Biochemistry of methanogenesis: a tribute to Marjory Stephenson

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Historical overview

In 1933, Stephenson & Stickland (1933a) published that they had isolated from river mud, by the single cell technique, a methanogenic organism capable of growth in an inorganic medium with formate as the sole carbon source.

\[
\begin{align*}
4\text{HCOO}^- + 4\text{H}^+ &\rightarrow 4\text{H}_2 + 3\text{CO}_2 + 2\text{H}_2\text{O} \\
\Delta G^o &= -144.5 \text{ kJ mol}^{-1}
\end{align*}
\]

Methane formation from formate was shown to occur in a stepwise manner, by the preliminary decomposition of formic acid into CO₂ and H₂ followed by a reduction of CO₂ by H₂, suggesting that formate was not an intermediate in the reduction of CO₂ to methane.

\[
\begin{align*}
\text{HCOO}^- + \text{H}^+ &\rightarrow \text{H}_2 + \text{CO}_2 \\
\Delta G^o &= -3.5 \text{ kJ mol}^{-1} \\
4\text{H}_2 + \text{CO}_2 &\rightarrow 4\text{H}_2\text{O} + \text{CH}_4 \\
\Delta G^o &= -131 \text{ kJ mol}^{-1}
\end{align*}
\]

Cell suspensions of the micro-organism catalysed the reduction of methylene blue with H₂, indicating that the methanogen contained an enzyme which activates molecular hydrogen.

\[
\text{H}_2 \rightarrow 2\text{e}^- + 2\text{H}^+ \quad F'_0 = -414 \text{ mV}
\]

This enzyme had been discovered by Stephenson & Stickland (1931a) 2 years before in a number of bacterial species and was named by them 'hydrogenase'.

The following review summarizes what we presently know about the biochemistry of methanogenesis, emphasis being put on the enzyme methyl-coenzyme M reductase catalysing the methane-forming reaction proper. It is a tribute to Marjory Stephenson, who discovered hydrogenase in these micro-organisms. Marjory Stephenson died 50 years ago on 12 December 1948 at the age of only 63 (Elsden & Pirie, 1949; Robertson, 1949; Woods, 1950; Elsden, 1981). She had worked for 30 years in the Cambridge Biochemical Laboratory headed by Frederick Gowland Hopkins (Stephenson, 1948) on various aspects of the chemistry of bacteria (Stephenson, 1932, 1933, 1934, 1935). The 3rd edition of her book on *Bacterial Metabolism* (Stephenson, 1949) is a treasury of knowledge very worth reading even today, 50 years later. Marjory Stephenson was the second woman to be elected to the Royal Society of London (Mason, 1992, 1996) and a cofounder of the Society for General Microbiology, of which she was the second president (Postgate, 1995).
In this review, with the exception of a few papers describing discoveries, only publications on the biochemistry of methanogenesis are cited that have appeared since 1993: these contain reference to earlier and often more important papers, many of which have come from the laboratories of A. J. Kluyver (Kamp et al., 1959), H. A. Barker (Barker, 1956), T. Stadtman (Stadtman, 1967; Grahame & Stadtman, 1993), R. Wolfe (Wolfe, 1991, 1996), J. D. Ferry (Ferry, 1993b, 1995, 1997a), G. Gottschalk (Deppenmeier et al., 1996), A. Klein (Sorgenfrei et al., 1997a), J. Reeve (Reeve et al., 1997) and G. Vogels (Keltjens & Vogels, 1993). A historical overview of methanogenesis has recently been published by Wolfe (1993). The reader is also referred to the book on Methanogenesis: Ecology, Physiology, Biochemistry & Genetics edited by Ferry (1993a), to the review by Daniels (1993) and by Blaut (1994) and three more recent reviews by the author (Weiss & Thauer, 1993; Thauer et al., 1993; Thauer, 1997).

**Methanogens: where they live, what they do and who they are**

Methane is an end product of the microbial decomposition of organic matter in anaerobic freshwater environments such as lake sediments and the intestinal tract of animals. Estimates are that presently about 1% of the plant material formed per year by photosynthesis from CO₂ is remineralized via methane, more than 10⁸ tons of the combustible gas being intermediately generated. Roughly two-thirds of the methane diffuses into aerobic zones, where it is oxidized by methanotrophic bacteria. A few per cent is buried, leading to the formation of methane deposits, but most of the rest escapes to the atmosphere where it is photochemically converted to CO₂. The concentration of atmospheric methane has increased steadily during the last 300 years from 0.7 p.p.m. to 1.7 p.p.m., owing probably in large part to the expanded cultivation of rice and ruminant livestock (Conrad, 1996). This is of concern since methane is a potent greenhouse gas.

In anaerobic freshwater sediments, plant material such as glucose from cellulose is completely decomposed to CO₂ and CH₄.

\[
\text{Glucose} \rightarrow 3\text{CO}_2 + 3\text{CH}_4 \hspace{1cm} \Delta G^o = -418.1 \text{ kJ mol}^{-1}
\]

This reaction is not catalysed by single micro-organisms but by syntrophic associations of micro-organisms. First the glucose is fermented to acetate, CO₂ and H₂ or to acetate, formate and H₂:

\[
\text{Glucose} + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{H}^+ + 2\text{CO}_2 + 4\text{H}_2 \hspace{1cm} \Delta G^o = -2157 \text{ kJ mol}^{-1}
\]

\[
\text{Glucose} + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{HCOO}^- + 4\text{H}^+ + 2\text{H}_2 \hspace{1cm} \Delta G^o = -2087 \text{ kJ mol}^{-1}
\]

These fermentations are brought about by strictly anaerobic bacteria and/or protozoa. In a second step, the products of glucose fermentation are then converted to methane, the rate of conversion being such that the concentrations of acetate (<1 mM), formate (<0.1 mM) and H₂ (<1 µM) in the anaerobic sediments remain very low (Zinder, 1993).

\[
\begin{align*}
\text{CH}_3\text{COO}^- + \text{H}^+ &\rightarrow \text{CO}_2 + \text{CH}_4 & \Delta G^o &= -36 \text{ kJ mol}^{-1} \\
4\text{H}_2 + \text{CO}_2 &\rightarrow \text{CH}_4 + 2\text{H}_2\text{O} & \Delta G^o &= -131 \text{ kJ mol}^{-1} \\
4\text{HCOO}^- + 4\text{H}^+ &\rightarrow \text{CH}_4 + 3\text{CO}_2 + 2\text{H}_2\text{O} & \Delta G^o &= -1445 \text{ kJ mol}^{-1}
\end{align*}
\]

The organisms mediating these methane-forming reactions were originally thought to be bacteria, but are now recognized as belonging to a separate phylogenetic domain, the Archaea. Methanoarchaea are highly specialized. They can only use acetate, H₂ and CO₂, formate and/or other C₁ compounds such as methanol, methylthiols and methylamines as energy substrates, the C₁ compounds being either disproportionated to CO₂ and CH₄ or reduced to methane with H₂ as exemplified for methanol:

\[
4\text{CH}_3\text{OH} + 2\text{H}_2\text{O} \rightarrow 3\text{CH}_4 + 1\text{CO}_2 + 4\text{H}_2\text{O} \hspace{1cm} \Delta G^o = -106.5 \text{ kJ (mol CH}_4\text{)}^{-1}
\]

\[
\text{CH}_3\text{OH} + \text{H}_2 \rightarrow \text{CH}_4 + \text{H}_2\text{O} \hspace{1cm} \Delta G^o = -112.5 \text{ kJ mol}^{-1}
\]

Methanogenesis is the only way that methanogenic archaea can obtain energy for growth and these are the only organisms known to produce methane as a catalytic end product.

Despite the high specialization, not all methanogens are phylogenetically closely related. This is reflected, for example, in differences in cell wall composition (Sprott & Beveridge, 1993; Kandler & König, 1998). Methanogens can also have very different optimal growth conditions; thus some thrive optimally at temperatures below 20 °C and others at or near the temperature of boiling water (Boone et al., 1993).

Methanogens taxonomically all belong to the archaeal kingdom of Euryarchaeota. They are classified in five orders each phylogenetically related to another as distantly as the Cyanobacteria to the Proteobacteria. The five orders are: Methanobacteriales, Methanococcales, Methanomicrobiales, Methanopyrales and Methanosarcinales. Of these, only the Methanosarcinales can ferment acetate to CO₂ and CH₄ and grow on methanol, methylthiols or methylamines as sole energy source. In turn, hyperthermophilic species are found only among the Methanobacteriales, Methanococcales and Methanopyrales (Boone et al., 1993).

The DNA sequence of the total genome has, until now, been determined for two methanoarchaea, for Methanococcus jannaschii (Bult et al., 1996) and for Methanobacterium thermoautotrophicum (strain A1) (Smith et al., 1997). Recently, the sequence for Archaeoglobus fulgidus has also been published (Klenk et al., 1997). This sulphate-reducing archaean is phylogenetically most closely related to the Methanosarcinales with which it has many biochemical features in common.
Table 1. Reactions and enzymes known to be involved in methane formation from acetate in the *Methanosarcinales* (Ferry, 1993b, 1995, 1997a)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme (gene)</th>
<th>Most recent literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate + CoA → acetyl-CoA + H₂O</td>
<td>Acetate kinase (ack) and phosphotransacetylase (pta) in <em>Methanosarcina</em> spp. or acetate thio kinase (acs) in <em>Methanosaeta</em> spp.</td>
<td>Latimer &amp; Ferry (1993); Singh-Wissmann &amp; Ferry (1995); Eggen et al. (1991a)</td>
</tr>
<tr>
<td>Acetyl-CoA + H₂SPT → CH₄-H₂SPT + CO₂ + CoA + 2[H]</td>
<td>Carbon-monoxide dehydrogenase/acetyl-CoA synthase</td>
<td>Grahame (1993); Sowers et al. (1993); Lu et al. (1994); Grahame &amp; DeMoll (1995, 1996); Eggen et al. (1991b, 1996); Grahame et al. (1996); Maupin-Furlow &amp; Ferry (1996a, b)</td>
</tr>
<tr>
<td>Methanosarcinales spp. as a multienzyme complex composed of five different subunits (Grahame &amp; DeMoll, 1996); Maupin-Furlow &amp; Ferry (1996b). CdhA and CdhC are predicted to be nickel iron-sulphur proteins and CdhD and CdhE to harbour the corrinoid prosthetic group.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ΔG⁰* values add up to −38 kJ mol⁻¹, which is very similar to the *ΔG⁰* = −36 kJ mol⁻¹ calculated for CH₃COO⁻ + H⁺ → CH₄ + CO₂ from free energy of formation data (Thauer et al., 1977). See also the first footnotes to Tables 2 and 3.

