Stereochemical analysis of the methyl transfer catalyzed by cobalamin-dependent methionine synthase from E. coli B

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is prohibitive[17] (theoretical calculations[18] place barriers between 62 and ~80 kcal mol^{-1} on this transformation), the strained nature of **1** suggests that it should be able to undergo ring opening to the triyne. Indeed, while **1b** is stable in the neat state to 400 °C (10 min, 50% recovery), flash pyrolysis (700 °C, 10^{-2} mm) leads to the desired isomerization (40%; clearly identifiable by spectral data, paticularly ^{13}C NMR[20]). This retrocyclization is the first which unravels a benzene ring to its three-component alkyne units in a purely thermal process without additional reagents which would be normally required to overcome thermodynamic obstacles.[19] Our observations lend support to the contention that **1b** may have been an intermediate in the flash-thermolytical decomposition of a tricinnoline to the same triyne.[20]

With **1b** and two of its derivatives at hand, a detailed investigation of their physical, chemical, and physiological properties will be the subject of future efforts.

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Supplementary Material Available: A listing of positional and thermal parameters and tables of bond lengths and angles and structure factors for **1a** (45 pages). Ordering information is given on any current masthead page.

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Sterochemical Analysis of the Methyl Transfer Catalyzed by Cobalamin-Dependent Methionine Synthase from *Escherichia coli* B

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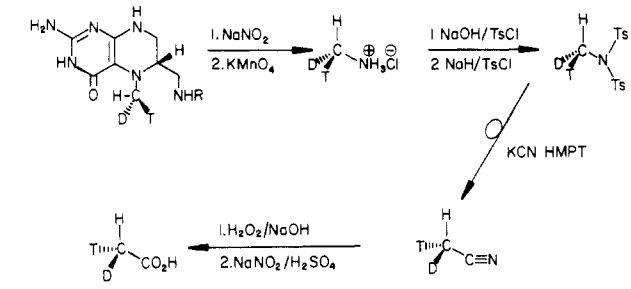
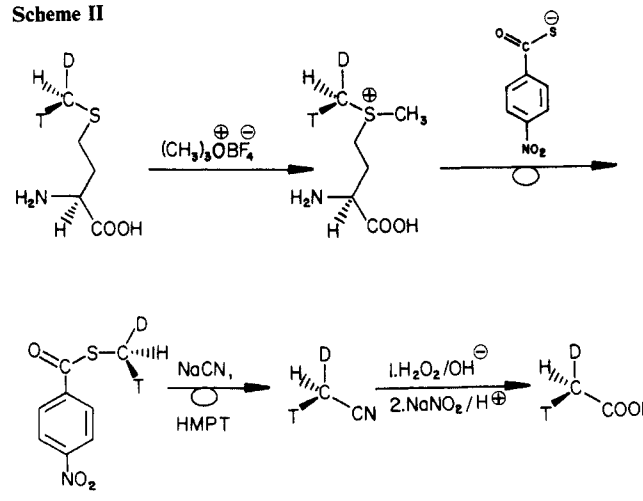
The last step in the biosynthesis of methionine involves transfer of the methyl group of N_5-methyltetrahydrofolate (5-CH_3-H_4-folate) or its polyglutamate analogue to the sulfur of homocysteine:

5-CH_3-H_4folate + $HSCH_2CH_2CH(NH_2)COOH$ → $CH_3SCH_2CH_2CH(NH_2)COOH$ + H_4folate

Scheme I



Scheme II



Two classes of methionine synthases are known to catalyze this reaction, one contains cobalamin as a cofactor and the other is cobalamin-independent.[1] The first class is found in mammalian tissues and the second class in plants, and microorganisms may have either one or both enzymes.

To further our understanding of the catalytic mechanism of methionine synthases, we studied the steric course of the methyl transfer from 5-CH_3-H_4folate to homocysteine catalyzed by the cobalamin-dependent enzyme from *E. coli*. The requsite substrate, 5-CH_3-H_4folate, carrying a chiral methyl group, was prepared by sequential reduction of 5,10-methenyltetrahydrofolate (5,10-CH^+-H_4folate) with NaB^2H_4 in the presence of diethylaniline, known to produce monodeuterated 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4folate) with over 80% stereoselectivity,[2] followed by further reduction of the 5,10-CH_2-H_4folate with a 2-fold excess of tritiated sodium borohydride in 50 mM Tris buffer, pH 7.5[3] (Scheme III). The diastereomer of opposite methyl configuration was generated from 5,10-CH_2-H_4folate produced by $NaBH_4$ reduction of 11-deuterio-5,10-CH^+-H_4folate. The 5-CH_3-H_4folate was purified by passage over a column of DEAE Sephadex and elution with a linear gradient of 0.2–2.0 M triethylammonium bicarbonate. Fractions containing 5-CH_3-H_4folate were pooled and concentrated by lyophilization. The two samples of 5-CH_3-H_4folate were degraded by diazotation and $KMnO_4$ oxidation to give methylamine[4,5] which by standard procedures[6] was converted stereospecifically to acetic acid for chirality analysis[7-9] of

