General introduction

Abbreviations used: AdoMet, S-adenosylmethionine; B_{12}-DMBI, cobalamin, 5,6-dimethylbenzimidazolylcobamide; B_{12}-HBI, 5-hydroxybenzimidazolylcobamide; CODH, carbon monoxide dehydrogenase; Ni/Fe-S, nickel/iron-sulfur component of CODH; Co/Fe-S, corrinoid/iron-sulfur component of CODH; DEAE, diethylaminoethyl; DMBI, 5,6-dimethylbenzimidazole; EPR, electron paramagnetic resonance; F_{430}, a nickel-tetrapyrrole derivative; H_4MPT, 5,6,7,8-tetrahydromethanopterin; H_4SPT, 5,6,7,8-tetrahydrosarcinapterin; HBI, 5-hydroxybenzimidazole; HS-CoA, coenzyme A; HS-CoM, coenzyme M, 2-mercaptoethanesulfonic acid; CH_3-S-CoM, methylated coenzyme M, 2-(methylthio)ethanesulfonic acid; HS-HTP, 7-mercaptoheptanoylthreonine phosphate; CoM-S-S-HTP, the heterodisulfide of HS-CoM and HS-HTP; MAP, methyltransferase activation protein; MFR, methanofuran; MT_1, methanol:5-hydroxybenzimidazolylcobamide methyltransferase; MT_2, Co-methyl-5-hydroxybenzimidazolylcobamide: HS-CoM methyltransferase; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate

1.1. Methanogenic archaea

The production of methane as a major catabolic product is unique to methanogens, that differ in a number of aspects from other micro-organisms. Phylogenetically, methanogens belong to the Archaea [84,85], the third grouping next to Eucarya and Bacteria (Fig. 1). Archaea are characterized by a number of specific properties. The membranes contain lipids composed of isoprenoids ether-linked to glycerol or other carbohydrates [16]. In addition, the cell walls are composed of proteins, glycoproteins, or polysaccharides [35], and lack peptidoglycan containing muramic acid [34]. Furthermore, archaea contain DNA-dependent RNA polymerases that are insensitive to the antibiotics rifampicin and streptolydigine [88]. In the archaea two major lineages can be distinguished (Fig. 1). One, comprises most of extremely thermophilic, sulfur-dependent micro-organisms for which the name crenarchaeota is suggested [85]. It is a physiologically

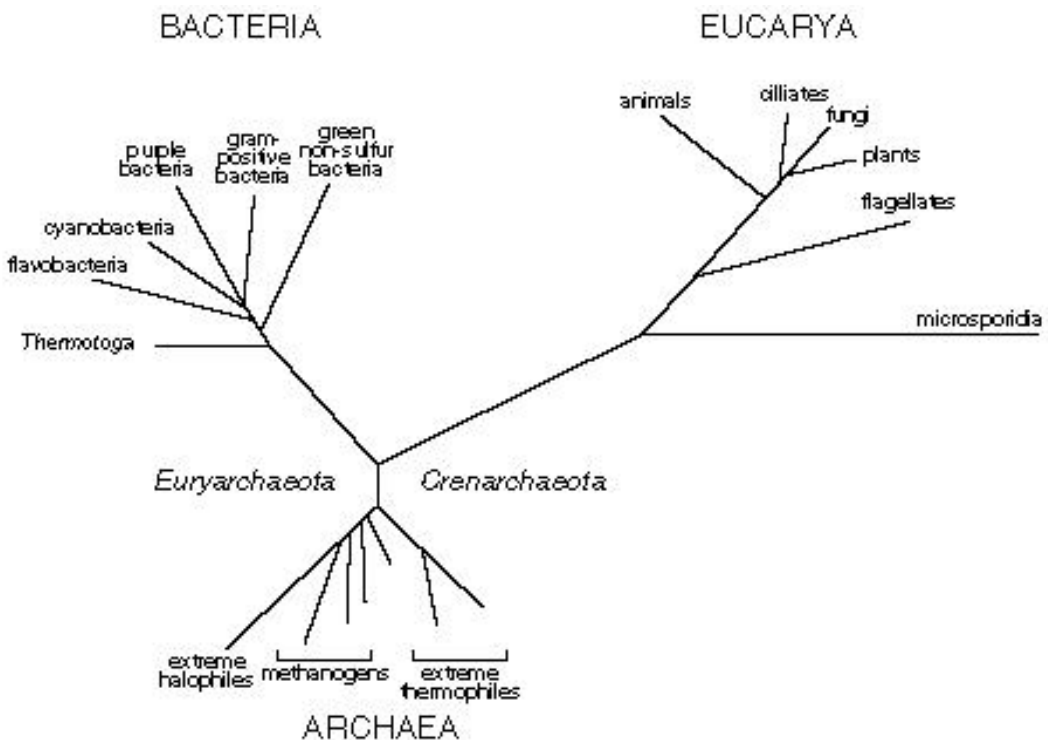

Fig. 1. Universal phylogenetic tree showing the three domains. Branching order and branch lengths are based on 16S rRNA sequence comparison [84,85].

Table 1. *Conversion of methanogenic substrates.*

					$\Delta G^{\circ\prime}$ (kJ/mol CH_4)
CO_2	+	$4H_2$	⟶	$CH_4 + 2H_2O$	-130.4
4HCOOH			⟶	$CH_4 + 3CO_2 + 2H_2O$	-119.5
4CO	+	$2H_2O$	⟶	$CH_4 + 3CO_2$	-185.5
$2CH_3CH_2OH$	+	CO_2	⟶	$CH_4 + 2CH_3COOH + H_2O$	-116[a]
CH_3COO^-	+	H^+	⟶	$CH_4 + CO_2$	-36.0
$4CH_3OH$			⟶	$3CH_4 + CO_2 + 2H_2O$	-106
CH_3OH	+	H_2	⟶	$CH_4 + H_2O$	-112.5
$4CH_3NH_2$	+	$2H_2O$	⟶	$3CH_4 + CO_2 + 4NH_3$	-76.7
$2(CH_3)_2NH$	+	$2H_2O$	⟶	$3CH_4 + CO_2 + 2NH_3$	-74.8
$4(CH_3)_3N$	+	$6H_2O$	⟶	$9CH_4 + 3CO_2 + 4NH_3$	-75.8
$4CH_3SH$	+	$2H_2O$	⟶	$3CH_4 + CO_2 + 4H_2S$	-51
$2(CH_3)S$	+	$2H_2O$	⟶	$3CH_4 + CO_2 + 2H_2S$	-52.2

[a] Other short-chain alcohols like isopropanol may also be utilized

relatively homogeneous group, whose niches are entirely thermophilic [84]. The other group is phenotypically heterogeneous, comprising the extreme halophiles, the sulfate-reducing species (the genus *Archeoglobus*), two types of thermophiles (the genus *Thermoplasma* and the *Thermococcus-Pyrococcus* group), and three methanogenic lineages (the *Methanococcales*, the *Methanobacteriales*, and the *Methanomicrobiales*). For this group the name euryarchaeota is proposed [85].

