A Model of the Cobalamin-independent Methionine Synthase Reaction[1]

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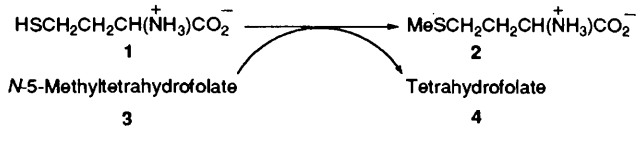
Homocysteine is converted to methionine *via* a nonenzymatic methyl transfer from a 5-methyltetrahydrofolate model bearing a positive charge at N(5).

The group of enzymes called methionine synthases catalyse the conversion of homocysteine **1** to methionine **2** utilizing 5-methyltetrahydrofolate **3** as an essential cofactor.[2] The overall chemical reaction involves the transfer of a methyl group from the cofactor to the thiol group of the substrate, with the concomitant formation of methionine **2** and tetrahydrofolate **4**. One class of methionine synthases, found in fungi and higher plants, causes the direct transfer of the methyl group (Scheme 1), while the second class of enzymes requires cobalamin as an intermediary methyl-carrier, taking the methyl group over from 5-methyltetrahydrofolate and delivering it to homocysteine.[2] In this communication we describe a nonenzymatic model of the reaction mediated by cobalamin-independent methionine synthase.

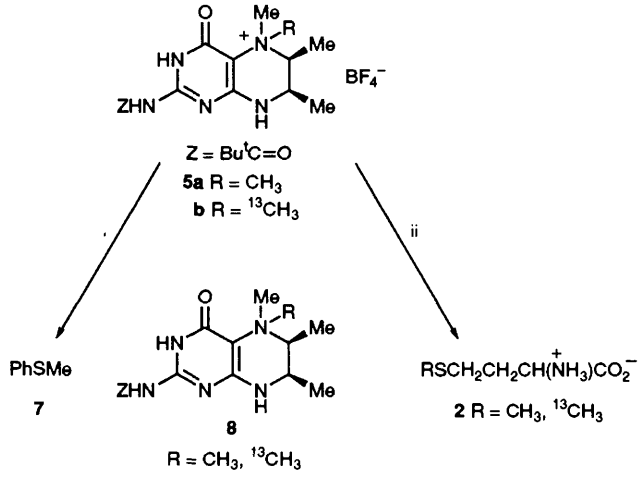
The direct transfer of the methyl group of cofactor **3** constitutes an overall nucleophilic displacement of a secondary amine by the thiolate residue of homocysteine **1**. Since the secondary amine anion corresponding to the tetrahydropterin moiety of **4** would be a poor leaving group, it has been suggested that the N(5) is activated either by oxidation, or *via* coordination with an electrophilic species in the active site of the enzyme.[3] It should be emphasized that thus far no redox (cofactor) system has been identified in association with the enzyme,[3] though the involvement of cysteine thiol residues, as functional redox centres cannot be excluded.

In order to develop a chemical precedent for the transfer of a methyl group from a (quaternized) nitrogen corresponding to N(5) of cofactor **3**, to thiols in general and homocysteine specifically, we have investigated the reactions of pterin salts **5a,b**,† with potassium thiophenolate **6** and the salt of homocysteine (Scheme 2).

Reaction of **5a** (1 equiv.) with freshly prepared potassium salt of **6** (2.2 equiv.), [18-crown-6 (1 equiv.), acetonitrile, 343 K, 24 h] resulted in a reaction mixture from which PhSMe **7** could be isolated and identified (^{1}H NMR, 200 MHz, $CDCl_3$, δ 2.49, s, PhSMe). The residue showed the presence of the demethylated pterin **8**, which was attested by signals at δ 2.55 (s, N*Me*) and 0.77 [d, *J* 6.7 Hz, C(6)-Me or C(7)-Me].



Scheme 1



Scheme 2 *Reagents*: i, $K^+{}^-SPh$, **6**; ii, $^-SCH_2CH_2CH(^+NH_3)CO_2^-$, **1**

† The synthesis of these salts will be described in detail elsewhere.