† From Thauer et al. (1977).

‡ From Thauer (1990); CO₂ in the gaseous state at 10⁵ Pa. *E*- was determined by Grahame & DeMoll (1995) to be −270 mV and *ΔG⁰* to be +28 kJ mol⁻¹, values not consistent with the overall thermodynamics of methanogenesis from acetate (see first footnote), from CO₂ and from methanol (Table 3). At first sight, a *ΔG⁰* = +41.3 kJ mol⁻¹ appears unrealistically endergonic. Note, however, that in the reaction four products are formed from two substrates. *ΔG*- is thus highly concentration dependent. At 0-1 M concentrations of all substrates and products (pH₁ = 10 Pa), *ΔG*- = −2.6 kJ mol⁻¹ and *E*- = −430 mV (Thauer, 1990).

§ Calculated for 2[H] = 1H₂ at 10⁵ Pa.

|| The nickel iron–sulphur corrinoid enzyme is isolated from *Methanosarcina* spp. as a multienzyme complex composed of five different subunits (Grahame & DeMoll, 1996; Maupin-Furlow & Ferry, 1996a, b); ferredoxin appears to be the physiological electron acceptor (Fischer & Thauer, 1990; Peer et al., 1994; Ferry, 1995, 1997a). The encoding genes in *Methanosarcina thermophila* are organized in a transcription unit, cdhABCXDE, X being an open reading frame of unknown function (Maupin-Furlow & Ferry, 1996b). CdhA and CdhE are predicted to be nickel iron–sulphur proteins and CdhB and CdhC are predicted to harbour the corrinoid prosthetic group.

¶ From Weiss et al. (1994).


** From Thauer et al. (1993).**

†† The enzyme is isolated as a multienzyme complex of three different subunits, McrABG, in an αβγδ configuration.

†‡ Membrane-associated enzyme purified from methanol-grown *Methanosarcina barkeri* is a complex of two different subunits, HdrDE (Künkel et al., 1997), and from acetate-grown cells is a complex of eight different subunits, HdrDE and EchABCDEF, Ech being an *E. coli* hydrogenase-3-type hydrogenase (Künkel et al., 1998). HdrE is a b-type cytochrome (Künkel et al., 1997, 1998).

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(Schwörrer et al., 1993a; Kunow et al., 1993, 1994, 1995; Thauer & Kunow, 1995; Vorholt et al., 1995, 1997a).

Noteworthy is a recent hypothesis for the origin of eukaryotic cells from strictly hydrogen-dependent autotrophic methanoarchaea (Martin & Müller, 1998; see also Vogel, 1998).

### Energy metabolism of methanoarchaea

The reactions and enzymes known to be involved in methane formation from acetate, from CO₂ and from methanol are summarized in Tables 1–3.

The energy metabolism of methanogens can be viewed...
Table 2. Reactions and enzymes known to be involved in methane formation from CO₂ (Thauer et al., 1993)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme (gene)</th>
<th>Most recent literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ + MFR + 2[H] → formyl-MFR</td>
<td>Formylmethanofuran dehydrogenase</td>
<td>Schmitz et al. (1994); Bertram &amp; Thauer (1994); Bertram et al. (1994a, b);</td>
</tr>
<tr>
<td></td>
<td>(fwhHFGDACB and fmdECB§)</td>
<td>Wasserfallen (1994); Hochheimer et al. (1995, 1996); Vorholt et al. (1996, 1997b);</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vorholt &amp; Thauer (1997); Holm &amp; Sander (1997)</td>
</tr>
<tr>
<td>Formyl-MFR + H₂MPT → formyl-H₂MPT + MFR</td>
<td>Formylmethanofuran: H₂MPT</td>
<td>Shima et al. (1995, 1996); Lehmacher (1994); Ermller et al. (1997a); Kunow et al. (1996)</td>
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<td></td>
<td>formyltransferase (fr)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methenyl-H₂MPT+ + F₄₂₀H₂ →</td>
<td>F₄₂₀-dependent methylene-H₂MPT</td>
<td>Klein et al. (1993b); Kunow et al. (1993);</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Methenyl-H₂MPT+ + H₂ →</td>
<td>H₂-forming methylene-H₂MPT</td>
<td>Schwörr et al. (1993b); Schleucher et al. (1994, 1995); Klein et al. (1995a, b);</td>
</tr>
<tr>
<td>methylene-H₂MPT + H⁺</td>
<td>dehydrogenase (hmtd)</td>
<td>Berkesell &amp; Thauer (1995); Hartmann et al. (1996a, b); Thauer et al. (1996)</td>
</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylen-H₂MPT + F₄₂₀ + H₂ →</td>
<td>F₄₂₀-dependent methylene-H₂MPT</td>
<td>Vaupel &amp; Thauer (1995); Nolling et al. (1995a); Kunow et al. (1993)</td>
</tr>
<tr>
<td>CH₃-H₂MPT + F₄₂₀</td>
<td>reductase (mer)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₂-H₂MPT + H-S-CoM →</td>
<td>Methyl-H₂MPT: coenzyme M</td>
<td>Stupperich et al. (1993); Gätterner et al. (1993, 1994); Weiss et al. (1994);</td>
</tr>
<tr>
<td></td>
<td>(mtrEDCBAFGH)</td>
<td></td>
</tr>
<tr>
<td>CH₂-S-CoM + H-S-CoB →</td>
<td>Methyl-coenzyme M reductase</td>
<td>Bonacker et al. (1993); Goubeaud et al. (1997); Shima et al. (1997); Ermller et al. (1997b); Becker &amp; Ragsdale (1998)</td>
</tr>
<tr>
<td>CoM-S-S-CoB + CH₃</td>
<td>(mcrBDCGAFH and mrtBDGA)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>heterodisulphide reductase (hdrA,</td>
<td>Hedderich et al. (1994); Setzke et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>hhdrBC)†</td>
<td></td>
</tr>
</tbody>
</table>

*ΔGº values from Thauer et al. (1993). They add up to −130.7 kJ mol⁻¹, which is almost identical to the ΔGº = −131.1 kJ mol⁻¹ calculated for 4H₂ + CO₂ → CH₄ + 2H₂O from free energy of formation data (Thauer et al., 1977).†From Bertram & Thauer (1994); determined at 60 °C; CO₂ in the gaseous state at 10⁶ Pa.‡Calculated for 2[H] = 1H⁺ at 10⁶ Pa. The direct electron donor is probably a polyferredoxin (Vorholt et al., 1996). The endergonic reduction of CO₂ with H₂ to formylmethanofuran is driven by the electrochemical Na⁺ potential involving reversed electron transport (Kaesler & Schönheit, 1989a, b).

§ fwh denotes the genes encoding the tungsten enzyme and fmd the molybdenum enzyme in Methanobacterium thermoautotrophicum (Hochheimer et al., 1996). The latter enzymes are isolated from Methanobacterium thermoautotrophicum as multisubunit complexes composed of the subunits FwdABC and FwdA+FmdBC, respectively. FwdB and FmdB contain bound molybdopterin-guanine dinucleotide and tungsten and molybdenum, respectively (Hochheimer et al., 1996).

| H₂ + F₄₂₀ → F₄₂₀H₂ | ΔGº = −11 kJ mol⁻¹ | the E⁰ of the F₄₂₀/F₄₂₀H₂ couple is −360 mV (Thauer et al., 1993) and that of the H⁺/H₂ couple is −414 mV (Thauer et al., 1977). |
to consist of two parts (Fig. 1): an oxidative part in which coenzyme M (H-S-CoM, 2-thioethanesulfonate) and coenzyme B (H-S-CoB, 7-thioheptanoylthreonine-phosphate) (for structures see Fig. 4) are oxidized to the heterodisulfide CoM-S-S-CoB; and a reductive part in which the heterodisulfide of coenzyme M and coenzyme B is re-reduced.

**Oxidative part**

In this part, methyl-coenzyme M is a central intermediate (Taylor & Wolfe, 1974a; b; Shapiro & Wolfe, 1980; Lovley et al., 1984). It is formed from coenzyme M and acetate, CO₂ or reduced C₁ compounds such as methanol, methylthiols and methylamines via the pathways shown in Fig. 2. Methyl-coenzyme M is subsequently reduced with coenzyme B to methane with the concomitant formation of the heterodisulfide of coenzyme M and coenzyme B (Bobik et al., 1987; Ellermann et al., 1988).

\[ \text{CH}_3\text{-S-CoM} + \text{H-S-CoB} \rightarrow \text{CH}_4 + \text{CoM-S-S-CoB} \]

As far as known the exergonic reaction, which is catalysed by methyl-coenzyme M reductase and which proceeds essentially irreversibly, is not coupled with energy conservation (Thauer et al., 1993).

In methanogenesis from CO₂ (Escalante-Semerena et al., 1984) and from acetate (Fischer & Thauer, 1989), N²⁺-methyltetrahydrodromethanopterin (CH₃-H₄MPT) or N²⁺-methyltetrahydroarabinopterin (CH₃-H₄SPT) is an intermediate in methyl-coenzyme M formation (Fig. 2). [Tetrahydrodromethanopterin (H₄MPT) is the tetrahydrofolate (H₄F) analogue found instead of H₄F in the *Methanobacteriales* (for structures see Fig. 3). In the other orders, modified forms of H₄MPT are present: tetrahydroarabinopterin in the *Methanosarcinales* and *Methanococcales* and tatiopterin and/or thermopterin in the *Methanomicrobiales* (Gorris & van der Drift, 1994; see also White, 1998).] The methyl group of CH₃-H₄MPT is transferred to coenzyme M in an exergonic reaction (\(\Delta G^\circ = -30 \text{ kJ mol}^{-1}\)) coupled with energy conservation via an electrochemical sodium potential across the cytoplasmic membrane (Becher et al., 1992a, b; Becher & Müller, 1994; Müller & Gottschalk, 1994; Lienard et al., 1996; Lienard & Gottschalk, 1998). Despite this fact, methyl-coenzyme M formation from acetate or CO₂ is most probably not associated with a net phosphorylation of ADP since the first step in methanogenesis from acetate (Table 1) and that from CO₂ (Table 2) are endergonic reactions which in order to proceed are predicted to consume more energy than is conserved in the methyl transfer reaction.

