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Model Studies of the Cobalamin-Dependent Methionine Synthase Reaction

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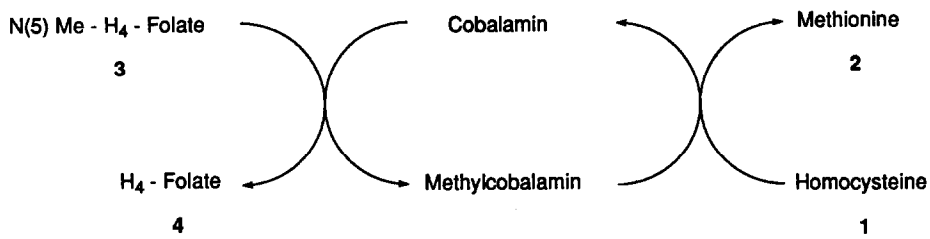
Abstract *: The cobalt atom of cobaloxime(I) and cobalamin(I) is methylated by a 5-methylpterinium salt acting as a model of the activated 5-methyltetrahydrofolate cofactor.*

Methionine synthases catalyze the conversion of homocysteine (**1**) to methionine (**2**) [Scheme I][2]. In this reaction, the source of the methyl group is the cofactor 5-methyltetrahydrofolate (**3**), which is concomitantly transformed to tetrahydrofolate (**4**). The enzymes found in microorganisms and mammals utilize, in addition to the cofactor **3**, the cobalamin system, which, acting as a shuttle, takes up the methyl group from the cofactor and delivers it to the substrate homocysteine. The overall process involves two steps: (i) transfer of the methyl moiety of **3** to the cobalt atom of cobalamin and (ii) donation of the cobalt-bound CH_3-unit of methylcobalamin to the thiol residue of **1**. While the nonenzymatic transfer of the methyl group from cobalamin to homocysteine has been reported[3a,b], there is a lack of a chemical precedent for the first step of the reaction. This communication describes a nonenzymatic transfer of the methyl group from a 5-methyl- tetrahydrofolate model to cobaloxime(I) and cobalamin(I).



Scheme I

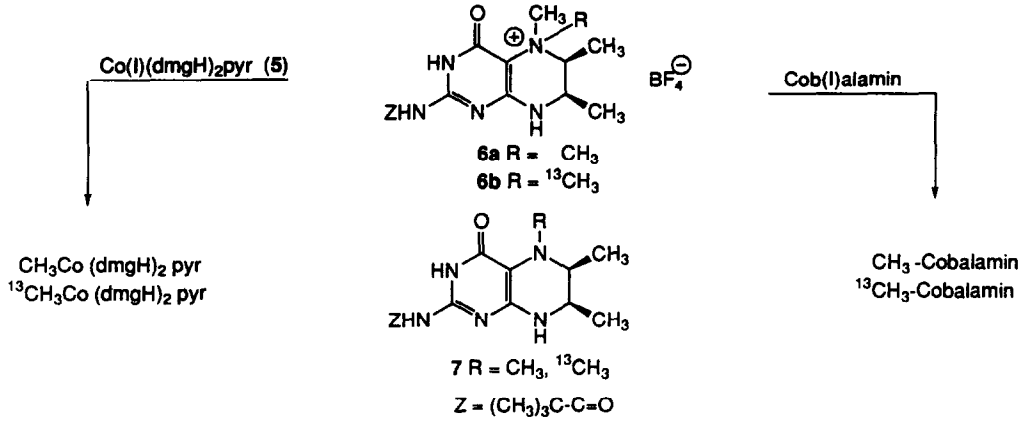
The first step of the cobalamin mediated methionine synthase reaction is written in general terms as a displacement of the tetrahydropteridine moiety by attack of Co(I) on the N(5)-methyl carbon of **3**. Since such a nucleophilic displacement of a secondary amine anion from a neutral tertiary amine is an unprecedented process, it has been suggested that the N(5)-position of the cofactor (**3**) is activated for the process, either via oxidation or by coordination with an electrophile/proton in the enzymic site[4]. Attempted alkyl transfers from a number of tertiary amines and quaternary salts to cobalamin or cobaloxime have not been successful[5]. One report, however, describes the transfer - to cobaloxime(I) - of a benzyl group from benzyltrimethylammonium iodide and claims a methyl transfer from an *in situ* generated ammonium species[6]. In order to develop a better insight into the mechanism of transfer of alkyl groups from ammonium salts to Co(I), we have investigated the reaction of a range of ammonium salts[7] with cobaloxime(I) **[Co(I)(dmgH)$_2$pyr] 5**. The reactions were carried out by allowing a (1:1) mixture of **5**, generated by basic disproportionation of Co(II), and the substrate salt, in