The methane-producing archaea can truly be termed cosmopolitan. Methanogens exhibit extreme habitat diversity. Species have been isolated from virtually every habitat in which anaerobic biodegradation of organic compounds occurs, including freshwater and marine sediments, digestive and intestinal tracts of animals and insects, and anaerobic waste digesters [89]. Additional isolates have also been obtained from extreme environments such as geothermal springs and both shallow and deep-sea hydrothermal vents [89]. Morphologically, methanogens are also divers and occur as rods, cocci, packets of cocci (sarcinas), filaments, spirilla, and angular plates [58]. The physiologically most salient feature is the extreme catabolic specialization of methanogens. As a group they can only use a small number of simple compounds for methanogenesis and growth (Table 1). Many methanogens use only one or two substrates. A major consequence of this substrate specialization is that in most anaerobic habitats, methanogens are dependent on other organisms for their substrates. Therefore, a food web of interacting groups of anaerobes is required to convert most

organic matter to methane. During the last 5 years, methanogens were isolated that where able to convert substrates like methane thiol, dimethylsulfide, tetramethylammonium, and secondary alcohols [48,67, 83]. The latter alcohols are solely used as electron donors. The broadest versatility in substrate use is displayed by the methyl-group utilizing methanogens, such as *Methanosarcina barkeri*. This organism is able to grow on H_2/CO_2, methanol, acetate, CO, and mono-, di- and trimethylamine. The conversion of methanol by *M. barkeri* strain MS (DSM 800) [13] is studied in this thesis.

1.2. Corrinoids

Since the pioneering studies of Barker and associates [43], methanogens are known to contain considerable amounts of corrinoids, compounds that are structurally related to vitamin B_{12} [42,51]. In order to discuss their role in methanogenesis from methanol, a short general introduction to the corrinoids seems appropriate.

Almost 50 years ago, the red cobalt complex vitamin B_{12} was firstly isolated as the antipernicious factor present in liver. By 1956 it was structurally characterized as a porphyrin-like nucleotide-containing complex, in which pyrroles are linked by three methine bridges and one direct bond [18]. This so-called corrin [90] contains a cobalt coordinated to the nitrogen atoms of the pyrroles (Fig. 2). The cobalt atom may occur in three redox states, Co(III), Co(II), and Co(I), which display quite different chemical properties. Co(III) is six-coordinated by four equatorial nitrogens of the tetrapyrroles and by two axial ligands. In vitamin B_{12} the lower or α-ligand is 5,6-dimethylbenzimidazole, whereas the upper or β-ligand is a cyanide group (Fig. 2). Corrinoids in which the base is bound to the cobalt atom are named "base-on". When coordination is absent or replaced by an exogenous ligand the designation "base-off" is used. One-electron reduction of Co(III) results in a pentacoordinated Co(II) corrinoid, which has only one axial ligand. Reduction of Co(II) results in the formation of the square-planar, four-coordinated Co(I) corrinoid, one of the strongest nucleophiles in nature [18]. No α- or β-ligands are present. Oxidoreductive conversions between the three redox states of cobalt are of key importance in the biochemistry of corrinoids [59].

The various corrinoids isolated over the years usually differ in their α-ligand (Fig. 2) [25]. The most abundant corrinoid encountered in nature, called cobalamin, contains 5,6-dimethylbenzimidazole as its base [25]. Methanogens do not contain cobalamins, but instead three

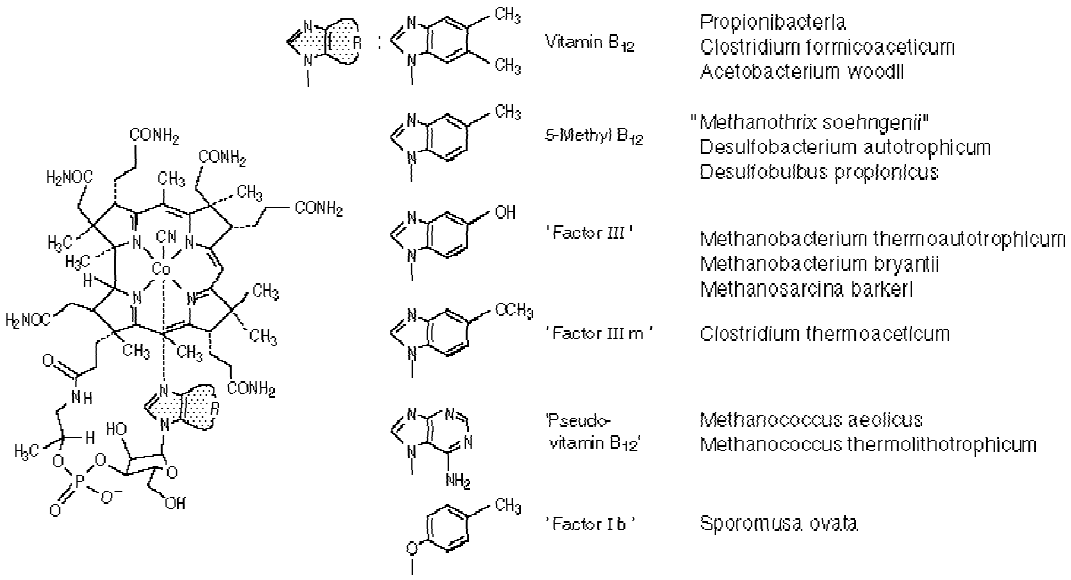

Fig. 2. Structure of corrinoids from bacterial origin showing the variability of the nucleotide portion.

other types of cobamides, base-containing corrinoids, have been found: factor III, pseudo-vitamin B_{12} and 5-methylbenzimidazolylcobamide. Factor III, which contains 5-hydroxybenzimidazole (HBI) as a base, is the prevalent cobamide found in the *Methanobacteriales* and the *Methanomicrobiales*. Its presence seems to be restricted to the methanogens [65]. Since the term factor III is also used to denominate an intermediate in cobyrinic acid biosynthesis, the abbreviation B_{12}-HBI is preferred [51]. Pseudo-vitamin B_{12}, containing adenine as a base, is the predominant cobamide of the *Methanococcales* though it is also found in *Bacteria* [65]. 5-Methylbenzimidazolylcobamide was isolated from "*Methanothrix*" *(Methanosaeta) soehngenii*, and this corrinoid is present in non-methanogens as well [41]. Obviously, cobamides of different structure occur in methanogens. Whether these variations in base composition effect the respective reactions of the cobamides, is not clear. It is notable, however, that vitamin B_{12} could be substituted for B_{12}-HBI in *Methanobacterium thermoautotrophicum* without affecting the growth rate [66].

