corrinoid possessing the same side-chains, including the nucleotide side-chain terminating in 5,6-dimethylbenzimidazole (usually coordinated to the Co), as in B₁₂ (cyanocobalamin); Cbi, cobinamide, differs from Cbl in lacking the aminopropanol and nucleotide on side-chain f; DTT, dithiothreitol; ME, 2-mercaptoethanol; RSH, any thiol; TG, thioglycolic acid.

‡ Me-Cbi was identified as the product from reaction at pH 6 by TLC in two solvent systems (II and III of ref. 20) against a sample prepared from Co^I as well as from its UV-VIS spectrum.

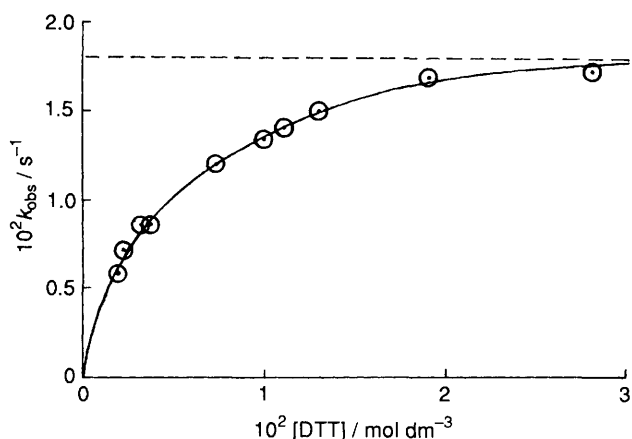


Fig. 1 Variation of the observed pseudo-first-order rate constant (k_{obs}) with the concentration of added DTT. For experimental conditions see text.

increasing small changes in the spectrum (fall in the max. at 475 nm and shift in the small max. from 405 to ca. 400 nm) which give an approximate value of the equilibrium constant K ca. $2 \times 10^2 \text{ dm}^3 \text{ mol}^{-1}$ in agreement with the kinetically determined value of 2.5×10^2 ; analogous $\text{Co}^{\text{II}}\text{-RSH}$ complexes are already known.¹⁸ The occurrence of side-reactions at $<10^{-2} \text{ mol dm}^{-3}$ MeI prevented the determination of any realistic equilibrium constant for adduct formation with MeI (but clearly $K > 10^2 \text{ dm}^3 \text{ mol}^{-1}$).

Our results establish methylation of $\text{Co}^{\text{II}}\text{-Cbi}$ by MeI in the presence of RSH as a third pathway distinct from those which involve reaction with $\text{Co}^{\text{I}5}$ [and would require a HO^- -dependent rate of reduction in accordance with eqn. (1)] and with Co^{II} alone (second order in Co^{II} concentration with $\text{Cbl} > \text{Cbi}$).¹³ They suggest that the third pathway involves reaction within some adduct of the $[\text{RSH}\text{-Co}^{\text{II}}]$ complex with MeI; the immediate products are presumably Me-Cbi and either $\text{I}^- + \text{RSH}^+ (\rightarrow \text{RS}^{\cdot} + \text{H}^+)$ or $\text{I}^{\cdot} + \text{RSH}$. The presence of a coordinated base explains the absence of any significant analogous reaction with $\text{Co}^{\text{II}}\text{-Cbl}$; the reactions reported⁶ for the Cbls were, for reasons not given, carried out at pH 2.5 where the base is protonated and displaced from coordination.³ These results underline the remarkable ability of the low-spin Co ion to form σ M-C bonds, which is probably associated with the low $3d\text{-}4s/4p$ promotion energy required

to allow good M-C orbital overlap.⁹ They also suggest the intriguing possibility that today's complex but efficient methyl transfer enzymes based on Co^{I} , which possess an unusual mechanism to generate the low potential required to form Co^{I} ,¹⁹ could have evolved from simpler but less efficient enzymes based on $\text{Co}^{\text{II}} + \text{RSH}$.

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