Table

Transfer of alkyl groups from quaternary ammonium salts to 5

Entry	Salt	Group Transferred	$RCo(dmgH)_2pyr$ Yield
1.	$PhN^+Me_3I^-$	Me	47%
2.	$PhCH_2N^+Me_3Cl^-$	$PhCH_2$	58%
3.	$p\text{-}MeOPhCH_2N^+Me_3Cl^-$	$p\text{-}MeOPhCH_2$	41%
4.	$p\text{-}MeOPhCH_2N^+Me_2CH_2PhCl^-$	$p\text{-}MeOPhCH_2$	47%
		$PhCH_2$	20%
5.	$p\text{-}NO_2PhCH_2N^+Me_3Cl^-$	--	--
6.	$p\text{-}NO_2PhCH_2N^+Me_2CH_2PhBr^-$	--	--
7.	$[DABCO]^+MeCl^-$	--	--
8.	$[DABCO]^+CH_2PhCl^-$	$PhCH_2$	54%
9.	$[DABCO]^+CH_2Ph(p\text{-}MeO)Cl^-$	$p\text{-}MeOPhCH_2$	52%
10.	$[DABCO]^+CH_2Ph(p\text{-}NO_2)Cl^-$	--	--
11.	$[DABCO]^+CHPh_2Br^-$	--	--
12.	$(CH_2)_5N^+MeCH_2PhBF_4^-$	$PhCH_2$	47%
13.	$(CH_2)_5N^+Me_2I^-$	--	--

DABCO = 1,4-diazabicyclo[2,2,2]octane



Scheme II

methanol, to stand (4 - 16 h) at room temperature, under strictly anaerobic conditions. The cobalt derivatives were isolated and identified by comparison (NMR) with authentic samples. The results of the reactions of **5** with these salts are described in the Table .

The results presented in the Table show the following trends. A methyl group is effectively transferred from the trimethylanilinium salt to the cobalt atom of **5** (entry 1). A benzyl group in an ammonium salt is transferred in preference to a similarly situated methyl substituent (compare entries 7 with 8 and 12 with 13). The *p*-methoxybenzyl substituent shows a better aptitude to transfer than the unsubstituted benzyl group (entries 3 and 4). The lack of reactivity of the benzhydryl salt (entry 11) is highly significant. It stresses the role of steric features, implying a mechanism in which the reaction is strongly inhibited by the presence of bulky groups. It also reveals, albeit indirectly, that mechanisms involving a carbenium ion (Ph_2CH^+) or a radical ($Ph_2CH\cdot$), which would be more stable than the corresponding benzyl intermediates, cannot be operative. In view of this, the results, except in the case of the *p*-nitrobenzyl salts, can be rationalized on the basis of a nucleophilic displacement reaction. The *p*-nitrobenzyl salts, where no transfer is observed (entries 5, 6 and 10), constitute an exception. In the first instance, it seems puzzling that the electron-withdrawing nitro group totally suppresses the reactivity towards the S_N2 substitution by the Co(I)-nucleophile. However, it should be noted that the benzyl protons of the ammonium salt substrates in entries 5, 6 and 10 are unusually acidic due to the combined effect of the charge on the adjacent nitrogen and the electron-withdrawing *p*-nitro substituent. This high acidity is evidenced by the ^{1}H-NMR spectra of the DABCO salts; the methylene protons of the benzyl, *p*-methoxybenzyl and *p*-nitrobenzyl substituents, being observed at d 5.09, 4.97 and 5.38, respectively.

