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# Stimulation of the methyltetrahydromethanopterin: coenzyme M methyltransferase reaction in cell-free extracts of *Methanobacterium thermoautotrophicum* by the heterodisulfide of coenzyme M and 7-mercaptoheptanoylthreonine phosphate

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**Abstract.** The conversion of formaldehyde to methylcoenzyme M in cell-free extracts of *Methanobacterium thermoautotrophicum* was stimulated up to 10-fold by catalytic amounts of the heterodisulfide (CoM-S-S-HTP) of coenzyme M and 7-mercaptoheptanoylthreonine phosphate. The stimulation required the additional presence of ATP, also in catalytic concentrations. ATP and CoM-S-S-HTP were mutually stimulatory on the methylcoenzyme M formation and it was concluded that the compounds were both involved in the reductive activation of the methyltetrahydromethanopterin: coenzyme M methyltransferase. Micromolar concentrations of benzyl viologen or cyanocobalamin inhibited the formaldehyde conversion; these compounds, however, strongly stimulated the reduction of CoM-S-S-HTP. The results described here closely resemble observations made on the activation and reduction of $CO_2$ to formylmethanofuran indicating that this step and the reductive activation of the methyltransferase are controlled by some common mechanism.

**Key words:** Methanobacterium thermoautotrophicum – Coenzyme M – 7-Mercaptoheptanoylthreonine phosphate – 5-Methyltetrahydromethanopterin: coenzyme M methyltransferase – Corrinoid enzyme – Reductive activation

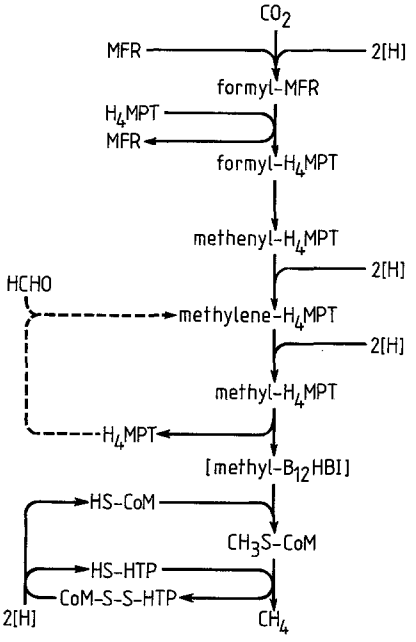


**Fig. 1.** Process of methanogenesis from $H_2$ and $CO_2$. The non-enzymatic synthesis of methylene-$H_4$MPT from formaldehyde is shown by a *dashed line*. *MFR*, methanofuran; *$H_4$MPT*, 5,6,7,8-tetrahydromethanopterin; *[$B_{12}$HBI]*, enzyme-bound 5-hydroxybenzimidazolyl cobamide; *HS-HTP*, 7-mercaptoheptanoylthreonine phosphate, *HS-CoM*, 2-mercaptoethanesulfonate

The process of methanogenesis from hydrogen and carbon dioxide is characterized by the participation of a set of unusual coenzymes (Fig. 1). Methanofuran (MFR), 5,6,7,8-tetrahydromethanopterin ($H_4$MPT) and coenzyme M (HS-CoM) have been shown to act as carriers of the $C_1$-moiety, derived from $CO_2$ (Keltjens and Van der Drift 1986; Rouvière and Wolfe 1988). The first step in methane production involves the reduction of $CO_2$ to the level of formate, resulting in the formation of formyl-MFR (Leigh et al. 1985). In the two subsequent reduction steps to the level of methanol, $H_4$MPT serves as the $C_1$-carrier via 5-formyl-$H_4$MPT, 5,10-methenyl-$H_4$MPT, 5,10-methylene-$H_4$MPT and 5-methyl-$H_4$MPT, respectively (Escalante-Semerena et al. 1984; Keltjens and

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*Abbreviations:* HS-CoM, Coenzyme M, 2-mercaptoethanesulfonate; $CH_3$S-CoM, methylcoenzyme M, 2-(methylthio)ethanesulfonate; $H_4$MPT, 5,6,7,8-tetrahydromethanopterin; MFR, methanofuran; HS-HTP, 7-mercaptoheptanoylthreonine phosphate; CoM-S-S-HTP, the heterodisulfide of HS-CoM and HS-HTP; BES, 2-bromoethanesulfonate; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonate; CN-Cbl, cyanocobalamin; HO-Cbl, hydroxycobalamin; HBI, 5-hydroxybenzimidazole; DMBI, 5,6-dimethylbenzimidazole