**Reductive part**

Since the generation of the heterodisulfide in the oxidative part of the energy metabolism apparently is not coupled with net ATP formation, the energy required for growth of methanogens must be generated in the reductive part, the exergonic reduction of the heterodisulfide (Fig. 1), and indeed it has been shown that the reduction of the heterodisulfide is coupled with phosphorylation of ADP via the chemiosmotic mechanism involving an electrochemical H⁺ potential as intermediate (Peinemann et al., 1990; Deppenmeier et al., 1990a, b, 1991; Sparling et al., 1993). Dependent on the methanogenic growth substrate, the electrons for the reduction derive from the oxidation of either H₂, the carbonyl group of acetyl-CoA, formate, ethanol or 2-propanol or of one of the reduced C₁ compounds methanol, methylthiols or methylamines. Under standard conditions, the free energy change associated with heterodisulfide reduction with H₂ is \(-40 \text{ kJ mol}^{-1}\), with formate is \(-43.5 \text{ kJ mol}^{-1}\), with ethanol (acetate as product) is \(-35 \text{ kJ mol}^{-1}\) and with methanol (CO₂ as product) is \(-34 \text{ kJ mol}^{-1}\), which is sufficient to drive the phosphorylation of 1 mol ADP (\(\Delta G^\circ = +31.8 \text{ kJ mol}^{-1}\)) (Thauer et al., 1977). The reduction of the heterodisulfide with acetyl-CoA (\(\Delta G^\circ = +0 \text{ kJ mol}^{-1}\)) (calculated from \(E^\circ\) values given in Table 1) and with 2-propanol (\(\Delta G^\circ = -15 \text{ kJ mol}^{-1}\)) is much less exergonic. Under the conditions in the cell, the free energy change \(\Delta G\) is, however, probably considerably more negative since the reduction is ‘pushed’ by irreversible formation of the heterodisulfide from methyl-coenzyme M and coenzyme B. Such a coupling is not unusual: e.g. in the arginine dihydrolase pathway operative in many bacteria and archaea, the energy-conserving formation of ornithine, CO₂ and NH₃ from citrulline (\(\Delta G^\circ = -19 \text{ kJ mol}^{-1}\)) is also ‘pushed’ by the preceding reaction, the irreversible hydrolysis of arginine to citrulline and NH₃ (\(\Delta G^\circ = -37.7 \text{ kJ mol}^{-1}\)) (Thauer et al., 1977). There is evidence that heterodisulfide reduction with H₂ is coupled with the synthesis of more than 1 mol ATP (Deppenmeier et al., 1996).

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*From Weiss et al. (1994).*

* Membrane-associated multi-enzyme complex composed of eight different subunits, MtrA-H, MtrA harbouring a corrinoid prosthetic group (Harms et al., 1995). Energy conservation proceeds via an electrochemical Na⁺ potential (Gärtner et al., 1994). For literature on the enzyme complex from *Methanosarcina* spp., see Table 1.

* mcr denotes the genes encoding the isoenzyme I and mrt the isoenzyme II. Both isoenzymes are isolated as multienzyme complexes composed of three different subunits, McrABG or MrtABG, in an \(\alpha_2\beta_2\gamma_2\) configuration. For literature on the enzyme complex from *Methanosarcina* spp., see Table 1.

* Calculated for \(2[H] = H_2\) at 10⁵ Pa.

* The enzyme is purified from *Methanobacterium thermoautotrophicum* in a complex with the F₄₃₃ non-reducing hydrogenase (Setzke et al., 1994).
Heterodisulphide reduction with H₂

The reduction of the heterodisulphide with the primary electron donors involves at least two enzymes, a dehydrogenase and a heterodisulphide reductase, and an electron transport chain connecting the two enzymes as schematically exemplified for heterodisulphide reduction with H₂:

\[ \text{H}_2 \rightarrow 2e^- + 2\text{H}^+ \]
\[ 2e^- + 2\text{H}^+ + \text{CoM}-\text{S-S-CoB} \rightarrow \text{H-S-CoM} + \text{H-S-CoB} \]

\[ E'_0 = -414 \text{ mV} \]

\[ E'_0 = -200 \text{ mV} \]
The heterodisulphide reductase (Hdr), first discovered by Hedderich & Thauer (1988), is a novel type of disulphide reductase catalysing the reduction of the heterodisulphide in two successive one-electron steps and probably involving thyl radical intermediates (Künkel et al., 1997; Heim et al., 1998). The enzyme complex, composed of at least two different subunits, has been characterized from *Methanobacterium thermoautotrophicum* (Hedderich et al., 1990, 1994; Setzke et al., 1994), from *Methanosarcina barkeri* (Heiden et al., 1993, 1994; Künkel et al., 1997, 1998) and from *Methanosarcina thermophila* (Peer et al., 1994). Heterodisulphide reductase HdrDE from *Methanosarcina* spp. is a cytochrome b (HdrE)-containing enzyme (Heiden et al., 1993, 1994; Künkel et al., 1997); methanophenazine is most probably the direct electron donor, the oxidation of reduced methanophenazine with heterodisulphide being coupled with energy conservation (Abken et al., 1998a, b; Bäumer et al., 1998). The enzyme HdrABC from *Methanobacterium* spp. is an iron–sulphur protein (Hedderich et al., 1994; Setzke et al., 1994). The direct electron donor is not yet known (see Nolling et al., 1995c).

Hydrogenotrophic methanogens can contain up to four different types of hydrogenases and of each type, several isoenzymes (Thauer et al., 1993; Sorgenfrei et al., 1997a, b; Künkel et al., 1998; for topology see Braks et al., 1994): (i) F$_{100}$-reducing Ni/Fe-hydrogenase (Vaupel & Thauer, 1998); (ii) F$_{100}$-non-reducing hydrogenase of unknown physiological electron acceptor—in *Methanosarcina* spp., one of the subunits from the enzyme is a cytochrome b (Deppenmeier, 1995; Deppenmeier et al., 1995; Kumazawa et al., 1994; Kenner & Zeikus, 1994a, b) and in *Methanobacterium* spp., which are devoid of cytochromes, the electron acceptor is possibly either a 44 kDa polyferredoxin (Reeve et al., 1989;
**Table 4.** Dehydrogenases catalysing the reduction of $F_{420}$ and the oxidation of $F_{420}H_2$ in the energy metabolism of methanogenic archaea

$F_{420}$ is a 5'-deazaflavin derivative found in relatively high concentration in methanogenic archaea (Gorris & van der Drift, 1994). $F_{420}$ in methanogens is converted to inactive $F_{380}$ by adenylation and $F_{380}$ to $F_{420}$ by deadenylation in response to different growth conditions (Vermeij et al., 1994, 1995, 1996).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Organism</th>
<th>Most recent literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reduction of $F_{420}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{420}$-reducing hydrogenase</td>
<td>All hydrogenotrophic methanogens</td>
<td>Sorgenfrei et al. (1997a, b); Vaupel &amp; Thauer (1998)</td>
</tr>
<tr>
<td>$F_{420}$-dependent formate dehydrogenase</td>
<td>Methanogens growing on formate</td>
<td>Nölling &amp; Reeve (1997); Grahame &amp; Stadman (1993)</td>
</tr>
<tr>
<td>$F_{420}$-dependent methylene-$H_4$MPT dehydrogenase</td>
<td>Methanogens growing on methanol, methylthiols or methylamines</td>
<td>Keltjens &amp; Vogels (1993)</td>
</tr>
<tr>
<td>$F_{420}$-dependent methylene-$H_4$MPT reductase</td>
<td>Methanogens growing on methanol, methylthiols or methylamines</td>
<td>Keltjens &amp; Vogels (1993)</td>
</tr>
<tr>
<td>$H_2$-forming methylene-$H_4$MPT dehydrogenase plus $F_{420}$-dependent methylene-$H_4$MPT dehydrogenase</td>
<td>Most hydrogenotrophic methanogens of the orders Methanobacteriales, Methanococcales and Methanopyrales</td>
<td>Thauer et al. (1996); Afting et al. (1998)</td>
</tr>
<tr>
<td>$F_{420}$-dependent alcohol dehydrogenase</td>
<td>Methanogenium liminatans, Methanococcus thermophilicus</td>
<td>Klein et al. (1996); Widdel &amp; Frimmer (1995); Berk et al. (1996)</td>
</tr>
<tr>
<td>NADP-dependent alcohol dehydrogenase plus $F_{420}$-dependent NADP reductase</td>
<td>Methanobacterium palustre, Methanococcus stipitatus</td>
<td>Berk et al. (1997); Widdel &amp; Frimmer (1995)</td>
</tr>
<tr>
<td>2. Oxidation of $F_{420}H_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{420}$-dependent methylene-$H_4$MPT dehydrogenase</td>
<td>Methanogens reducing CO$_2$ to methane</td>
<td>See Table 2</td>
</tr>
<tr>
<td>$F_{420}$-dependent methylene-$H_4$MPT reductase</td>
<td>Methanogens reducing CO$_2$ to methane</td>
<td>See Table 2</td>
</tr>
<tr>
<td>$F_{420}H_2$ dehydrogenase complex</td>
<td>Methanothermobacterium mazei, Methanobacterium tindarius</td>
<td>Haase et al. (1992); Abken &amp; Deppenmeier (1997); Abken et al. (1998a, b)</td>
</tr>
<tr>
<td>$F_{420}$-dependent hydrogenase</td>
<td>Methanogens of the orders Methanococcales and Methanobacteriales growing on formate and lacking $F_{420}H_2$, dehydrogenase complex</td>
<td>Vaupel (1993)</td>
</tr>
</tbody>
</table>

Hedderich et al., 1992; Nölling et al., 1995c) or a 45 kDa flavoprotein (Wasserfallen et al., 1995; Nölling et al., 1995c); (iii) *Escherichia coli* hydrogenase-3-type Ni/Fe-hydrogenase of unknown physiological electron acceptor (Kinkel et al., 1998); and (iv) a metal-free hydrogenase, the $H_2$-forming methyleneetetrahydro-methanopterin dehydrogenase, which together with the $F_{420}$-dependent methyleneetetrahydromethanopterin dehydrogenase catalyses the reduction of $F_{420}$ with $H_2$ (Thauer et al., 1996; Reeve et al., 1997). Both enzymes are induced during growth under conditions of nickel deprivation (Afting et al., 1998).