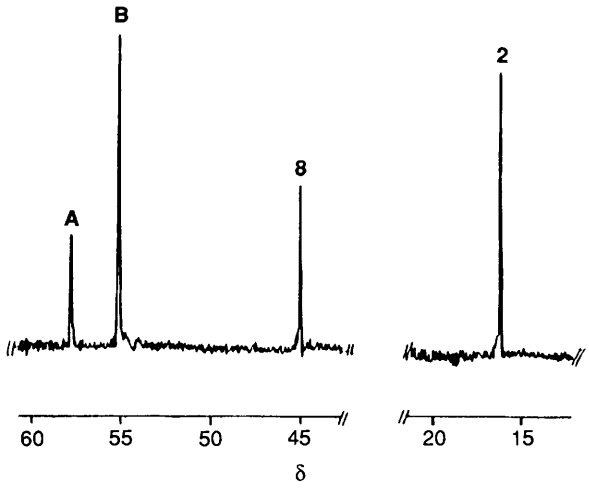


Fig. 1

HPLC analysis of the reaction mixture showed the formation of the thio ether **7** in 57% yield.‡ It should be pointed out that thio ether **7** is a volatile product, so that its actual yield is presumably higher than the observed value. These results, consequently, reflect a very substantial amount of methyl transfer.

When homocysteine **1** (2 equiv.) was employed as the substrate, reaction with the salt **5a** (1 equiv.), NaOH (4 equiv.), in ethanol–water 10 : 1 resulted in a mixture whose ^{1}H NMR spectrum (200 MHz, 0.1 mol dm^{-3} NaOD/D_2O) showed clearly recognizable signals for **2** (δ 2.10, s, S*Me*), **8** [0.75, d, *J* 6.7 Hz, C(6)-Me or C(7)-Me] and the disulfide corresponding to **1**. From the integration of these signals, a 40% transfer of the methyl group from the salt could be estimated. It is noteworthy that the intensities of the signals for **2** and **8** gave a ratio of 1 : 1 for the two products.

The transfer of the methyl group from the pterin salt **5a** to homocysteine **1** was further confirmed by the synthesis of ^{13}C-labelled salt **5b** (which consists of a mixture of two 'isotopomers'§ **A** and **B**) and studying its reaction with **1**, under the previously described conditions. The ^{13}C spectrum (50.32 MHz, 0.1 mol dm^{-3} NaOD/D_2O) of the reaction mixture (Fig. 1) contained only four signals, which were highly informative and significant. The ^{13}C signals at δ 57.7 and 55.0 originate from the unreacted isotopomers (**A** and **B**) of salt **5b**; the signal at δ 45.0 represents the demethylation product of the salt, namely **8**, and finally the signal at δ 16.0 attested to the formation of methionine.¶ These results unambiguously demonstrate that a labelled methyl group had been transferred from **5b** to **1** with the formation of methionine **2**.

Concerning the mechanistic details of the substitution reaction of ammonium salts by thiols, it should be noted that a study of a series of structurally designed salts[4] suggests the involvement of an electron transfer step and radical intermediates. This aspect of the investigation will be presented elsewhere.

The results of the present model studies support a mechanism of the cobalamin-independent methionine synthase reaction, in which a positively charged N(5)-pterin moiety of cofactor **3** (5-methyltetrahydrofolate) delivers its methyl group to the thiol residue of homocysteine.

Received, 22nd February 1993; Com. 3/01053H

References

1 Taken in part from the forthcoming doctorate thesis of E. Hilhorst, University of Amsterdam.
2 R. G. Matthews, in *Folates and Pterins*, eds. R. L. Blakeley and S. J. Benkovic, Wiley, 1984, vol. 1, ch. 13, pp. 497–553.
3 R. G. Matthews and J. T. Drummond, *Chem. Rev.*, 1990, **90**, 1275.
4 Unpublished results from this laboratory.

‡ Using an authentic sample of PhSMe **7**, a standard plot was prepared (column: Polygosil 60 C 18; 250 mm, i.d. 4 mm, 10 μm; 80 : 20 MeOH–H_2O; 1.5 cm^3 min^{-1}; λ 254 nm).

§ The term 'isotopomers' has been suggested in (an as yet unpublished) draft 8 of the IUPAC document 'Basic Terminology of Stereochemistry'. Personal communication, Dr L. Maat, Delft Technical University. The two isotopomers can be distinguished by the ^{1}H and ^{13}C NMR spectra of the salt.

¶ Compared with an authentic sample of a similarly labelled methionine.