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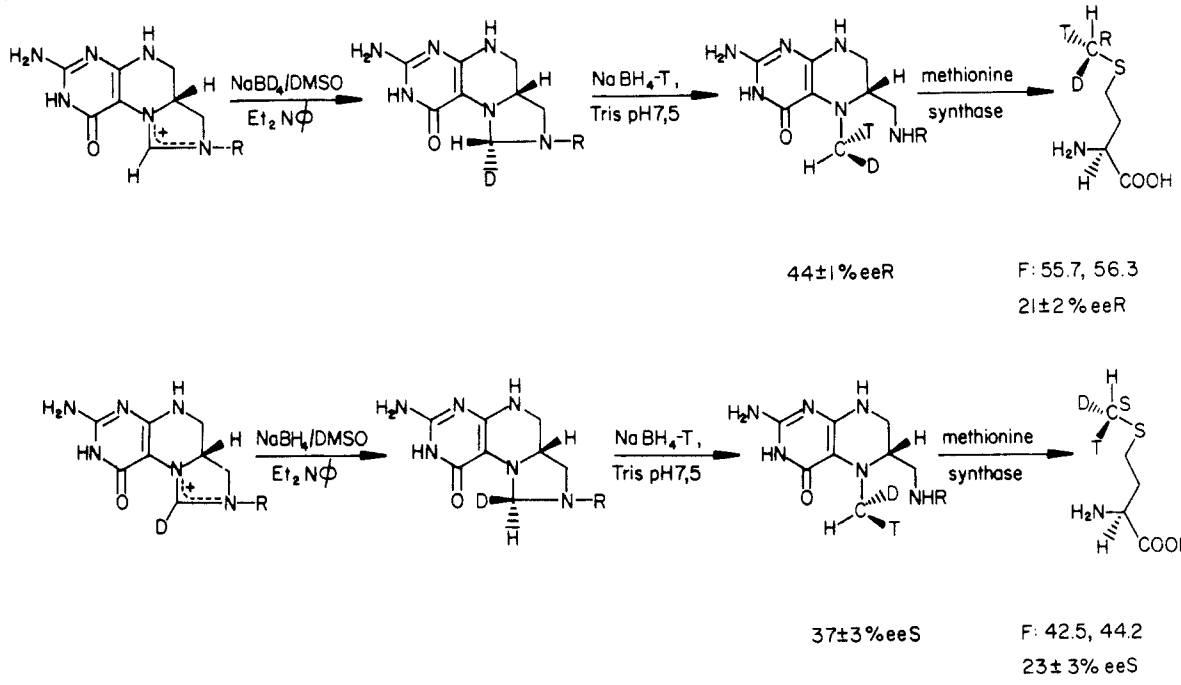
Scheme III



the methyl group (Scheme I). *F* values[9] of 37.2 and 37.5 (duplicate analyses) for the acetate from the first sample and 59.8 and 61.4 from the second indicated predominant *S* and *R* configuration, respectively, of the two acetate specimens. Since the degradation sequence involves one inversion of configuration in the cyanide displacement step to give acetonitrile, it follows that the first and the second 5-CH_3-H_4folate sample contained 44 ± 1% ee *R* and 37 ± 3% ee *S* isomer, respectively (Scheme III). Hence the reduction of 5,10-CH_2-H_4folate had occurred stereoselectively, exhibiting a 2–3-fold preference for attack syn to C_6-H of the 5,10-CH_2-H_4folate molecule.

Samples of the chiral methyl-*R* and methyl-*S* 5-CH_3-H_4folate were then incubated with purified cobalamin-dependent methionine synthase from *E. coli*[10] in 0.1 M potassium phosphate buffer, pH 7.2, in the presence of 25 mM dithiothreitol, 50 μM aquocobalamin, 0.5 mM homocysteine, and 19 μM *S*-adenosylmethionine to give methionine in about 80% yield. Methionine was separated from residual 5-CH_3-H_4folate by passage over a column of AG1X8 (Bio-Rad) and then purified by HPLC on an ODS column equilibrated with 0.1 M ammonium acetate, pH 3.55. Methionine eluted at 5–7 min,[11] and residual ammonium acetate was removed by lyophilization. The purified methionine samples were degraded by the procedure of Arigoni and coworkers[12,13] (Scheme II) to recover the methyl group as acetate for chirality analysis. This degradation sequence involves two inversions of the methyl group configuration, one in the displacement of methyl from *S*-methylmethionine by *p*-nitrothiobenzoate anion and another in the reaction of methyl *p*-nitrothiobenzoate with cyanide to give acetonitrile. Thus, the configuration of the acetate from the degradation directly reflects that of the methionine methyl group. *F* values of 42.5 and 44.2 (duplicate experiments) for the material derived from methyl-*S*-5-CH_3-H_4folate and 56.3 and 55.7 for that from methyl-*R*-5-CH_3-H_4folate indicated that the product of the enzyme reaction contained methyl groups of predominantly the same configuration as the substrate (Scheme III).

The results demonstrate that the cobalamin-dependent methionine synthase from *E. coli* transfers the methyl group of 5-CH_3-H_4folate stereoselectively to the sulfur of homocysteine to generate methionine with net retention of configuration. The observed steric course is consistent with the postulated mechanism of the reaction, which invokes two sequential transfers of the methyl group, one from 5-CH_3-H_4folate to cobalt to generate enzyme-bound methylcobalamin and a second from cobalt to sulfur to produce methionine. There is a substantial decrease in the chiral purity of the methyl group during the overall process. Since the degradation procedures for both CH_3-H_4folate and methionine are highly stereospecific,[4,5,12,13] it follows that the enzymatic methyl transfer is accompanied by nearly 50% racemization. This observation may provide a clue for the further mechanistic analysis of the reaction catalyzed by cobalamin-dependent methionine synthase.

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β-Eliminations in Isonitriles

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The physical organic chemistry of the nitrile group has been the subject of intensive investigations[1] which have shown that is

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