There are indications that at least two of the hydrogenases can be involved in heterodisulphide reduction with $H_2$; with inside-out vesicles from *Methanosarcina mazei* it has been shown that both $F_{420}$-dependent (Deppenmeier et al., 1990a, b) and $F_{420}$-independent (Deppenmeier et al., 1991) reduction of the heterodisulphide with $H_2$ are coupled with the phosphorylation of ADP (Deppenmeier et al., 1996; Abken et al., 1998a, b).

**Heterodisulphide reduction with $F_{420}H_2$**

Cell extracts of *Methanosarcina mazei* and of *Methanobacterium tindarius* catalyse the reduction of heterodisulphide with reduced $F_{420}$, the coenzyme of many dehydrogenases in methanogenic archaea (Table 4). ($F_{420}$ is a 5'-deazaflavin derivative with an $E_{m}^{\circ} = -360$ mV present in relatively high concentrations in these organisms; Gorris & van der Drift, 1994.) For the reduction of the heterodisulphide with $F_{420}H_2$, besides heterodisulphide reductase an additional enzyme is required: *Methanosarcina* spp. (Deppenmeier et al., 1990a, b; Abken & Deppenmeier, 1997) and *Methanobacterium tindarius* (Deppenmeier et al., 1990b; Haase et al., 1992) have been shown to contain a membrane-associated $F_{420}H_2$ dehydrogenase complex catalysing...
the reduction of methanophenazine with $F_{430}H_2$, the reduction being coupled with phosphorylation of ADP (Abken et al., 1998a, b; Bäumer et al., 1998). Such an $F_{430}H_2$ dehydrogenase complex is also found in Archaeoglobus sp. (Knunov et al., 1993, 1994; Klenk et al., 1997), but is apparently lacking in Methanococcus spp. (Bult et al., 1996; and Methanobacterium sp. (Smith et al., 1997) as deduced from the genome sequences. In methanogens lacking the $F_{430}H_2$ dehydrogenase complex and not growing on $H_2$ and CO$_2$, heterodisulphide reduction with $F_{430}H_2$ probably proceeds via $H_2$ as intermediate (Vaupel, 1993).

**Coupling with ATP synthesis**

As indicated, methyl transfer from $N^8$-methyltetrahydromethanopterin to coenzyme M in the oxidative part of the energy metabolism is coupled with the build-up of an electrochemical Na$^+$ gradient (Becher et al., 1992a, b; Lienard et al., 1996). In turn, the reduction of the heterodisulphide in the reductive part is coupled with electrogenic $H^+$ translocation (Peinemann et al., 1990; Deppenmeier et al., 1990a, b, 1991; Sparling et al., 1993). *Methanosarcina* spp. have been shown to contain an $H^+$-translocating $\Delta\varphi$ ATPase (Wilm et al., 1996; Inatomi, 1996; Ruppert et al., 1998) and there is indirect evidence also for the presence of an Na$^+$-translocating $F,F_0$ ATPase (Becher & Müller, 1994). *Methanobacterium* spp. and *Methanococcus* spp. harbour only one $\Delta\varphi$ ATPase as deduced from the genome sequences (Bult et al., 1996; Smith et al., 1997). It is not known whether this ATPase is Na$^+$- or $H^+$-translocating. There is evidence for both (Chen & Konisky, 1993; Sparling et al., 1993; Smigan et al., 1994). Methanogens contain an Na$^+$/$H^+$ antiporter capable of interconversion of the electrochemical $H^+$ and Na$^+$ potentials (Schönheit & Beimborn, 1983; Deppenmeier et al., 1996).

The stoichiometry of coupling of methanogenesis with ADP phosphorylation appears not to be constant. Evidence is available that during growth of methanogens on $H_2$ and CO$_2$, the growth yield [g (mol CH$_4$)$^{-1}$] increases with decreasing $H_2$ concentrations (Schönheit et al., 1980; Morgan et al., 1997; for regulation see Pihl et al., 1994; Nölling et al., 1995a, b; Nölling & Reeve, 1997), indicating that at low $H_2$ concentrations coupling of heterodisulphide reduction with ADP phosphorylation is tighter than at high $H_2$ concentrations. With decreasing $H_2$ concentrations the free energy change associated with CO$_2$ reduction to methane decreases from $-131$ kJ mol$^{-1}$ at $10^5$ Pa $H_2$ to only approximately $-30$ kJ mol$^{-1}$ at the $H_2$ concentrations prevailing in methanogenic ecosystems where the $H_2$ partial pressure is only between 1 and 10 Pa.

**Other heterodisulphide-generating reactions**

Methyl-coenzyme M reduction with coenzyme B is not the only reaction in which the heterodisulphide is regenerated. Most methanogens contain a fumarate reductase which catalyses the reduction of fumarate with H-S-CoM and H-S-CoB to succinate and CoM-S-S-CoB (Bobik & Wolfe, 1989; Heim et al., 1998). This reaction is involved in the biosynthesis of 2-oxoglutarate, which, in the methanogens containing the fumarate reductase, is synthesized via reductive carboxylation of succinyl-CoA (Tersteegen et al., 1997; Thompson et al., 1998).

**Analogy between heterodisulphide reduction and sulphur reduction**

Many anaerobic bacteria and archaea can grow with elemental sulphur as terminal electron acceptor in the energy metabolism (Schauder & Kröger, 1993). Sulphur reduction to H$_2$S is coupled with energy conservation as evidenced by growth on $H_2$ and S$^0$. In elemental sulphur, which at room temperature is S$_2$, the sulphur atoms are connected via S-S bonds just as the two sulphurs in heterodisulphide. The redox potential of the S$^0$/H$_2$S couple and that of the heterodisulphide/H$_2$S-CoM/H$_2$-CoB couple are both in the order of $-200$ mV (Thauer et al., 1977). Methanogens and sulphur-reducing micro-organisms thus have in common that they both live at the expense of S-S bond reduction. Methanogens differ, however, from sulphur reducers in that they are independent of an external sulphur source as electron acceptor since they can reoxidize the 'reduced sulphur' with CO$_2$ or one of the other methanogenic carbon substrates, which themselves are concomitantly reduced to methane.

**Methyl-coenzyme M, an intermediate unique to methanogenesis**

Coenzyme M and methyl-coenzyme M have, until now, only been found in methanogenic archaea (Balch & Wolfe, 1979). The same holds true for the enzymes catalysing the formation of methyl-coenzyme M (Table 5) and for methyl-coenzyme M reductase. All the other enzymes and coenzymes involved in methanogenesis, maybe with exception of coenzyme B, are also present in sulphate-reducing archaea (Vorholt et al., 1995, 1997a; Klenk et al., 1997). Recently, tetrahydromethanopterin and tetrahydromethanopterin-dependent enzymes were even found in a methylo trophic $\alpha$-proteobacterium (Chistoserdova et al., 1998), indicating that some of the methanogenic enzymes and coenzymes are not as unique to the archaeal kingdom as previously thought. Also of interest is the recent finding that several non-methanogens, including *Bacillus subtilis*, contain genes encoding a putative heterodisulphide reductase, the function of which remains to be elucidated (Heim et al., 1998).

Coenzyme M is the smallest coenzyme known to date. The enzymes catalysing its methylation and reductive demethylation are highly specific for its structure. 2-Selenoethanesulphonate and 3-thiopropionate can in part substitute for H-S-CoM in its function, 3-thiopropanesulphonate cannot (Gunsalus et al., 1978; Wackett et al., 1987; Tallant & Krzycki, 1996, 1997).
Table 5. Reactions and enzymes known to be involved in the formation of methyl-coenzyme M (Keltjens & Vogels, 1993)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme system</th>
<th>Most recent literature</th>
</tr>
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<tbody>
<tr>
<td>CH₃H₂MPT + H-S-CoM → CH₃-S-CoM + H₂</td>
<td>N⁵-Methyl-H₂MPT:coenzyme M methyltransferase (energy conserving) (MtrA–H)</td>
<td>Stupperich et al. (1993); Gärtner et al. (1993, 1994); Weiss et al. (1994); Harms et al. (1995); Harms &amp; Thauer (1996a, 1997, 1998); Lu et al. (1995); Lienard et al. (1996); Lienard &amp; Gottschalk (1998)</td>
</tr>
<tr>
<td>CH₂NH₂ + H-S-CoM → CH₂H₂S-CoM + NH₃</td>
<td>Monomethylamine:coenzyme M methyltransferase</td>
<td>Burke &amp; Krzycki (1995, 1997); Burke et al. (1998)</td>
</tr>
<tr>
<td>CH₃NH₂ + H-S-CoM → CH₃H₂S-CoM + CH₃NH₃</td>
<td>Dimethylamine:coenzyme M methyltransferase (MtbA + MtbB + ?)</td>
<td>Wassenaar et al. (1996, 1998)</td>
</tr>
<tr>
<td>CH₃H₂S-CoM + CH₃NH₃</td>
<td>Trimethylamine:coenzyme M methyltransferase</td>
<td>Ferguson &amp; Krzycki (1997); Wassenaar et al. (1996)</td>
</tr>
<tr>
<td>CH₃H₂S-CoM + CH₃NH₃</td>
<td>Tetramethylamine:coenzyme M methyltransferase</td>
<td>Asakawa et al. (1998); Tanaka (1994)</td>
</tr>
<tr>
<td>CH₃H₂S-CoM + CH₃NH₃</td>
<td>Methylthiol:coenzyme M methyltransferase (MsaB)</td>
<td>Paul &amp; Krzycki (1996); Tallant &amp; Krzycki (1996, 1997)</td>
</tr>
</tbody>
</table>

