

## Stereochemical Course of Methyl Transfer from Methanol to Methyl Coenzyme M in Cell-Free Extracts of *Methanosarcina barkeri*

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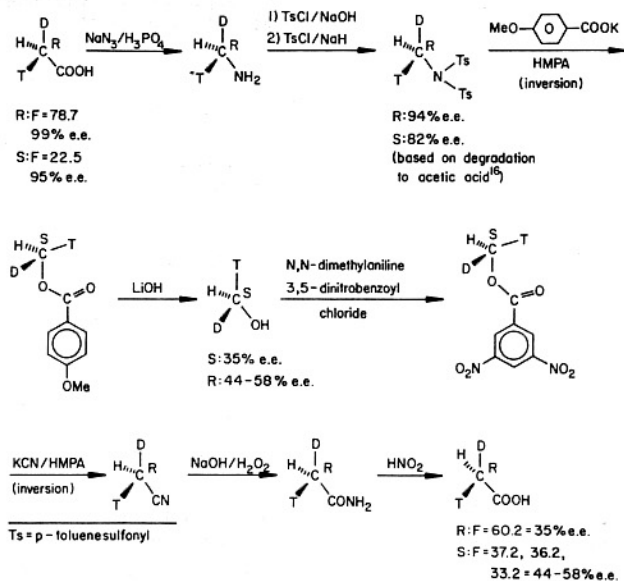
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The majority of methanogens produce methane from CO<sub>2</sub> and H<sub>2</sub>;<sup>1,2</sup> however, some species, such as *Methanosarcina barkeri*, can utilize methanol, methylamines, or acetic acid to produce methane and cellular carbon compounds.<sup>3,4</sup> The conversion of methanol to methane was originally thought to involve transfer of the methyl group to the cobalt of vitamin B<sub>12</sub> followed by reduction of the resulting methylcobalamin.<sup>5,6</sup> However, more recent work, following the discovery of coenzyme M as a methyl carrier in methanogens,<sup>7</sup> points to the involvement of two methyltransferases, MT<sub>1</sub> and MT<sub>2</sub>, which convert CH<sub>3</sub>OH to CH<sub>3</sub>-SCoM,<sup>8</sup> followed by reduction of the latter to methane by methylreductase.<sup>9</sup> MT<sub>1</sub> is a corrinoid enzyme which requires reductive activation,<sup>10</sup> and MT<sub>2</sub> can transfer the methyl group from a free or bound methylated corrin to coenzyme M. The conversion of CH<sub>3</sub>OH to CH<sub>3</sub>-SCoM, therefore, appears to involve two sequential transfers of the methyl group, each presumably occurring with inversion of configuration,<sup>11</sup> predicting that the overall reaction proceeds with net retention of methyl group configuration. Alternate reaction sequences or mechanisms, e.g., free-radical intermediates, might result in opposite stereochemistry or in significant degrees of racemization.

To probe the steric course of methyl coenzyme M formation we synthesized (*R*)- and (*S*)-[<sup>2</sup>H,<sup>3</sup>H]methanol from (*S*)- and (*R*)-[<sup>2</sup>H,<sup>3</sup>H]acetic acid<sup>14</sup> as shown in Scheme I. Samples of the methanol and the intermediate methyliditosylimide were degraded to acetic acid<sup>15,16</sup> to determine their chiral purity by using the chirality analysis method of Cornforth et al.<sup>17</sup> and Arigoni and co-workers.<sup>18</sup> It is evident from the data in Scheme I that the conversion of methyliditosylimide to methanol was accompanied by substantial racemization, probably due to displacement of the methyl group from methyl *p*-methoxybenzoate by the *p*-meth-

### Scheme I. Synthesis and Configurational Analysis of Chiral Methanol



oxybenzoate anion as a side reaction. Nevertheless the chiral purity of the methanol was sufficient for the following experiment.