Over the years several β-ligands have been described, including water, CN^-, OH^-, NO_2^-, SO_3^- [25,50], but only two have been shown to perform a coenzyme function, viz. methyl-B_{12} and 5-deoxyadenosyl-B_{12} [18]. The latter derivative, also known as coenzyme B_{12}, serves as a cofactor in various enzymatic reactions in which a hydrogen atom is displaced by a substituent on an adjacent carbon atom [2]. The C-C

rearrangement reactions proceed via a radical reaction induced by the protein-bound coenzyme [45]. As yet, this type of B_{12}-mediated reaction has not been encountered in methanogens and will therefore not be considered further. The methylated corrinoids are catalysts in biological methyl transfer reactions [44,59] that will be discussed below in some detail.

1.3. Biochemistry of methanogenesis

The results of the investigations on the biochemistry of methanogenesis have been thoroughly reviewed during the past decade [7,21, 22,36,69,82]. In this process, three basic metabolic pathways can be distinguished: the pathway from H_2/CO_2, from acetate, and from methanol. Methanogenic processes using other substrates are less well studied but seem to have many properties in common with these basic pathways [81]. In the following sections an overview will be given of the methanogenic metabolism of *M. barkeri*, notably methanogenesis from methanol. Methane production from acetate, H_2/CO_2, methylamines, and methylsulfides will only be briefly discussed with emphasis on the methyltransferase reactions involved.

1.3.1. Methanol reduction to methane

In 1938 Schnellen [54] obtained a pure culture of a sarcina-like methanogen, which he named *Methanosarcina barkeri* [13] and which was able to grow at the expense of methanol. From that time on, many attempts were made to elucidate the pathway by which methanol was reduced to methane. After the discovery that methylcobalamin could serve as a precursor for methanogenesis in extracts of *M. barkeri*, Blaylock and Stadtman [10] suggested that methanol fermentation might involve an enzyme-catalyzed transfer of the methyl group from methanol to cobalamin, followed by a reductive methylation to methane. Later they reported the enzyme-catalyzed formation of methylcobalamin from cob(I)alamin and methanol [11], and the system involved was resolved into four components [9,12]: a high-molecular weight corrinoid-containing enzyme, a ferredoxin-like compound, a protein with unknown function, and a heat- and acid-stable cofactor. The formation of methylcobalamin from methanol and electrochemically prepared cob(I)alamin required the presence of those four components as well as ATP and H_2. Another indication of the possible

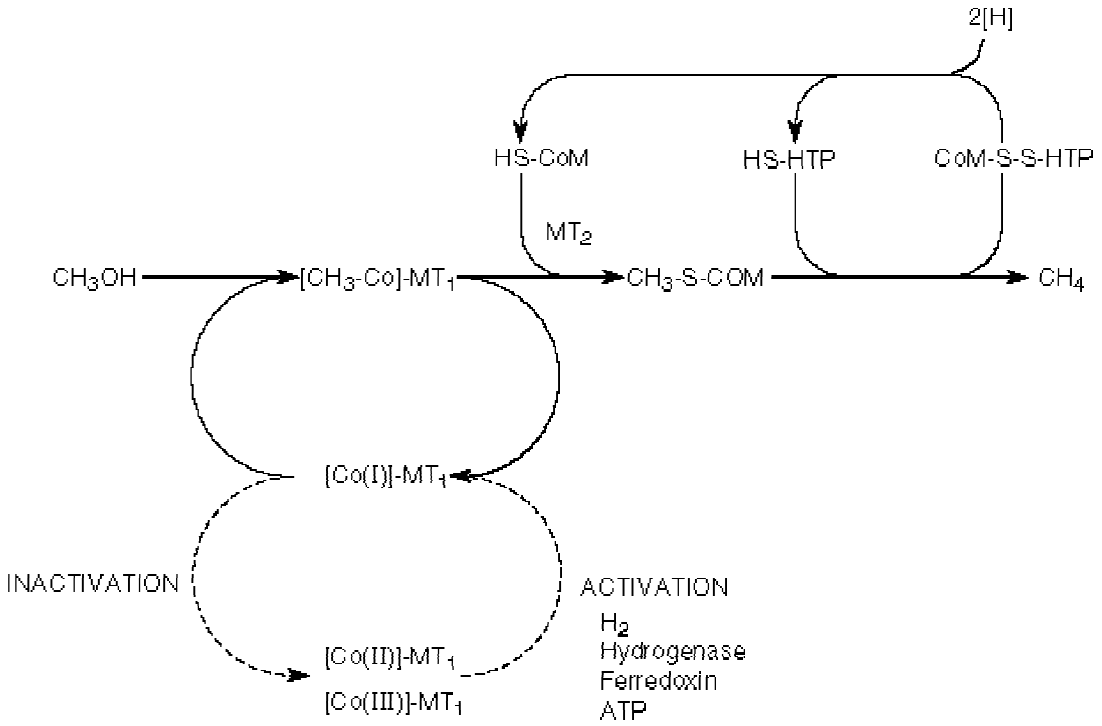

Fig. 3. Scheme of methanogenesis from methanol. The cycle of activation-inactivation of methanol:5-hydroxybenzimidazolylcobamide methyltransferase (MT_1) is indicated by the dashed lines. [Co(III)], [Co(II)], and [Co(I)] represent the various oxidation states of the cobalt of the corrinoid prosthetic groups of MT_1. MT_2, Co-methyl-5-hydroxybenzimidazolylcobamide:coenzyme M methyltransferase; HS-CoM, coenzyme M; HS-HTP, 7-mercaptoheptanoylthreonine phosphate.

involvement of corrinoids was the presence of rather copious amounts of B_{12}-HBI. In *M. barkeri* grown on methanol 4.1 nmol of corrinoid per milligram of cell dry weight was found [42]. After the discovery of coenzyme M (HS-CoM) and its central role as methyl carrier in methanogenesis, the role of B_{12} in the process became uncertain [55]. Studies on the corrinoid protein of *M. barkeri* continued, though quite some doubt remained [86]. The work of Van der Meijden et al. definitely established a role for B_{12}-HBI in the conversion of methanol [72]. Today, the role of corrinoids in methanogenesis is, finally, fully appreciated.

A scheme of the reactions involved in the conversion of methanol to methane in *M. barkeri* is presented in Fig. 3. Reduction of methanol to methane proceeds via methylated coenzyme M (CH_3-S-CoM) [57], which is synthesized from methanol and 2-mercaptoethanesulfonic acid (HS-CoM) by the combined action of two methyltransferase [73]. The first methyltransferase, methanol:B_{12}-HBI methyltransferase (MT_1),

contains a corrinoid which is methylated by methanol, when it is present in its fully reduced Co(I) state. Next, the methyl group is transferred to HS-CoM by Co-methyl-5-hydroxybenzimidazolyl-cobamide:HS-CoM methyltransferase (MT_2). Both enzymes are insensitive to 2-bromoethanesulfonic acid, the typical inhibitor of the CH_3-S-CoM reductase system.