* From Weiss et al. (1994).
† For activity in cell extracts the presence of a methyltransferase-activating protein (MAP), reduced ferredoxin and ATP are additionally required (Daas et al., 1993, 1994, 1996a, b). MtaA (= MT2-M), MtBA (= MT2-A), MtqA and MtsA exhibit methylcobalamin:coenzyme M methyltransferase (MT2) activity (Grahame, 1989; Yeliseev et al., 1993; Ferguson et al., 1996; Wassenaar et al., 1996) and show sequence similarity (LeClerc & Grahame, 1996; Harms & Thauer, 1996b; Paul & Krzycki, 1996; Asakawa et al., 1998). MtaA and MtBA are zinc enzymes (Sauer & Thauer, 1997); MtC, MtMc, MtqC and MtsB have been shown to be corrinoid proteins harbouring 6-hydroxybenzimidazolyl cobamide as prosthetic group.
‡ Obtained from ΔG° for methylamine reduction to methane with H₂ calculated from the free energies of formation from the elements tabulated in Thauer et al. (1977) and from ΔG° of −85 kJ mol⁻¹ for methyl-coenzyme M reduction with H₂ to CH₄ and coenzyme M (Thauer et al., 1993).
§ Also catalyses the methylation of coenzyme M with methylmercaptropropanol (95%), methylmercaptropropionate (80%), mercaptomethanol (10%), methylmercaptoethanol (8%) and methyl iodide (170%) (Tallant & Krzycki, 1997) and the methylation of mercaptethanol (27%) and 2-mercaptopropanol (66%) (Tallant & Krzycki, 1996).
Methyl-coenzyme M reductase and of its isoenzyme

Methyl-coenzyme M reductase (MCR) has an apparent molecular mass of approximately 300 kDa. It is composed of three different subunits, α (MCRα), β (MCRβ) and γ (MCRγ), in an αβγβγ arrangement (Gunsalus & Wolfe, 1980). The enzyme contains 2 mol of tightly but not covalently bound coenzyme F₄₃₀ mol⁻¹ (Ellenson et al., 1982), which is a nickel porphyrin, the structure of which is shown in Fig. 5. The enzyme as purified generally also contains 2 mol H-S-CoM (Hartzell et al., 1987) and 2 mol H-S-CoB (Noll & Wolfe, 1986). The enzyme can be reconstituted from its subunits in the presence of coenzyme F₄₃₀, albeit with very low specific activity (Hartzell & Wolfe, 1986).

**Fig. 5. Structure of coenzyme F₄₃₀ in the Ni(II) oxidation state**

(Methanobacterium thermoautotrophicum, Methanobacterium fervidus and Methanococcus jannaschii (probably all members of the Methanobacteriales and Methanococcales) have been shown to contain besides MCR a genetically distinct isoenzyme of methyl-coenzyme M reductase designated MRT (Rospert et al., 1990; Brenner et al., 1993; Lehmacher & Klenk, 1994; Bult et al., 1996; Nolling et al., 1996). The expression of the two isoenzymes is differentially regulated by the growth conditions (Bonacker et al., 1992; Pihl et al., 1994; Reeve et al., 1997; Pennings et al., 1997).

The genes encoding the three subunits of the isoenzyme I (MCR) form a transcription unit (mcrBDCGA) which additionally contains two open reading frames encoding two polypeptides, McrC and McrD, of molecular masses below 20 kDa (Cram et al., 1987; Bokranz & Klein, 1991). It has been proposed and evidence has been reported that the physiological electron donor for methyl-coenzyme M reduction could possibly be a larger molecule which contains coenzyme B covalently bound through a mixed anhydride linkage to a sugar moiety (Sauer et al., 1990; Sauer, 1991). This is presently no longer considered to be very likely. Recently, methyl-coenzyme M reductase was isolated from Methanobacterium thermoautotrophicum under very mild conditions and then crystallized. The purified enzyme contained coenzyme B bound in its active site. In the crystal structure, the threonine phosphate moiety of coenzyme B could clearly be resolved. The phosphate group of the threonine phosphate moiety was not covalently bound to any other molecule (Ellermann et al., 1997b).
Coenzyme F₄₃₀, the prosthetic group of methyl-coenzyme M reductase

The first report on coenzyme F₄₃₀ (Fig. 5) is by Gunsalus & Wolfe (1978), who observed in cell extracts of Methanobacterium thermoautotrophicum a low-molecular-mass non-fluorescent compound with an absorption maximum at 430 nm. In 1979, Schönheit and others discovered that growth of methanogens is dependent on nickel. Diekert et al. (1980a) and Whitman & Wolfe (1980) then showed that the nickel was in part required for the synthesis of F₄₃₀, in which it was incorporated, and that F₄₃₀ contained mol⁻¹ 1 mol nickel. In the same year, biosynthetic evidence was provided by Diekert et al. (1980b) that F₄₃₀ is a nickel tetrapyrrole, the structure of which was subsequently elucidated in collaboration with the group of A. Eschenmoser at the ETH Zürich (Pfalz et al., 1982; Färber et al., 1991). Finally, in 1982 it was found that F₄₃₀ is the prosthetic group of methylcoenzyme M reductase (Ellefson et al., 1982). Until now, coenzyme F₄₃₀ has been found only in methanogenic archaea in which it is always present (Diekert et al., 1981) and in which its only function appears to be the prosthetic group of methylcoenzyme M reductase.

The presence of a ligand nickel atom is the striking feature of coenzyme F₄₃₀. Nickel brings up to five of different metals found in the centre of biological cyclic tetrapyrroles. Up to the discovery of F₄₃₀, the different metals known to occur were iron in haems and in sirohaem, magnesium in chlorophylls, cobalt in corrinoids and copper in turacin, the pigment of turaco bird feathers. Another striking feature is the state of reduction of the pyrrole ring system. One encounters the interesting fact, possibly related to its anaerobic evolution and function, that coenzyme F₄₃₀ is by far the most highly reduced or saturated amongst all known tetrapyrroles (Fig. 6). Coenzyme F₄₃₀ has only five double bonds, of which two pairs are conjugated, but they are separated by two single bonds and so do not form a larger conjugated bond system. The yellow colour of coenzyme F₄₃₀ in contrast to the red colour of porphyrins and corrinoids, is no doubt related to this low degree of conjugation (Friedmann et al., 1991).

Biosynthetically, coenzyme F₄₃₀ is derived from dihydrosirohydrochlorin, which is also the biosynthetic precursor of sirohaem and B₅₆ (Thauer & Bonacker, 1994; Hungerer et al., 1996).

Coenzyme F₄₃₀, properties relevant to the catalytic mechanism of methyl-coenzyme M reductase

Coenzyme F₄₃₀ is released from methyl-coenzyme M reductase upon denaturation with perchloric acid or trichloroacetic acid. In the released coenzyme, nickel is in the Ni(II) oxidation state. For the understanding of the function of coenzyme F₄₃₀ in methylcoenzyme M reductase, the following properties of the non-protein-bound cofactor are considered to be of importance (Jaun, 1993; Won et al., 1993; Telser, 1998; see also Stolzenberg & Zhang, 1997; Pandiyan et al., 1997; Schmid et al., 1996, 1997; Jäger & Rudolph, 1997). They are discussed in comparison to those of cobalamin (B₁₂) (Kräutler et al., 1998) (Fig. 7).

F₄₃₀ as isolated in the Ni(II) oxidation state can be reduced to the Ni(I) oxidation state. In aqueous solution, the standard redox potential Eₒ of the Ni(II)/Ni(I) couple is between −600 mV and −700 mV (Jaun & Pfaltz, 1986; Holliger et al., 1993) and is thus in the same
Fig. 7. Properties of protein-free coenzyme F₄₃₀ (a) and of cobalamin (b) explaining why the former is a good methyl group reduction catalyst and cobalamin a good methyl group transfer catalyst. CH₃X, methyltosylate, methylhalides or methylsulphonium ions (Jaun, 1991). With methyltosylate, primary methyl-Ni(II) F₄₃₀ is formed spontaneously, yielding a methyl-Ni(II) metalloorganic compound (Jaun & Pfaltz, 1988; Lin & Jaun, 1991). With methyltosylate, primarily methyl-Ni(II) F₄₃₀ must be formed (Jaun, 1993), just as methylcob(III)alamin is formed upon methylation of cob(I)alamin. Methyl-Ni(II) F₄₃₀ is, however, a very labile compound which is predicted to be almost instantaneously reduced to methyl-Ni(II) F₄₃₀ by any electron donor available (Jaun, 1993). Upon methylation of Ni(II) F₄₃₀, only methyl-Ni(II) F₄₃₀ rather than methyl-Ni(III) F₄₃₀ can be detected (Lin & Jaun, 1991). Based on the E₀' of the Ni(III) F₄₃₀/Ni(II) F₄₃₀ redox couple (Jaun, 1990), the E₀' of the methyl-Ni(III)/methyl-Ni(II) couple is estimated to be much more positive than 0 V. In this property, F₄₃₀ differs significantly from cobalamin, the methylated form of which [methylcob(III)alamin] is stable and resistant towards reduction to methyl-cob(II)alamin with electron donors more positive than −650 mV. Methylation of Ni(I) F₄₃₀ to methyl-Ni(III) F₄₃₀ is a nucleophilic substitution which is predicted to proceed with inversion of stereoconfiguration (Lin & Jaun, 1992).

Methyl-Ni(II) F₄₃₀ spontaneously protonolyses to yield CH₄ and Ni(II) F₄₃₀ (Lin & Jaun, 1991). It is an electrophilic substitution which is predicted to proceed with retention of stereoconfiguration. To the contrary, methylcob(II)alamin dissociates to a methyl radical and cob(I)alamin rather than to protonolysate to methane and cob(II)alamin.

These properties of F₄₃₀ indicate that the nickel porphrinoid is a good methyl group reduction catalyst and differs in this property from cobalamin, which is a good methyl transfer catalyst. They are probably relevant to the catalytic mechanism of methyl-coenzyme M reductase: there is evidence that the enzyme only becomes active upon reduction of its prosthetic group from the Ni(II) to the Ni(I) oxidation state (Goubeaud et al., 1997). Furthermore, there is evidence that methylcoenzyme M reductase catalyses the reduction of the methyl group of methyl-coenzyme M to methane with inversion of stereoconfiguration, consistent with Ni(I) F₄₃₀ methylation and methyl-Ni(II) F₄₃₀ protonolysis as steps in the catalytic cycle (Ahn et al., 1991).

