*by Piet J.H. Daas*

# Corinnoid Enzymes in Methanogenic Biotransformation

The formation of methane is terminal step in the anaerobic microbial degradation of organic matter. The gas is produced by a select group of micro-organisms, the methanogenic bacteria. Though most methanogens are only able to use hydrogen and carbon dioxide as their substrates for growth, some species show a broader substrate range. The latter, the so-called methylotrophs, may grow on a series of methylated one-carbon compounds, like methanol, the various methylamines and dimethylsulfide, as well as acetate. At the conversion of these compounds the organisms use a collection of different methyltransferases. Common property of the methyltransferases is the presence of a corrinoid ($B_{12}$) prosthetic group as the catalytic centre. $B_{12}$ is a tetrapyrrole cobalt complex with 5,6-dimethylbenzimidazole as the usual nitrogenous base. In methanogenic bacteria the base is typically substituted for 5-hydroxybenzimidazole (HBI) *(Fig. 1)*. In this report we will confine our discussion to a single methyltransferase (system), the methanol:coenzyme M methyltransferase from *Methanosarcina barkeri*.

The methanol:coenzyme M methyltransferase system catalyses the methyl group transfer from methanol to 2-mercaptoet-hanesulfonic acid (coenzyme M, HS-CoM) *(reaction 1)*. The reaction product, 2-methylthioethanesulfonic acid (methylcoenzyme M, $CH_3S$-CoM), is the substrate in the methane-forming step.

$CH_3OH+HS\text{-}CoM \dashrightarrow CH_3S\text{-}CoM+H_2O$
*(reaction 1)*

Previous studies in our laboratory showed that in fact two distinct methyltransferases were involved in reaction (1): methanol:5-hydroxybenzimidazolylcobamide methyltransferase (MT1) and Co-methyl-$B_{12}$:HS-CoM methyltransferase (MT2).[1] In the reaction MT1 accepts the methyl group from methanol and binds it to $B_{12}$-HBI. The transfer of the corrinoid-bound methylgroup to HS-CoM is subsequently brought about by MT2, a 38 kDa polypeptide. In order to proceed, the reaction requires the additional presence of catalytic amounts of ATP, a reducing system (hydrogen, hydrogenase and ferredoxin) and an enzyme called Methyltransferase Activation Protein (MAP).[2] The requirement of the latter has to do with the special chemical properties of the corrinoid. Corrinoids are nature's most powerful nucleophiles, which makes them ideal catalysts in methyl group activation. This only holds, if cobalt is present in the super-reduced Co(I) oxidation state. Oxidation to Co(II) or Co(III), which will inevitably occur, even under vigorously anaerobic *in vitro* and *in vivo* conditions, renders the coenzyme inactive. MT1 is routinely purified as a 122 kDa protein composed of two subunits in an $\alpha 2\beta$ configuration; per mol enzyme 2 mol $B_{12}$-HBI are found. Though the purification is performed in an anaerobic glove box ($O_2$ <0.2 ppm), MT1 is isolated in an inactive form with $B_{12}$-HBI in the Co(III) state. Incubation of the as-isolated MT1 with hydrogen, hydrogenase and *M. barkeri* ferredoxin, a 12 kDa dimeric electron carrier with two cubic [4Fe-4S] clusters per subunit having a midpoint redox potential ($E_m$) of -340 mV,[3] enabled the reduction of Co(III) to Co(II). The latter species is paramagnetic, which makes the enzyme amenable to Electron Paramagnetic Resonance (EPR) spectroscopy. EPR analysis of the incubated MT1 showed HBI to be directly coordinated with Co(II), the so-called 'base-on' state.[4] In this oxidation state MT1 is still inactive.

Activation of MT1 must involve a further reduction of 'base-on' Co(II) to Co(I). Electrochemical studies with free $B_{12}$-HBI demonstrated that the reduction takes place at an extreme low $E_m$=-592 mV.[4] These studies also indicated that Co(II)-$B_{12}$-HBI is more easy to reduce ($E_m$= -500 Mv) if present in the uncoordinated 'base-off' state. At this point MAP, which now has been purified as a 59 Kda polypeptide with ATPase activity,[5] could play a role. It is thought that MAP catalyses the ATP-dependent conversion of 'base-on'- to 'base-off'-Co(II)-$B_{12}$HBI, which makes the coenzyme more accessible to reduction with hydrogen ($E_m$= -414 mV).

Studies on the specific mechanism of the reductive activation of MT1 may not only be interesting from a fundamental scientific point of view. Preliminary investigations indicated that the same mechanism is operative in other corrinoid-containing methyltransferases from *M. barkeri* as well.[5] Moreover, it is known that pure cultures of methanogenic bacteria are able to perform the reductive dehalogenation of solvents like chloroform.[6] Chloroform is degraded *via* $CH_2Cl_2$ and $CH_3Cl$ into methane. The sequence of reactions could be chemically brought about with free $B_{12}$ as the catalyst.[7] Each dehalogenation reaction was accompanied with the oxidation of Co(I) into Co(III), which subsequently had to be reconverted into Co(I) by a potent chemical reductant. *In vivo* the reductive dehalogenation may be realized by the corrinoid methyltransferases.[6] Here, the discussed activation mechanism might be active in keeping the corrinoid in the Co(I) state.


## In Summary

Methanogenic bacteria harbour a collection of corrinoid-containing methyltransferases used in the conversion of methylated one-carbon compounds. In order to keep the enzymes active, a sophisticated reductive activation system is required. This system was investigated in some detail for the methanol:coenzyme M methyltransfer reaction in *Methanosarcina barkeri*. It is suggested that the activation system is also operative in anaerobic reductive dehalogenation reactions that can be carried out by methanogens.


## Literature


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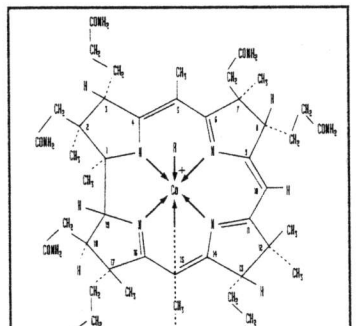

**Figure 1**
*Structure of 5-hydroxybenzimidazolyl cobamide ($B_{12}$-HBI), the characteristic corrinoid of methanogenic bacteria. In the Figure the central cobalt atom is present in the 6-coordinated Co(III) oxidation state. The sixth ligand (R) may be a methyl group.*

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