MT_1 has been purified from *M. barkeri* by a factor 5.7, as determined from the increase of the specific corrinoid content [76]. Nevertheless, the enzyme preparation was over 90% pure, which indicates MT_1 comprises of about 15% of the soluble protein in the cell-extract. Purification required strict anoxic conditions and, even then, major loss in activity occurred. Native MT_1 showed an apparent molecular mass of 122 kDa and an $\alpha_2\beta$ configuration with two subunits of 34 kDa and one subunit of 53 kDa [76]. Per molecule of protein 3.4 molecules of B_{12}-HBI were found. The corrinoid was tightly, but not covalently bound to the holoenzyme, and it was completely lost upon dissociation of the enzyme into its subunits by treatment with SDS and 2-mercaptoethanol [76]. For stability, the enzyme required the presence of divalent cations, preferably Mg^{2+}. MT_1 is only active if the corrinoids are present in the fully reduced Co(I) state [75]. In the presence of viologen dyes, flavins, or oxygen oxidation of the reduced corrinoids readily occurs. Yet, reactivation is possible by the combined action of ATP and a reducing system consisting of H_2, hydrogenase, and ferredoxin [74,77]. ATP is required in only catalytic amount. Its role remained, however, unknown [56,72,76,78]. GTP, CTP and, to some extent, UTP were able to substitute for ATP in the resolved system [76]. In crude extracts, hydrogen could be replaced by CO [76] and pyruvate plus coenzyme A (HS-CoA) [74], but not by the powerful reducing agent titanium(III)-citrate [78]. Addition of a protein fraction which was eluted just in front of MT_1 during a first DEAE-cellulose purification step, stimulated the overall methyltransferase reaction when added together with hydrogenase and ferredoxin [76]. The fraction contained at least one protein component, called component S. Activity was sensitive to boiling and oxygen. The protein involved was not further characterized [68].

As a result of the MT_1 reaction, an enzyme containing a firmly bound methyl-corrinoid is obtained [75]. Methylated MT_1 and non-protein-bound methylcobalamin both are substrates of the second methyltransferase MT_2 [73]. The protein is highly specific for HS-CoM as methyl group acceptor [73,87]. MT_2 is insensitive to oxygen and is composed of a single polypeptide with a molecular mass of 38 kDa [87]. In *M. barkeri* two isoenzymes are present that differ in electrophoretic and immunological properties and that can be separated by hydroxylapatite

chromatography [27]. The N-terminal amino acid sequences of the isoenzymes are only 55% similar [87]. Isoenzyme I predominates in methanol-grown cells (about 90%), whereas isoenzyme II is mainly present (about 80%) in cells grown on H_2/CO_2, trimethylamine or acetate [27,87]. For the MT_2 isoenzyme I a function in the conversion of methanol and for the MT_2 isoenzyme II a function in the conversion of trimethylamine has been suggested [87].

In the final step of methanogenesis CH_3-S-CoM is reduced to methane by the methylreductase system. This system has been extensively studied in *M. thermoautotrophicum* [70]. In this organism it consists of four enzymes or enzyme complexes catalyzing the following reactions. (i) The reduction of CH_3-S-CoM by 7-mercaptoheptanoylthreonine phosphate (HS-HTP) which results in the generation of methane and the heterodisulfide of HS-CoM and HS-HTP (CoM-S-S-HTP). (ii) The

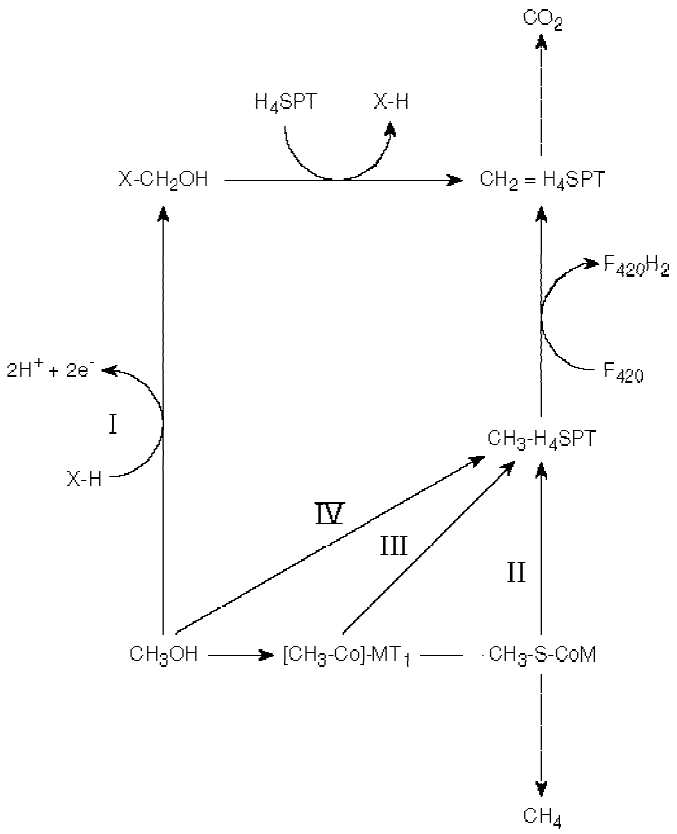

Fig. 4. Alternative pathways for the methanol methyl group oxidation. Roman numbers next to arrows indicate the different routes proposed in the text. H_4SPT, 5,6,7,8-tetrahydrosarcinapterin; F_{420}, a 8-hydroxy-5-deazaflavin derivative; X, unknown one-carbon carrier suggested by Blaut and Gottschalk [8].

reduction of a still unknown electron acceptor by hydrogenase. (iii) Transfer of the electrons of the unknown electron acceptor to a still unknown electron donor. (iv) Reduction of CoM-S-S-HTP by the unknown electron donor. Reaction (iv) and probably also reaction (iii) play a role in energy conservation [46]. The enzyme methylcoenzyme M reductase catalyzes the reductive demethylation of CH_3-S-CoM, reaction (i), and contains, as prosthetic group, 2 molecules of a nickel-tetrapyrrole called F_{430}. In cell-free and resolved systems methylcoenzyme M reductase is generally almost completely inactive. The enzyme can be reactivated to a few percent of the *in vivo* activity in an H_2- and ATP-dependent enzyme-catalyzed reaction [30]. The activation mechanism is poorly understood. Since active methylcoenzyme M reductase contains F_{430} in the highly reduced Ni(I) state [53] activation might imply the reduction of as-isolated Ni(II) into Ni(I).