**Activation of methyl-coenzyme M reductase by reduction to the Ni(I) oxidation state**

Methyl-coenzyme M reductase isolated from cells of methanogenic archaea is more or less inactive (Gunsalus & Wolfe, 1980; Brenner et al., 1993). The enzyme purified from *Methanobacterium thermoautotrophicum* ΔH requires the addition of at least two proteins, designated A2 and A3a, of ATP and of Ti(III) citrate for activation *in vitro*. Even in their presence, however, only less than 0.1% of the specific activity *in vivo* is reached.

\[
\text{MCR (inactive)} \xrightarrow{A_2, A_{3a}, \text{ATP}} \text{MCR (0-1\% active)}
\]

The sequence of *atu*, the A2-encoding gene, predicts that A2 is a 59 kDa ATP-binding protein related to the ABC family of proteins that participates in energy-dependent transport processes (Kuhner et al., 1993).

It was mentioned above that MCR as isolated in its inactive form contains tightly bound 2 mol coenzyme M (Hartzell et al., 1987). When in the presence of the activating system (A proteins, ATP and an electron donor), the inactive enzyme was incubated with [methyl-¹⁴C]methyl-coenzyme M and component B, the coenzyme M moiety of methyl-coenzyme M was found to slowly exchange into the enzyme-bound coenzyme M during turnover, 150–160 mol CH₄ (mol enzyme)⁻¹ having to be formed before the exchange into the 2 mol bound coenzyme M was complete (Hartzell et al., 1987). In the absence of the activating system and of component
B or in the presence of 2-bromoethanesulphonate, neither an exchange nor methane formation was observed. Apparently exchange was dependent on enzyme turnover. The finding of complete exchange indicates that all the MCR molecules present must have been activated under the assay conditions. The finding that only 0.1% of the maximal possible specific activity was reached (see above) and that it took more than 100 turnovers for complete exchange indicates that the MCR molecules present were not all activated at the same time and that they lost activity again after several turnovers.

The enzyme purified from the Marburg strain of Methanobacterium thermoautotrophicum shows residual activity (0.1 U mg⁻¹) in the absence of A2, A3a and ATP (Ankel-Fuchs & Thauer, 1986). In the presence of an appropriate reductant, the specific activity even increases to 1 U mg⁻¹, which is 2% of that expected from in vivo data (Ellermann et al., 1989). EPR spectroscopic analysis revealed that these enzyme preparations were essentially EPR silent, as were the cells from which the enzyme was purified. This indicates that the inactive methyl-coenzyme M reductase preparations contained F₄300 in the Ni(II) oxidation state.

A first breakthrough was the finding that the specific activity of methyl-coenzyme M reductase was much higher in extracts prepared from cells of Methanobacterium thermoautotrophicum reduced with 100% H₂ prior to harvest. Such reduced cells and the methyl-coenzyme M reductase enriched from such cells exhibited a nickel-based EPR spectrum designated MCRred₁ and the spin concentration of the signal correlated with the specific activity. The highest spin concentration reached was between 10 and 20% and the highest specific activity was between 10 and 20 U per mg purified methyl-coenzyme M reductase (Rospert et al., 1991; Bonacker et al., 1993).

The final breakthrough was the finding that purified methyl-coenzyme M reductase in the MCRox₁ state (see below) can be activated to a specific activity of 100 U mg⁻¹ by reduction with Ti(III) citrate at pH 10. The reduced enzyme exhibited an axial MCRred₁ signal with a spin concentration near to 100% (Goubeaud et al., 1997).

\[
\text{MCRox₁ (inactive)} \xrightarrow{\text{Ti(III):pH 10}} \text{MCRred₁ (100% active)}
\]

Methyl-coenzyme M reductase with a specific activity of 100 U mg⁻¹ shows an EPR spectrum very similar and a characteristic UV/visible spectrum almost identical to that of non-protein-bound coenzyme F₄300 in the Ni(I) oxidation state (Goubeaud et al., 1997). From this finding, it is concluded that the MCRred₁ signal of methyl-coenzyme M reductase is derived from its Ni(I) reduced prosthetic group. From the correlation of the specific activity with the MCRred₁ spin concentration it is deduced that the prosthetic group of methyl-coenzyme M reductase has to be in the Ni(I) oxidation state for the enzyme to be active.

**EPR-signal-exhibiting forms of methyl-coenzyme M reductase: MCRred₁, MCRred₂, MCRox₁, and MCRox₂**

As already indicated, active methyl-coenzyme M reductase exhibits the EPR spectrum MCRred₁. The signal is induced in cells of Methanobacterium thermoautotrophicum growing on 80% H₂/20% CO₂ by gassing with 100% H₂. The axial signal builds up within 10 min. Upon further gassing with 100% H₂, a second methyl-coenzyme M reductase derived signal appears, designated MCRred₂, which is rhombic rather than axial (Albracht et al., 1988; for a Ni(I) macrocyclic complex exhibiting a rhombic EPR spectrum see Suh et al., 1996). Cell extracts of such reduced cells also show a high specific activity and also exhibit the MCRred₁ signal. Upon addition of coenzyme M to the cell extracts, the MCRred₁ signal increases; upon addition of methyl-coenzyme M, the MCRred₂ signal is converted to the MCRred₁ signal (Rospert et al., 1991, 1992).

When gassing of growing Methanobacterium thermoautotrophicum cultures is switched from 80% H₂/20% CO₂ to 80% N₂/20% CO₂, a methyl-coenzyme M reductase derived EPR signal is induced which differs significantly from the MCRred₁ and MCRred₂ signals and which was designated MCRox₁ (Albracht et al., 1986, 1988). This signal is also induced when the cultures of Methanobacterium thermoautotrophicum growing on 80% H₂/20% CO₂ are supplemented with Na₂S (50 mM) (Becker & Ragsdale, 1998). The methyl-coenzyme M reductase in the MCRox₁ form can be purified. The pure enzyme shows only very low specific activity but can be activated by reduction with Ti(III) citrate at pH 10 to the MCRred₁ form (Goubeaud et al., 1997) (see above).

When cultures of Methanobacterium thermoautotrophicum growing on 80% H₂/20% CO₂ are supplemented with Na₂SO₃ (10 mM) or Na₂S₂O₅ (10 mM), a methyl-coenzyme M reductase derived novel EPR signal is induced which is designated MCRox₂. The enzyme in this form can be isolated but is inactive. It cannot be activated by reduction with Ti(III) (Becker & Ragsdale, 1998).

**EPR-silent forms of methyl-coenzyme M reductase: MCRsilent, MCRred₁-silent, MCRred₂-silent, MCRox₁-silent and MCRox₂-silent**

Different EPR-silent forms of methyl-coenzyme M reductase have to be discriminated. These forms all have in common that they show very little activity and are not susceptible to activation by reduction with Ti(III) at pH 10 to the MCRred₁ form. (Residual activity may be due to the presence of traces of EPR active forms.)

The methyl-coenzyme M form referred to as MCRsilent is the one obtained by isolation from cells of Metha-
anobacterium thermoautotrophicum harvested without prior gassing with 100 % H₂ or 20 % CO₂/80 % N₂. The forms designated MCR(red1 silent), MCR(red2 silent), MCR(ox1 silent) and MCR(ox2 silent) are generated spontaneously from the respective EPR active forms when stored under strictly anaerobic conditions at 4 °C: the spin concentration of the different EPR signals decreases by 50 % within only a few hours. In the presence of traces of O₂, the MCR(red1 and MCR(red2 signals are instantaneously quenched (Rospert et al., 1991, 1992) whereas the decay rate of the MCR(ox) signals is similar under anaerobic and aerobic conditions (Goubeaud et al., 1997; Becker & Ragsdale, 1998). The latter finding suggests that the MCR(ox) forms of methyl-coenzyme M reductase could contain F₄₃₀ in the Ni(III) oxidation state. Interestingly, the two MCR(ox) forms exhibit a UV/visible spectrum more closely related to that of Ni(II) F₄₃₀ than to Ni(I) F₄₃₀ or Ni(III) F₄₃₀ (Jaun, 1990, 1993).

Crystal structure of methyl-coenzyme M reductase in the MCR(ox1 silent) state

First crystals of methyl-coenzyme M reductase were obtained in 1991 by Rospert (1991) for the isoenzyme I from Methanobacterium thermoautotrophicum (Marburg). From this time on the enzyme from various methanogens and from various different preparations was crystallized and tested for diffraction. Beginning 1996, the first good crystals were obtained from a highly purified preparation of methyl-coenzyme M reductase isoenzyme I in the MCR(ox1 state from Methanobacterium thermoautotrophicum (Marburg strain) (Shima et al., 1997). The crystal structure was solved in the MCR(ox1 silent state to 1.4 Å resolution (1 Å = 0.1 nm) (Ermler et al., 1997b; see also Ferry, 1997b; and Cammack, 1997). The primary structure of the enzyme had previously been resolved by Bokranz et al. (1988).

Methyl-coenzyme M reductase in the MCR(ox1 silent state contains bound 2 mol coenzyme F₄₃₀, 2 mol reduced coenzyme M and 2 mol reduced coenzyme B as revealed by the X-ray structure.

Overall structure

It is characterized by a series of α helices arranged in a compact form with an ellipsoidal shape of about 120 by 85 by 80 Å. The subunits are mutually tightly associated, as indicated by extended interface areas, particularly between subunits α and α′ and subunits β and β′, and by the fact that, except for subunits γ and γ′, each subunit contacts all other subunits of the multisubunit complex (Ermler et al., 1997b).

Two independent active sites

There are two binding sites, roughly 50 Å apart, for coenzyme F₄₃₀, coenzyme M and coenzyme B, forming two separated structurally identical active sites. In each binding site the three coenzymes are embedded inside a narrow channel, 30 Å long, and at the narrowest part 6 Å in diameter, extending from the protein surface deeply into the interior of the protein complex (see the diagram in Fig. 11). The channel and the coenzyme-binding sites are formed by residues of subunits α, α′, β and γ (and equivalently α′, α′, β′ and γ′), indicating that one trimer is not sufficient to accomplish the enzymic reaction (Ermler et al., 1997b).