While Co(I) of cobaloxime is an extremely powerful nucleophile (n = 13.8)[8,9] and is expected to exhibit nucleophilic properties dominantly; it is quite conceivable that towards the benzyl protons of the *p*-nitro- benzyl salts, Co(I) acts as a "base". Experimental support for this was seen in the fact that cobaloxime(I) **5**, generated by standard procedures and recognized by its characteristic colour, was quenched instantaneously upon addition of the nitrobenzyl salts. In case of entry 10, the presence of the starting *p*-nitrobenzyl salt could be demonstrated after quenching of the reaction mixture. These observations make it unlikely that an electron-transfer from Co(I) to the *p*-nitrophenyl substituent is playing a role. Furthermore, it has been shown in this laboratory[1], that in the reactions of the same *p*-nitrobenzyl salts with thiolate anions, where electron-transfer is involved, very significant transfer of the *p*-nitrobenzyl substituent to the thiolate sulphur takes place. It is also pertinent to draw attention to the fact that in entry 6, none of the expected transfer of the unsubstituted benzyl group was observed. The aforementioned observations and the results described in the Table lead us to regard the transfer of an alkyl substituent from an ammonium salt, to Co(I), as a nucleophilic substitution process, in which the nucleophilic cobalt displaces a tertiary amine, from the relevant carbon.

In order to develop a model of 5-methyltetrahydrofolate **3**, for the methyl transfer to cobalamin(I), we selected pterinium salt **6a**[7] (Scheme II), as a cofactor analogue, in which the MeN(5)-position is activated by coordination with a methyl group. One of the N-methyl groups of **6a** was labelled with ^{13}C, to give the salt **6b**[7], in which the fate of the methyl transfer to the Co(I) could be followed by ^{13}C-NMR; without recourse to isolation of the reaction products. It should be noted that salt **6b** consists of a mixture of two "isotopomers"[7,10] which can be distinguished by their NMR spectra.

Reactions of **6a,b** with, *in situ* generated, cobaloxime(I) at 293 K or 313 K, resulted in reaction mixtures in which the formation of the Co-$^{12}CH_3$ and Co-$^{13}CH_3$ derivatives of **5** could be readily identified by NMR. In the reaction with **6a**, a signal at d 0.81 corresponded to the methyl transfer product, while in the case of the reaction with **6b**, both labelled and unlabelled methylcobaloximes were formed. This reflects the fact that **6b** can donate either of the two (labelled or unlabelled) methyl groups. The unlabelled product again showed the expected signal at d 0.81,whereas the Co-$^{13}CH_3$ appeared as a doublet (J = 139.3) of which one peak at d 0.47 is distinctly visible, while the second one falls under other signals. The reaction of **6b** with freshly generated cobalamin(I) in a thoroughly degassed solution, was carried out in the dark, [NaOH, EtOH/H_2O, 293 K] at pH 9.3. After the colour change (to red) had indicated the formation of Co(III), the reaction mixture was frozen, lyophilized and the residue dissolved in D_2O and subjected to ^{13}C-NMR spectral analysis. The most significant signal was that at d 10.6, which can be assigned to the Co-$^{13}CH_3$ group, by comparison with the spectrum of an

authentic sample of methylcobalamin. Signals were also observed for the unreacted salt **6b** (d 58.7 and 55.8, 2 x N^+-CH_3, due to the two isotopomers) and its demethylated pterin product **7** (d 47.0, N-Me). These results constitute the first chemical precedent for the initial half of the cobalamin-dependent methionine synthase reaction.

REFERENCES AND NOTES

* To whom enquiries may be sent.

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