1.3.2. Methanol oxidation to CO_2

In absence of hydrogen, *M. barkeri* oxidizes part of the methanol to CO_2 to generate the reducing equivalents needed for the reduction of the remainder of the methanol to methane. The oxidation route of methanol is not fully understood. Early experiments with [^{14}C]methanol revealed incorporation of label in "yellow fluorescent compound" as one of the intermediates in methanol oxidation [15]. Following its structural elucidation the compound is now known as 5,10-methenyl-5,6,7,8-tetrahydrosarcinapterin (methenyl-H_4SPT) [71] (see below). Experiments with whole cells of *M. barkeri* and *Methanosarcina mazei* strain Gö1 conclusively showed that a reaction between methanol and the formal redox level of formaldehyde is driven by a sodium-motive force [46]. The reaction (sequence) remains to be established and only a number of possible alternatives may be formulated (Fig. 4). One alternative (I) is the oxidation of methanol by use of a methanol dehydrogenase and an unknown one-carbon carrier [8]. In this case methanol would enter the oxidation pathway at the level of methylene-H_4SPT. However, the presence of methanol dehydrogenase has never been demonstrated. Other alternatives could be the MT_1-dependent activation of methanol (III) or CH_3-S-CoM synthesis (II), followed by the methyl group transfer to tetrahydrosarcinapterin (H_4SPT) (see below) or a direct methylation of H_4SPT by methanol catalyzed by a novel methyltransferase (IV) [37,46]. The oxidation of methyl-H_4SPT then follows the reversed CO_2 reduction route discussed in the next section.

1.3.3. Methanogenesis from H_2 and CO_2

The conversion of CO_2 to methane can formally be considered to proceed through the stages of formate, formaldehyde, and methanol. During this process the carbon atom remains bound to three different C_1-carriers (Fig. 5). In the first step carbon dioxide is reduced and bound to the first carrier, named methanofuran (MFR), yielding formyl-methanofuran. Hereafter, the formyl group is transferred to the next C_1-carrier 5,6,7,8-tetrahydrosarcinapterin (H_4SPT). This compound is a structural analog of tetrahydrofolate and is used as a one-carbon carrier at the formyl, formaldehyde, and methyl level. In *M. thermoautotrophicum* 5,6,7,8-tetrahydromethanopterin (H_4MPT) is used, which is nearly identical to H_4SPT except for the absence of one glutamyl group in the sidechain [71]. 5-Formyl-H_4SPT is subsequently dehydrated to yield 5,10-methenyl-H_4SPT, which is reduced via 5,10-methylene-H_4SPT to 5-methyl-H_4SPT .

The transfer of the methyl group of methyl-H_4SPT to HS-CoM is catalyzed by an integral membrane protein [23] and proceeds in a two-step process in which methyl-B_{12}-HBI is an intermediate [49,79]. The enzyme complex catalyzing the overall reaction has been isolated from *M. thermoautotrophicum* strain Marburg and ΔH [26,38]. The former protein had an apparent molecular mass of 670 kDa and was composed of seven different subunits of 34, 28, 24, 23, 21, 13, and 12 kDa. It contained 7.6 mol B_{12}-HBI, 37 mol non-heme iron, and 34 mol acid-labile sulfur per mol of enzyme. The corrinoid was bound to the 23-kDa polypeptide [26]. Comparison of the N-terminal amino acid sequences of the subunits to the DNA-sequence of a 3.5-kb transcription unit of the *M. thermoautotrophicum* strain Marburg chromosome revealed that the 21-kDa, 23-kDa, and 24-kDa polypeptides constitute an integral part of the enzyme [63]. No such evidence is available for the four other polypeptides. However, the preparation was inactive when one of these four polypeptides was missing [26]. The purified enzyme from *M. thermoautotrophicum* strain ΔH was isolated as a 100-kDa protein, according to non-denaturating polyacrylamide gel electrophoresis (PAGE), and contained 0.2 mol B_{12}-HBI per mol of protein. When the protein was boiled in sample buffer and analyzed with SDS-PAGE polypeptides of 35, 33, and 31 kDa were observed [38]. However, Gärtner et al. [26] demonstrated that the polypeptide pattern of the purified methyltransferase drastically changed by this treatment. The enzyme of *M. barkeri* has not been purified so far.

The methyl-H_4SPT:HS-CoM methyltransferase reaction is dependent on reducing conditions and is stimulated by ATP [79]. In cell-free

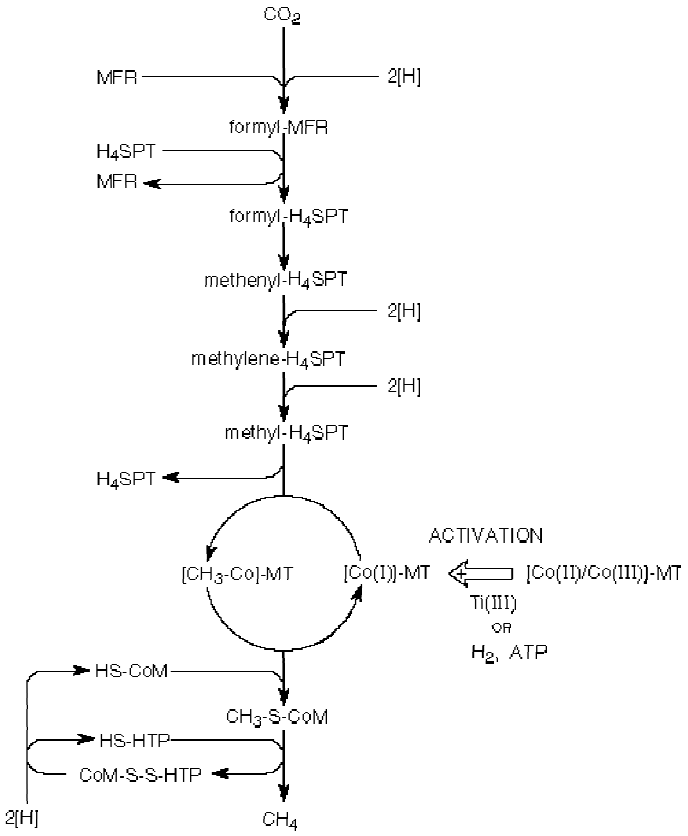

Fig. 5. Scheme of methanogenesis from H_2 and CO_2 in *Methanosarcina barkeri*. The activation of methyl-H_4SPT: HS-CoM methyltransferase (MT), indicated by the open arrow, is accomplished by H_2 and ATP that can be replaced by titanium(III)citrate. MFR, methanofuran.

extracts of *M. thermoautotrophicum* stimulation is accomplished by the combined action of ATP and CoM-S-S-HTP [39,40]. Ti(III)citrate is able to substitute for these requirements [26,38,40,79]. Ti(III)citrate, reducing conditions and ATP are all involved in generating the Co(I) form of the corrinoid [23,40,79]. Hereafter, the protein is able to accept the methyl group of methyl-H_4MPT [23,38,79]. The precise function of ATP in this process is unclear.

