

Methyl Transfer from Methylcobalamin to Thiols. A Reinvestigation[†]

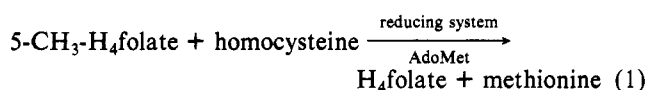
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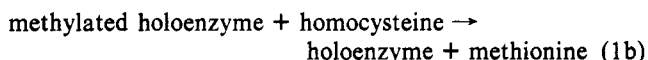
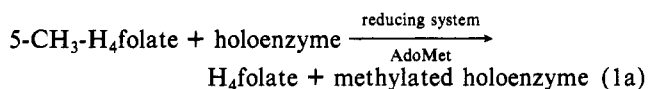
ABSTRACT: The methyl transfer from methylcobalamin to thiols has been reinvestigated. By use of methylcobalamin selectively enriched with ¹³C in the methyl moiety, the methyl transfer to thiols was followed by ¹³C NMR. The methyl transfer occurs in aqueous mildly alkaline (pH 8–12) solution, even in the complete absence of oxygen. ³¹P NMR and EPR studies demonstrate that cob(II)alamin is the final corrinoid product. However, the pH dependence of the methyl-transfer reaction from methylcobalamin to β-mercaptoethanol is consistent only with a nucleophilic displacement of the methyl group by a thiolate anion, resulting in the heterolytic cleavage of the carbon–cobalt bond. Difference visible spectroscopic measurements of the reaction mixture suggest that cob(I)alamin is formed as an intermediate.

Methionine synthetase (5-methyltetrahydropteroyl-L-glutamate:L-homocysteine S-methyltransferase, EC 2.1.1.13) is a cobalamin-dependent enzyme that catalyzes the methyl transfer from 5-methyltetrahydrofolate (5-methyl-H₄folate) to homocysteine (Taylor, 1982; Matthews, 1984).



Reaction 1 requires a reducing system such as FMNH₂ and dithiothreitol and S-adenosylmethionine as a cofactor.

Taylor & Weissbach (1967, 1968) demonstrated that the cobalamin–enzyme complex is methylated when [5-¹⁴C]-methyl-H₄folate is incubated with a stoichiometric amount of holoenzyme, a reducing system, and AdoMet but without homocysteine. They further showed that the labeled holoenzyme is able to transfer the methyl group to homocysteine. These observations clearly indicate that reaction 1 consists of two half-reactions (1a and 1b).



The methylated holoenzyme was shown to be a methylcobalamin–protein complex, and thus, the first half-reaction involves the transfer of the methyl group from 5-CH₃-H₄folate to the cobalt atom of the cobalamin. In the second half-reaction the methyl group of methylcobalamin is transferred to the thiol of homocysteine. Indeed, Guest and co-workers (1962) found that preparations of the holoenzyme that catalyze reaction 1 also catalyze the transfer of the methyl group from methylcobalamin to homocysteine. Taylor & Weissbach (1966) demonstrated that methyl iodide is able to replace AdoMet in reactions 1 and 1a and suggested that the holoenzyme is inactive until it is primed by reductive methylation (reducing system and AdoMet).

More than 20 years ago, Guest and co-workers (1962) showed that methyl transfer from methylcobalamin to ho-

mocysteine occurs slowly in the absence of methionine synthetase.

Johnson and co-workers (1963) reported that methionine is formed when methylcobalamin is photolyzed in the presence of homocysteine and suggested that a similar enzyme-catalyzed homolysis of the carbon–cobalt bond is not “improbable”. Schrauzer (1968) first postulated that the cobalamin-dependent methionine synthesis involves a nucleophilic attack of a thiolate anion on the carbon–cobalt bond to generate the methyl thioether and cob(I)alamin. Schrauzer and co-workers (1967, 1968) demonstrated the formation of methionine from methylcobaloxime and homocysteine in alkaline methanol. A radical mechanism was eliminated because they observed that methyl radicals generated in the presence of thiols gave primarily methane. These conclusions were questioned by several laboratories. Agnes and co-workers (1971) were unable to observe methyl transfer from methylcobalamin to thiols under strictly anaerobic conditions. Methyl transfer was rapid only in the presence of oxidizing agents, and they concluded that the reaction involves a one-electron oxidation attack on methylcobalamin and not a direct reaction with either thiolate anion or thiol. Frick et al. (1976) suggested that the demethylation of methylcobalamin by coenzyme M involves a thiol-promoted homolytic cleavage of the carbon–cobalt bond to cob(II)alamin and the thioether. They also found that the rate of demethylation was very slow under strictly anaerobic conditions, that it was enhanced in the presence of oxygen, and that the reaction rate was not affected by pH in the range 7.0–14.2. In contrast, Brown & Kallen (1972) were unable to detect any methyl thioether or methane as products of the reaction between methyl(aquo)cobaloxime and mercaptoethanol or mercaptoacetate. Their observations indicated that the reaction of these thiols with methyl(aquo)cobaloxime involves only axial ligation and not carbon–cobalt bond cleavage. In a subsequent publication, Schrauzer & Stadlbauer (1974) repeated their earlier experiments in methanol and in aqueous solution and concluded that methylation of mercaptide ions by alkylcobalamins and reactive alkylcobaloximes does occur “provided that the concentration of NaOH is between 0.1 and 1 M and thiol is present in considerable excess to assure high concentrations of thiolate anion”.

In an attempt to reconcile these divergent conclusions from well-established laboratories, we have reinvestigated the methyl transfer from methylcobalamin to thiols using several spectroscopic techniques. Our results demonstrate that methyl

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