Binding of coenzyme F₄₃₀

F₄₃₀ is bound at the bottom of the 30-Å-long channel such that its tetrapyrrole plane points with its front face (reduced pyrrole rings A, B, C and D clockwise) (see Fig. 5) roughly towards the mouth of the channel, whereas its rear face points to the channel bottom. Thus only the front face is accessible to the substrate. Interestingly, in sulphite reductase the prosthetic group sirohaem, which as F₄₃₀ is biosynthetically derived from dihydrosirohydrochlorin, is only accessible from the rear face (Cranne et al., 1995, 1997a, b). The tetrapyrole ring of coenzyme F₄₃₀ is bound in a rather flat conformation to the enzyme as predicted for the free coenzyme F₄₃₀ in the hexagonally coordinated Ni(II) state (Farber et al., 1991; Jaun, 1993). The Ni atom present as Ni(II) sits almost exactly in the tetrapyrole plane and is coordinated to six ligands arranged in a nearly optimal octahedral configuration. The four equatorially located nitrogen atoms of the tetrapyrole ring have distances to the nickel of 2-14 Å for ring A, 2-11 Å for ring B, 2-10 Å for ring C and 1-99 Å for ring D (Fig. 8). As the fifth ligand the side chain oxygen of Glnₙ¹⁴⁷ protrudes from a long loop to the rear face of F₄₃₀ and approaches the Ni(II) atom to 2-3 Å (Fig. 9). The 1-45 Å electron density map demonstrates that the oxygen and not the nitrogen of the side chain Glnₙ¹⁴⁷ is the axial ligand (Ermler et al., 1997b) as to be expected thermodynamically and as seen also in other proteins (Roach et al., 1997). Glnₙ¹⁴⁷ is embedded in the protein matrix such that it will probably not be able to move away when F₄₃₀ is reduced from the Ni(II) to the Ni(I) oxidation state in which the Ni(I) is preferentially tetraplanarly coordinated (Jaun, 1993; Telser et al., 1997). By the enforced axial fifth ligand the nucleophilicity of the Ni(I) in F₄₃₀ is predicted to be increased, facilitating the nucleophilic substitution of the methyl group of coenzyme M.

Binding of coenzyme M

In MCR(ox1 silent), the sixth coordination site of nickel, located in front of the tetrapyrole ring plane of F₄₃₀ is occupied by the thiol group of coenzyme M, which is positioned almost parallel to the tetrapyrole plane in contact with its front face (Fig. 9). The thiol group binds axially to the nickel (2-42 Å distance) and interacts with the hydroxyl group of Tyrₙ²³³ and Tyrₙ⁶⁸ and a water molecule that bridges coenzyme M and B. The ethyl moiety is embedded between the lactam ring of the Ni porphinoid and the phenyl ring of Pheₙ⁴⁴. Coenzyme M is anchored to the polypeptide chain by its negatively charged sulphonate group, forming a salt bridge to the
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Fig. 8. Coenzyme F₄₃₀ in the Ni(II) oxidation state as viewed by looking from outside through the 30-Å-long channel into the active site pocket of methyl-coenzyme M reductase (MCRoxl-silent). The front face (reduced pyrrole rings A, B, C and D clockwise) is seen. Structure at 1.45 Å resolution.

Fig. 9. Coenzyme M located in front of the tetrapyrole ring plane of F₄₃₀ with its thiol group bound to Ni(II) in the active site of methyl-coenzyme M reductase (MCRoxl-silent). Structure at 1.45 Å resolution. The oxygen of Gln¹⁴⁷ is seen to approach the Ni from the rear face of F₄₃₀ to 2.3 Å.

Fig. 10. Heterodisulphide of coenzyme M and coenzyme B bound via an oxygen of its sulphonate group to Ni(II) in the active site of methyl-coenzyme M reductase (MCRoxl-silent). Structure at 1.75 Å resolution.

Binding of coenzyme B

With its elongated conformation, coenzyme B fits accurately into the most narrow segment of the channel formed by residues of subunits α, α' and β (see also the diagram in Fig. 11a). Coenzyme B is anchored to the protein mainly by salt bridges between the negatively charged L-threonine phosphate moiety and five positively charged amino acids. The heptanoyl arm is in van der Waal's contact with several hydrophobic residues. The thiol group of coenzyme B is positioned at a distance of 8.7 Å from the nickel of F₄₃₀ and 6.2 Å from the coenzyme M thiol sulphur. It interacts with the side chain of nitrogen of Asn₂⁴⁸¹, the main chain peptide nitrogen of Val²⁴⁸² and the bridging water molecule mentioned above. Asn₂⁴⁸¹ is within hydrogen bond distance of the sulphur that is presumed to replace the backbone carbonyl oxygen of the modified Gly²⁴⁴⁵ (see below).

Five modified amino acids in the active site region

The electron density map revealed five modified amino acids located in subunits α and α' at or very near the active site region, 1-N-methyl-His²⁵⁷, 4-methyl-Arg²⁷¹, 2-methyl-Gln²⁰⁰, S-methyl-Cys²⁵² and Gly²⁴⁴⁵, where

guanidinium group of Arg¹²⁹, a hydrogen bond to the peptide nitrogen of Tyr²⁴⁴ and a hydrogen bond to a water molecule connected to the peptide oxygen of His³⁶⁴ (Ermler et al., 1997b).
the carbonyl oxygen appears to be substituted by sulphur (Ermel et al., 1997b).

**Exclusion of bulked water from the active site**

When coenzyme B is bound to the enzyme the active site is shielded from bulk solvent. The sole water molecule found in the active site region of MCRox1-silent between coenzyme M and coenzyme B should be displaced after binding of the more bulky methyl-coenzyme M. The active site is lined up by an annular arrangement of Phe230, Tyr233, Phe345, Phe361 and Tyr367 flanked further by hydrophobic and aromatic residues (Ermel et al., 1997b). These amino acids are completely conserved in all methyl-coenzyme M reductases (Nölting et al., 1996). Methane formation from methyl-coenzyme M and coenzyme B thus takes place in a greasy pocket and probably does not involve water molecules. These conditions would allow for radical intermediates. Solvent-inaccessible active sites coated by non-polar aromatic residues and attainable by a channel have been observed in several radical-based enzymes such as galactose oxidase (Ito et al., 1991), prostaglandin H2 synthase 1 (Picot et al., 1994) and methylmalonyl-CoA mutase (Mancia et al., 1996).

**Crystal structure of methyl-coenzyme M reductase in the MCRox2-silent, MCRred2-silent and MCRox1-silent states**

The structure of the MCRox1-silent state was refined in the resolution range 2.0-10.0 Å with the use of the MCRox1-silent structure for initial phase determination (Ermel et al., 1997b). The two enzyme states exhibit nearly identical overall structures. The model of the MCRox1-silent structure is mainly distinguished from that of MCRox1-silent by binding of the oxidized instead of the reduced forms of coenzyme M and coenzyme B (Fig. 10). A superposition of the structures reveals that the reduced coenzyme B in MCRox1-silent and the coenzyme B moiety of the heterodisulphide in MCRox1-silent align perfectly except that the sulphur is turned slightly towards the tetrapyrrole plane of F430, resulting in a 90° rotation of coenzyme M. In contrast to coenzyme B, coenzyme M has moved more than 4 Å away from its position in the MCRox1-silent state. The thiol group is shifted perpendicular and the sulphonate group parallel to the tetrapyrrole plane of F430, resulting in a 90° rotation of coenzyme M. In this position, one oxygen atom of the sulphonate is axially coordinated with the nickel and the sulphur is turned slightly towards the hydroxyl group of Tyr367 and the third to a water molecule located at the former binding site of the sulphonate.

The structures of methyl-coenzyme M reductase in the MCRred2-silent and MCRox2-silent states have, in the meantime, also been resolved but have not yet been completely refined. Available information indicates that in the MCRred2-silent state, the active site of methyl-coenzyme M reductase is probably devoid of both coenzyme M and coenzyme B. The 30-Å-long channel leading into the active site pocket is more open and the residues of the α- , α'-, β- and γ-subunits forming the channel are more flexible. In the MCRox2-silent state, methyl-coenzyme M reductase appears to contain bound coenzyme B but not coenzyme M (W. Grabarse, unpublished results).

**Catalytic mechanism proposed for methyl-coenzyme M reductase**

The crystal structures display inactive states of methyl-coenzyme M reductase with coenzyme F430 in the Ni(II) oxidation state. Nevertheless, the arrangement of the coenzymes and the protein environment, combined with the catalytic properties of the enzyme and the chemical properties of free coenzyme F430, allow for conclusions about the active site and the catalytic mechanism (Ermel et al., 1997b).

First the structure of the enzyme–substrate complex and of the enzyme–product complex and their formation and dissociation, respectively, will be discussed. Then two alternative mechanisms for the conversion of the enzyme–substrate to the enzyme–product complex will be proposed.

**Enzyme-substrate/product complex**

In Fig. 11, diagrams of the enzyme–substrate complex and of the enzyme–product complex are given. The prosthetic group is shown in the Ni(I) oxidation state. This is deduced from the finding that methyl-coenzyme M reductase only exhibits activity when assayed in its Ni(II) F430 reduced form (either MCRred1 or MCRred2 state) (Rospert et al., 1991, 1992; Goubeaud et al., 1997).

The diagram in Fig. 11 shows that the prosthetic group of methyl-coenzyme M is accessible only through a narrow channel and only by methyl-coenzyme M (Ermel et al., 1997b). Through this channel methyl-coenzyme M must enter before coenzyme B binds since upon coenzyme B binding the channel is completely locked. This is consistent with an ordered ternary complex kinetic mechanism displayed by the enzyme (Bonacker et al., 1993).

Coenzyme B is able to penetrate the 30-Å-long channel only with its aliphatic arm and its arm is not long enough for its thiol group at the end to reach the Ni of F430. A distance of 8.7 Å remains. A direct reaction of the coenzyme B thiol group with nickel, as has previously been proposed (Jaun, 1990; Berkessel, 1991), can therefore be excluded. Methyl-coenzyme M is, however, anchored in the active site to the protein matrix with its sulphonate group relative to the prosthetic group such that both the methyl group and the thioether sulphur could directly interact with the Ni(II). In the MCRox1-silent structure, coenzyme M probably mimics the binding position of methyl-coenzyme M with respect to the binding mode of the sulphonate moiety but
presumably not with respect to the binding mode of the thiol group. A Ni-S-CoM intermediate in the catalytic cycle is not attractive because of the long distance of 6.2 Å between the sulphurs of coenzyme M and coenzyme B. Model building studies indicate, however, that the two sulphurs of coenzyme M and coenzyme B come in van der Waal’s contact when the methyl group of methyl-coenzyme M is placed in van der Waal’s distance of the potentially attacking nickel (Fig. 11a) (Ermel et al., 1997b). Therefore, a Ni-CH₃ intermediate proposed from free coenzyme F₄₃₀ studies (Lin & Jaun, 1991; Jaun, 1993) appears to be compatible with the steric requirements of the active site.