The standard free energy change ($\Delta G^{\circ\prime}$) associated with the methyl-H_4SPT:HS-CoM methyltransferase reaction is -29.7 kJ/mol [36]. At first it was considered unlikely that this would be a site for energy conservation, since there was no precedent for energy conservation being coupled to a methyl group transfer reaction. Experiments with inverted vesicles of *M. mazei* strain Gö1, however, clearly demonstrated that this reaction was coupled with the generation of a sodium motive force [6].

Since the transfer of the methyl group of methyl-H_4MPT to hydroxy-cobalamin was also associated with sodium translocation [5], it was assumed that the transfer reaction from methyl-H_4MPT to the membrane-bound corrinoid is the actual exergonic step promoting the extrusion of sodium. The sodium-motive force thus generated can be converted into a proton-motive force by means of a sodium-proton antiporter, thus allowing synthesis of ATP by proton-translocation [46]. Immunological evidence exists that the purified proteins catalyzing the methyl-H_4MPT:HS-CoM methyltransferase reaction are identical to the membrane-bound corrinoid proteins of unknown function already described in 1986 [62]. Interestingly, membrane-associated corrinoids are found in all methanogens tested so far [14].

As a result of the methyl-H_4MPT:HS-CoM methyltransferase reaction CH_3-S-CoM is produced which is reduced to methane as described above (section 1.3.1.).

1.3.4. Methanogenesis from acetate

Conversion of acetate by methanogens proceeds by the cleavage of the molecule into a methyl group and a carbonyl group (Fig. 6). The methyl group is reduced to methane with electrons derived from oxidation of the carbonyl group to CO_2. The overall $\Delta G^{\circ\prime}$ is -36 kJ/mol for this reaction which is nearly equal to the energy required for synthesis of one molecule of ATP under standard conditions (+32 kJ/mol). This makes acetate a poor substrate for growth. Methanogenesis from acetate starts by converting acetate to acetyl-CoA at the expend of one ATP per acetate [21]. This is a considerable investment considering the small amount of energy available for ATP synthesis. Hereafter, acetyl-CoA is converted by the carbon monoxide dehydrogenase (CODH) complex. Although the name suggest otherwise the primary function of CODH is cleavage of acetate. The enzyme complex not only catalyzes cleavage of the C-C and C-S bond of acetyl-CoA, it also oxidizes CO to CO_2 (hence its name) and transfers the methyl group to H_4SPT (Fig. 6).

In *Methanosarcina thermophila* the CODH complex consists of five subunits which can be resolved into two enzymic components: a 200-kDa CO-oxidizing nickel/iron-sulfur (Ni/Fe-S) component which contains 89 and 19 kDa subunits, and a 100 kDa corrinoid/iron-sulfur (Co/Fe-S) component, which contains 60 and 58 kDa subunits [1]. The fifth subunit of the complex (71 kDa) has not been characterized. The Ni/Fe-S component cleaves the C-C and C-S bond of acetyl-CoA at the

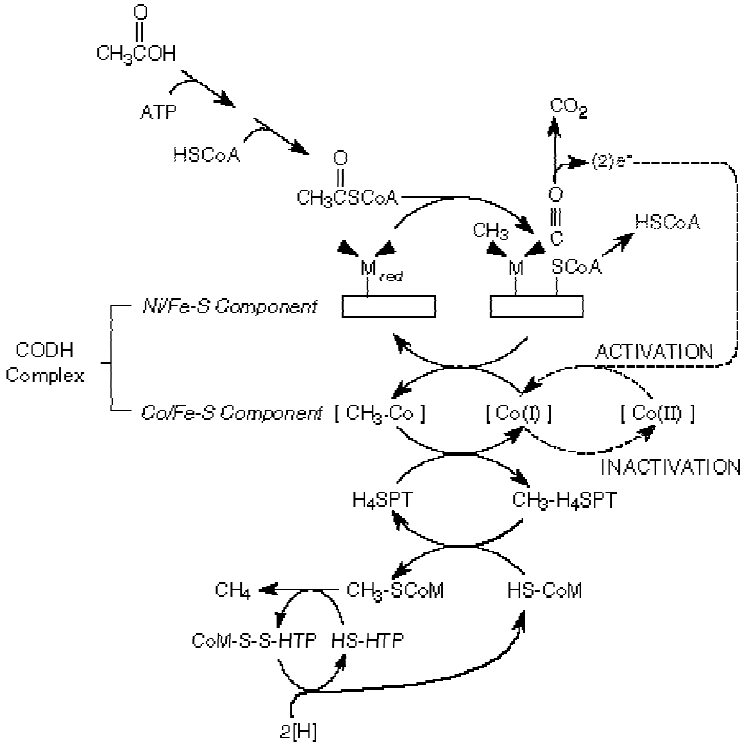

Fig. 6. Scheme of methanogenesis from acetate in *Methanosarcina thermophila* as proposed by Ferry [22]. The M above the rectangle represents the active site metal center of the Ni/Fe-S component. The cycle of activation-inactivation of the Co/Fe-S component is indicated by the dashed lines. [Co(II)] and [Co(I)] represent the oxidation states of the cobalt of the corrinoid prosthetic group of the Co/Fe-S component. CODH, carbon monoxide dehydrogenase.

Ni-Fe site. Hereafter, the methyl group can be transferred to the corrinoid of the Co/Fe-S component. Electron paramagnetic resonance (EPR) spectroscopy demonstrated that the Co/Fe-S component maintained the 5-hydroxybenzimidazolyl base of the cob(II)amide uncoordinated to the central cobalt atom [32]. Activation requires a reduction to the Co(I) state with a midpoint redox potential ($E^{0'}$) of -486 mV (pH 7.8) and is accomplished by electrons donated directly by the CO-oxidizing Ni/Fe-S component [1,32]. In *M. barkeri* an $E^{0'}$ of -426 mV has been reported for this reduction [29]. Since the CO/CO_2 couple has an $E^{0'}$ of -517 mV, reduction of the cobalt center of the Co/Fe-S component would be exergonic. Indeed, ATP is not required for reduction of the cobalt to the Co(I) state.

The Ni/Fe-S component is also able to reduce the 4Fe-4S cluster of the Co/Fe-S component ($E^{0'}$= -502 mV at pH 7.8) [1,32]. The role of

the Fe-S cluster is unknown but it is suggested that it may be involved in electron transfer from the Ni/Fe-S component to the corrinoid [32]. With the cobalt in the fully reduced Co(I) state the Co/Fe-S component is able to accept the methyl group from the Ni/Fe-S component and a methylated corrinoid intermediate is produced [1,80]. The methyl group is subsequently transferred to H_4SPT [24,28]. The C_1-unit of methyl-H_4SPT is converted to CH_3-S-CoM and methane as described in section 1.3.3. and 1.3.1., respectively.