Samples of the chiral methanol (R: 9.32·10<sup>6</sup> and 1.15·10<sup>7</sup> dpm; S: 2.1·10<sup>7</sup> dpm) were converted to methyl coenzyme M in a cell-free extract obtained from *Methanosarcina barkeri* strain 227 cells grown on methanol.<sup>19,20</sup> The extract was activated by preincubation with 7.5 mM ATP and 15 mM MgCl<sub>2</sub> in 20 mM TES buffer, pH 7.2, for 15 min at 37 °C under 40 psi hydrogen pressure<sup>10</sup> and then incubated with the chiral methanol (0.5–1 mM), 2 mM dithiothreitol, 18 mM coenzyme M, and 0.25 mM bromoethanesulfonic acid (to inhibit methyl reductase) for 5–6 h at 37 °C under 40 psi H<sub>2</sub>. Labeled methyl coenzyme M was isolated from the reaction mixture, in 30–40% yield, by passage through an AG 50 W-X8 (H<sup>+</sup>) column and subsequent TLC on cellulose plates (MeOH/1,3-dioxolane/NH<sub>4</sub>OH/H<sub>2</sub>O 3:6:1:1, R<sub>f</sub> 0.72).<sup>22</sup> The samples plus nonlabeled carrier material were degraded, as shown in Scheme II,<sup>12</sup> to recover the methyl group as acetic acid for chirality analysis. This degradation procedure involves two S<sub>N</sub>2 displacements at the methyl group, and hence the configuration of the acetic acid corresponds<sup>23</sup> directly to that of the methyl coenzyme M. As shown in Scheme II, methanol of 44–58% ee R configuration gave methyl coenzyme M which, upon degradation, produced acetic acid of 33–38% ee R configuration; the methyl coenzyme M from methanol of 35% ee S configuration gave acetic acid of 42% ee S configuration.

The results show clearly that the transformation of the methyl group of methanol into methyl coenzyme M proceeds with net retention of methyl group configuration and without significant racemization.<sup>24</sup> This is consistent with a mechanism proposed by Vogels and co-workers<sup>8</sup> in which the methyl group is transferred from methanol first to the cobalt of the corrinoid enzyme MT<sub>1</sub> and then to the sulfur of coenzyme M. This resembles the transfer

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(11) Studies on a substantial number of different methyltransferases have provided convincing evidence that single transfers of methyl groups by S<sub>N</sub>2 processes proceed with inversion of configuration.<sup>12,13</sup>

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(20) The cells were grown by using methanogen growth medium<sup>21</sup> under a 40% CO<sub>2</sub>:60% N<sub>2</sub> atmosphere with 100 mM methanol.

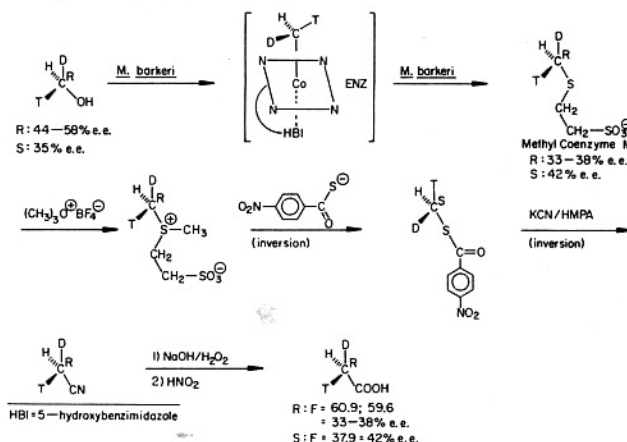
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(23) It was suggested by a perceptive reviewer that the methylsulfonium ion derived from methyl coenzyme M might rearrange to the methyl ester of methyl coenzyme M prior to displacement of the methyl group by the thioacid anion, adding one more inversion step to the sequence. This can be ruled out with a high probability, because such a process would lead to racemization unless the rearrangement were very fast relative to the displacement reaction. The sulfonium salt prepared in nonradioactive trial experiments was fully characterized and showed no tendency to undergo conversion to the methyl ester.

(24) In our hands<sup>25</sup> the chirality analysis of acetic acid is reproducible to ±2 F values of ±7% ee.

**Scheme II. Steric Course of the Enzymatic Synthesis of Methyl Coenzyme M from Methanol and Configurational Analysis of Methyl Coenzyme M**



of the methyl group of methyltetrahydrofolate to homocysteine, catalyzed by the  $B_{12}$ -dependent methionine synthase from *E. coli*, which we have demonstrated also occurs with net retention of methyl group configuration.<sup>26</sup> Both reactions pose the same question of how a relatively inert bond, the C–O bond of methanol in the present case or the C–N bond of methyltetrahydrofolate in the case of methionine synthase, is cleaved in the transfer of a methyl group.

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