From the structure of the enzyme–substrate complex shown in Fig. 11(a) it is evident that the H⁺ required for methane formation from methyl-coenzyme M is lastly provided by coenzyme B. The MCRₙₓₙ₁ₛᵣₜₒₜₜ structure reveals an interaction between the thiol group of coenzyme B and two hydrogen donors, the amide and peptide nitrogen of Asn₄₄₈ and Val₄₈₅, which would facilitate the cleavage of the H⁺ and permit the presence of a thiolate anion. However, the coenzyme B sulphur is probably too far away from the methyl group of methyl-coenzyme M for direct hydrogen transfer. Tyr₃₃₅ and Tyr₇₆₇ are positioned in the active site such that they could mediate this transfer (Ermel et al., 1997b).

The structure of the enzyme–product complex shown in Fig. 11(b) considers that the heterodisulphide cannot leave the enzyme when the sulphonate oxygen of the coenzyme M moiety binds to the Ni(II) atom of F₄₃₀ as in the MCRₙₓₙ₁ₛᵣₜₒₜₜ state. Coordination of the sulphonate oxygen to nickel is prevented when nickel is present as nucleophilic Ni(I) (Jaun, 1993; Telser et al., 1997). The repulsion between Ni(I) and the sulphonate oxygen might even be used as a driving force to push the heterodisulphide out of the channel. It is not evident at present where, and how the methane gets out (Ermel et al., 1997b).

Both mechanisms of the conversion of the enzyme–substrate complex (Fig. 11a) to the enzyme–product complex (Fig. 11b) in the following to be discussed are based on the assumption that the methyl group of methyl-coenzyme M is transferred to Ni(I) of the prosthetic group before being reduced to methane and that the methyl group of methyl-coenzyme M has to be somehow activated in order that the transfer to Ni(I) F₄₃₀ can proceed (Jaun, 1993).

**Mechanism 1**

It is assumed that methyl transfer from methyl-coenzyme M to Ni(I) yields CH₃-Ni(III) F₄₃₀ involving protonation of methyl-coenzyme M by Tyr₃₃₅ or Tyr₇₆₇ to the sulphonium cation in the transition state. A precedent for such a protonation is found in the crystal structures of ortho-hydroxyphenyl- or ortho-hydroxybenzyl thioethers, giving rise to 5- and 6-membered rings, respectively, but also in the crystal structures of 3,4-dihydro-6-hydroxy-4,5,7,8-pentamethyl-2H-1-(benzothio)pyran (d S-H = 2.55 Å, O-H-S = 148 °) (Burton et al., 1985) and 2,3,7,8-tetrahydroxanthrene (d S-H = 2.53 Å, O-H-S = 167 °) (Mansel & Klar, 1992.) The H⁺ would be replenished by the dissociation of coenzyme B.

\[
\text{CH}_3\text{-S-CoM} + \text{H}^+ + \text{Ni(I)} \rightarrow \text{CH}_3\text{-Ni(III)} + \text{H}_2\text{O} + \text{S-CoB} \]

The CH₃-Ni(III) F₄₃₀ formed by methylation of Ni(I) F₄₃₀ with methyl-coenzyme M is a strong one-electron oxidant and is therefore predicted to oxidize the protonated leaving group to the coenzyme M thyl radical cation.

\[
\text{CH}_3\text{-Ni(III)} + \text{H}_2\text{O} \leftrightarrow \text{CH}_3\text{-Ni(III)} + \text{H}^+ \rightarrow \text{CH}_3\text{-Ni(II)} + \text{H}_2\text{O} + \text{S-CoB} \]

\[
\text{CH}_3\text{-Ni(III)} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{-Ni(II)} + \text{H}_2\text{O} + \text{S-CoB} \]

\[
\text{CH}_3\text{-Ni(III)} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{-Ni(II)} + \text{H}_2\text{O} + \text{S-CoB} \]

\[
\text{CH}_3\text{-Ni(III)} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{-Ni(II)} + \text{H}_2\text{O} + \text{S-CoB} \]

\[
\text{CH}_3\text{-Ni(III)} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{-Ni(II)} + \text{H}_2\text{O} + \text{S-CoB} \]

\[
\text{CH}_3\text{-Ni(III)} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{-Ni(II)} + \text{H}_2\text{O} + \text{S-CoB} \]

\[
\text{CH}_3\text{-Ni(III)} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{-Ni(II)} + \text{H}_2\text{O} + \text{S-CoB} \]
CH$_3$-Ni(II) F$_{430}$ spontaneously protonolyses to CH$_4$ and Ni(II) F$_{430}$. The H$^+$ could be provided by the coenzyme M thyl radical cation, which is more acidic than coenzyme M.

CH$_3$-Ni(II) F$_{430}$ + H$^+$ → CH$_4$ + Ni(II) F$_{430}$

The catalytic cycle in the alternative mechanism is therefore assumed to start with the formation of the coenzyme B thyl radical by one electron oxidation of coenzyme B.

H-S-CoB + X → 'S-CoB + XH

The electron acceptor X cannot be Ni(II) F$_{430}$ as has been proposed since the enzyme is only active in its Ni(I) reduced form (Goubeaud et al., 1997). Therefore, there must be a second redox active group capable of oxidizing coenzyme B to the coenzyme B thyl radical, and indeed there is evidence for two independent redox active groups in methyl-coenzyme M reductase (Rospert, 1991; Rospert et al., 1992). This group could be the modified Gly$^{244}$, a thioglycine, which interacts with the thiol group of coenzyme B via the side chain nitrogen of Asn$^{248}$ and which could be susceptible to reduction to the thioethyl radical X$^-$ (Buckel & Keese, 1995; Buckel, 1996). Probably the protonated thioketyl would be reduced to the protonated thioethyl (XH) since this reaction is energetically more favourable than deduced from the thermodynamics of the reduction of ketones to ketyl in the protonated and unprotonated forms (Lenz & Giese, 1997).

The coenzyme B thyl radical would react with methyl-coenzyme M, generating the above-mentioned sulphuranyl radical. From the sulphuranyl radical, the methyl radical would be transferred to Ni(I) F$_{430}$, generating the heterodisulphide and CH$_3$-Ni(I) F$_{430}$ which would protonolyse to Ni(II) F$_{430}$ and CH$_4$.

CoM-S(Ch$_3$)-'S-CoM + Ni(II) F$_{430}$ →

CoM-S-S-CoB + CH$_3$-Ni(II) F$_{430}$

CH$_3$-Ni(II) F$_{430}$ + XH → Ni(II) F$_{430}$ + CH$_4$ + X$^-$

The catalytic cycle would be closed by electron transfer from X$^-$ to Ni(II) F$_{430}$.

X$^-$ + Ni(II) F$_{430}$ → X + Ni(I) F$_{430}$

Via endergonic XH dissociation to X$^-$ + H$^+$, the two last reactions could be thermodynamically tightly coupled.

The salient feature of mechanism 2 is that it involves transient methyl radical intermediates and does not involve CH$_3$-Ni(II) F$_{430}$ as intermediate in the catalytic cycle. Transient methyl radical intermediates could possibly account for the presumed methylation of His$^{2257}$, Arg$^{2271}$, Gln$^{2460}$ and Cys$^{442}$.

Future studies will have to show which of the proposed catalytic mechanisms is correct or whether both are wrong. Unfortunately, one can only disprove but not prove a catalytic mechanism (Stinson, 1995).

### From hydrogenase to methyl-coenzyme M reductase

It has been a long way from the first demonstration of an enzyme, of hydrogenase, in methanogens in 1933 by Stephenson & Stickland (1933a) to the crystal structure of methyl-coenzyme M reductase in 1997 and there is still a good way to go until the biochemistry of methanogenesis is completely understood. Many ques-
tions remain to be answered. For methyl-coenzyme M reductase, the most pertinent questions are, what functions do the five amino acid modifications in the enzyme have and how are these modifications brought to the understanding of the catalytic mechanism of the reductase, the most pertinent questions are, what about

The work of my group on the biochemistry of methanogenesis started with growth experiments and the discovery that growth of methanogens is dependent on nickel (Schönheit et al., 1979). This led to the finding that coenzyme F₄₈₀ is a nickel porphinoid (Diekert et al., 1980a, b), that carbon monoxide dehydrogenase from methanogens contains nickel (Hammel et al., 1984) and that the hydrogenases first described by Marjory Stephenson (Stephenson & Stickland, 1931a) are nickel enzymes (Graf & Thauer, 1981; Albracht et al., 1982; Albracht, 1994) (for recent reviews on nickel enzymes including methyl-coenzyme M reductase see Cammack & van Vliet, 1998; Maroney et al., 1998). Later we found that methanogens also contain a metal-free hydrogenase (Zirngibl et al., 1990; Thauer et al., 1996) and just recently that methanogens contain a fourth type of hydrogenase (Künkel et al., 1998) that shows sequence similarity to hydrogenase 3 (Sauter et al., 1992) and 4 (Andrews et al., 1997) of the formate hydrogenlyase from Escherichia coli, an enzyme complex first studied in detail in the laboratory of Marjory Stephenson now almost 70 years ago (Stickland, 1929; Stephenson & Stickland, 1932, 1933b; Woods, 1936). With respect to my research, I, therefore, feel myself in the scientific tradition of Marjory Stephenson. And there is even a genealogical connection: I learned to grow methanogens from Greg Zeikus when he was in Marburg for a sabbatical (Zeikus et al., 1977). He himself had obtained his training from Ralph Wolfe in Urbana (Zeikus & Wolfe, 1972), who discovered methyl-coenzyme M reductase (Wolfe, 1991). The first work of Ralph Wolfe on methanogens is together with Sidney Elsdon (Knight et al., 1966), who had been a student of Marjory Stephenson in the Cambridge Biochemistry Laboratory (Wolfe, 1991) where in 1933 the modern era of methanogenesis began (Wolfe, 1993). I am therefore very much indebted to the Society for General Microbiology for having chosen me to give the 1998 Marjory Stephenson Lecture.

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