1.3.5. Methanogenesis from methylamines and methylsulfides

At the biochemical level, methane formation from mono-, di-, and trimethylamine is only poorly investigated. When grown on trimethylamine *M. barkeri* contains a trimethylamine:HS-CoM methyltransferase activity [47]. By the action of this enzyme(system) dimethylamine and CH_3-S-CoM are produced. The reaction requires the presence of ATP and H_2, which suggests a reaction mechanism analogously to the MT_1/MT_2 system (section 1.3.1.). The second methyltransferase could be MT_2 isoenzyme II [87]. Besides dimethylamine, monomethylamine is also observed as a transient intermediate during the conversion of trimethylamine by cell extracts [47]. This and the fact that methanol-cultured cells are unable to convert mono-, di-, or trimethylamine could suggest that these three substrates are each converted by distinct inducible enzymes.

A selected number of methylotrophic methanogens are capable of growth on dimethylsulfide. Methane thiol is transiently produced during growth on dimethylsulfide but can also be used as a substrate for growth [48]. Cell extracts of methanol- and trimethylamine-cultured cells are unable to convert dimethylsulfide or methane thiol, which suggests that these substrates are converted by distinct inducible enzymes [48].

1.4. Methyltransferase reactions in non-methanogens

Methyl group transfers are associated with a number of key cellular functions, including regulation of gene expression, protein synthesis, and metabolic processes such as neurotransmitter biosynthesis. While the bulk of biological methyl transfer reactions use S-adenosyl-methionine (AdoMet), a condensation product of ATP and methionine, as the methyl donor [33], some reactions employ methyl-corrinoids.

Besides their function in methanogenesis (see above), corrinoid-dependent methyltransferases are also involved in biosynthesis of methionine and acetate [44]. The methyl transfer reactions concerned with the production of the latter two compounds will be discussed briefly.

1.4.1. Methionine synthesis

Cobalamin-dependent methionine synthase (EC 2.1.2.13) catalyzes the transfer of the methyl group from 5-methyl-tetrahydrofolate to homocysteine, to produce tetrahydrofolate and methionine. During turnover, the cobalamin cofactor of methionine synthase shuttles between methylcobalamin and cob(I)alamin [4]. As isolated from *Escherichia coli*, the 136-kDa protein is inactive, with cobalt in the Co(II) form. Activation requires catalytic amounts of the highly reactive methyl donor AdoMet and a reducing system with a redox range of about -350 mV [3]. The inactive Co(II) state of the enzyme has an $E^{0'}$ as high as -526 mV for the Co(II)-to-Co(I) reduction [3]. Here, AdoMet is the driving force for the thermodynamically unfavorable reduction of cob(II)alamin: it facilitates the reduction by trapping traces of cob(I)alamin formed as methylcobalamin [3]. It should be noted that neither AdoMet nor its methanogenic structural counterpart S-adenosylmethylcoenzyme M are involved in the reductive activation of corrinoid-dependent methanogenic methyltransferases [78].

The crystal structure of a 27-kDa methylcobalamin-binding fragment of methionine synthase revealed that the 5,6-dimethylbenzimidazole ligand of the corrinoid is not coordinated to the cobalt, but is kept instead in an hydrophobic pocket [19,20]. The lower ligand position is substituted by histidine 759 of the protein. It is suggested that the protein is able to modulate the reactivity of the corrinoid prosthetic group in this way [19].

1.4.2. Acetate synthesis

The acetogenic bacteria produce acetate from two more or less reduced C_1-compounds via the energy-yielding reductive acetyl-CoA pathway [17]. The central enzyme in this pathway is CODH, which synthesizes acetyl-CoA from a methyl group donated by a Co/Fe-S protein, a carbonyl group (CO), and HS-CoA. In fact, the reaction is identical but oppositely directed to the cleavage of acetyl-CoA during

methanogenesis from acetate (section 1.3.4.). The methyl group bound to the cobamide of the Co/Fe-S protein is derived from methyl-tetrahydrofolate. Synthesis of acetate has been most thoroughly studied in *Clostridium thermoaceticum* [52]. Here, the 88-kDa Co/Fe-S protein is isolated with the cob(II)amide in an inactive "base-off" form [52]. Activation requires a reduction to the Co(I) state with an $E^{0'}$ of -504 mV [31]. Electrons derived from the oxidation of CO to CO_2 catalyzed by CODH ($E^{0'}$ = -517 mV) can accomplish this reduction [52]. ATP is not required for the activation process.

In the acetogen *Sporomusa ovata* corrinoid-dependent methyltransferases are also involved in synthesis of methyl-tetrahydrofolate from methanol and methoxybenzoates. Methyl transfer from methanol to tetrahydrofolate is catalyzed by a 40-kDa corrinoid-containing protein [60,64]. Conversion of 3,4-dimethoxybenzoate requires a different methyltransferase. Both reactions are strictly dependent on Ti(III)citrate and catalytic amounts of ATP, that may be required for the reduction of the cobalt of the corrinoid cofactors to the Co(I) form [64]. The corrinoid of the 40-kDa methyltransferase has a *p*-cresolyl-group as its lower ligand, which cannot coordinate to cobalt [61]. Instead, a histidine ligand from the protein ligates to the cobalt atom [60,61]. It has been suggested that this lends the cob(I)amide a remarkable stability towards oxidation [60,61].

OUTLINE OF THE THESIS

Although the stimulating effect of ATP on methanogenesis from methanol was already reported in 1963, the exact mechanism by which this universal energy carrier acts upon that process is still unknown. To elucidate this mechanism the strictly ATP-dependent methanol:HS-CoM methyltransferase reaction was studied in *M. barkeri* strain MS.

In Chapter 2 the enhanced resolution of the components involved in the methanol:HS-CoM methyltransferase reaction is reported. Here, it is revealed that besides MT_1, MT_2, hydrogenase, and ferredoxin, one more protein is required for the overall reaction and more specifically in the activation of MT_1. The component is called Methyltransferase Activation Protein (MAP).

The purification and characterization of the ferredoxin concerned with the activation of MT_1 is described in Chapter 3. The redox properties of the 2[4Fe-4S] cluster of the protein are extensively studied.

To figure out what makes B_{12}-HBI the preferred candidate as a prosthetic group for methanogenic corrinoid proteins as opposed to the ubiquitously occurring cobalamin (B_{12}-DMBI), the electrochemistry of the former corrinoid will be studied in Chapter 4. It is demonstrated here that the HBI base is the weaker ligand, and thus favors "base-off" formation as compared to the DMBI base of cobalamin.

In Chapter 5 the purification of MAP is reported. The protein was shown to interact with ATP during the activation of MT_1. Moreover, MAP became phosphorylated by catalyzing the transfer of the γ-phosphoryl group of ATP, which elucidated the function of ATP during the activation of MT_1.

Chapter 6 describes the activation mechanism of MT_1. Here, the function of MAP and ATP is discussed in relation to the reduction of the corrinoids of MT_1 to the Co(I) level.

In addition, the first step in the oxidation of methanol was studied (Chapter 7). The methyl group of methanol was shown to be directly transferred to H_4MPT. The reaction was catalyzed by a membrane associated protein.

Finally, in Chapter 8 results with respect to the conversion of methanol by *M. barkeri* and the reductive activation of corrinoid containing methyltransferases in methanogens are summarized as conclusively remarks.

REFERENCES

1. Abbanat DR, Ferry JG (1991) Resolution of component proteins in an enzyme complex from *Methanosarcina thermophila* catalyzing the synthesis or cleavage of acetyl-CoA. Proc Natl Acad Sci USA 88:3272-3276

2. Babior BM (1988) The mechanism of adenosylcobalamin-dependent rearrangements. Biofactors 1:21-26

3. Banerjee RV, Harder SR, Ragsdale SW, Matthews RG (1990) Mechanism of reductive activation of cobalamin-dependent methionine synthase: an electron paramagnetic resonance spectroelectrochemical study. Biochemistry 29:1129-1135

4. Banerjee RV, Matthews RG (1990) Cobalamin-dependent methionine synthase. FASEB J 4:1450-1459

5. Becher B, Müller V, Gottschalk G (1992) N^5-Methyl-tetrahydromethanopterin: coenzyme M methyltransferase of *Methanosarcina* strain Gö1 is an Na^+-translocating membrane protein. J Bacteriol 174:7656-7660

6. Becher B, Müller V, Gottschalk G (1992) The methyl-tetrahydromethanopterin: coenzyme M methyltransferase of *Methanosarcina* strain Gö1 is a primary sodium pump. FEMS Microbiol Lett 91:239-244

7. Blaut M (1994) Metabolism of methanogens. Antonie van Leeuwenhoek 66:187-208

8. Blaut M, Gottschalk G (1984) Coupling of ATP synthesis and methane formation from methanol and molecular hydrogen in *Methanosarcina barkeri*. Eur J Biochem 141:217-222

9. Blaylock BA (1968) Cobamide-dependent methanol-cyanocob(I)alamin methyltransferase of *Methanosarcina barkeri*. Arch Biochem Biophys 124:314-324

10. Blaylock BA, Stadtman TC (1963) Biosynthesis of methane from the methyl moiety of methylcobalamin. Biochem Biophys Res Commun 11:34-38

11. Blaylock BA, Stadtman TC (1964) Enzymic formation of methylcobalamin in *Methanosarcina barkerii* extracts. Biochem Biophys Res Commun 17:475-480

12. Blaylock BA, Stadtman TC (1966) Methane biosynthesis by *Methanosarcina barkeri*. Properties of the soluble enzyme system. Arch Biochem Biophys 116:138-152

13. Bryant MP, Boone DR (1987) Emended description of strain MS^T ($DSM\ 800^T$), the type strain of *Methanosarcina barkeri*. Int J Syst Bacteriol 37:169-170

14. Dangel W, Schulz H, Diekert G, König H, Fuchs G (1987) Occurrence of corrinoid-containing membrane proteins in anaerobic bacteria. Arch Microbiol 148:52-56

15. Daniels L, Zeikus, JG (1978) One-carbon metabolism in methanogenic bacteria: Analysis of short-term fixation products of $^{14}CO_2$ and $^{14}CH_3OH$ incorporated into whole cells. J Bacteriol 136:75-84

16. De Rosa M, Gambacorta A (1988) The lipids of archaebacteria. Prog Lipid Res 27:153-175

17. Diekert G, Wohlfarth G (1994) Metabolism of homoacetogens. Antonie van Leeuwenhoek 66:209-221

18. Dolphin D (1982) B_{12}, Vol 1. John Wiley and Sons, New York.

19. Drennan CL, Huang S, Drummond JT, Matthews RG, Ludwig ML (1994) How a protein binds B_{12}: a 3.0 Å X-ray structure of B_{12}-binding domains of methionine synthase. Science 266:1669-1674

20. Drummond JT, Huang S, Blumenthal RM, Matthews RG (1993) Assignment of enzymatic function to specific protein regions of cobalamin-dependent methionine synthase from *Escherichia coli*. Biochemistry 32:9290-9295

21. Ferry JG (1992) Methane from acetate. J Bacteriol 174:5489-5495

22. Ferry JG (1993) Methanogenesis. Chapman and Hall, New York London

23. Fischer R, Gärtner P, Yeliseev A, Thauer RK (1992) N^5-Methyltetrahydro-methanopterin:coenzyme M methyltransferase in methanogenic archaebacteria is a membrane protein. Arch Microbiol 158:208-217

24. Fischer R, Thauer RK (1989) Methyltetrahydromethanopterin as an intermediate in methanogenesis from acetate in *Methanosarcina barkeri*. Arch Microbiol 151:459-465

25. Friedrich, W. (1975) Fermente-Hormone-Vitamine, Band III/2, Vitamin B_{12} und verwante Corrinoide, George Thieme Verlag, Stuttgart.

26. Gärtner P, Ecker A, Fischer R, Linder D, Fuchs G, Thauer RK (1993) Purification and properties of N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase from *Methanobacterium thermoautotrophicum*. Eur J Biochem 213:537-545

27. Grahame DA (1989) Different isozymes of methylcobalamin:2-mercaptoethanesulfonate methyltransferase predominate in methanol- *versus* acetate-grown *Methanosarcina barkeri*. J Biol Chem 264:12890-12894

28. Grahame DA (1991) Catalysis of acetyl-CoA cleavage and tetrahydrosarcinapterin methylation by a carbon monoxide dehydrogenase-corrinoid enzyme complex. J Biol Chem 266:22227-22233

29. Grahame DA (1993) Substrate and cofactor reactivity of a carbon monoxide dehydrogenase-corrinoid enzyme complex: stepwise reduction of iron-sulfur and corrinoid centers, the corrinoid $Co^{2+/1+}$ redox midpoint potential, and overall synthesis of acetyl-CoA. Biochemistry 32:10786-10793

30. Gunsalus RP, Wolfe RS (1978) ATP activation and properties of the methyl coenzyme M reductase system in *Methanobacterium thermoautotrophicum*. J Bacteriol 135:851-857

31. Harder SA, Lu W-P, Feinberg BF, Ragsdale SW (1989) Spectroelectrochemical studies of the corrinoid/iron-sulfur protein involved in acetyl coenzyme A synthesis by *Clostridium thermoaceticum*. Biochemistry 28:9080-9087

32. Jablonski PE, Lu W-P, Ragsdale SW, Ferry JG (1993) Characterization of the metal centers of the corrinoid/iron-sulfur component of the CO dehydrogenase enzyme complex from *Methanosarcina thermophila* by EPR spectroscopy and spectroelectrochemistry. J Biol Chem 268:325-329

33. Kagan RM, Clarke S (1994) Widespread occurrence of three sequence motifs in diverse S-adenosylmethionine-dependent methyltransferases suggests a common structure for these enzymes. Arch Biochem Biophys 310:417-427

34. Kandler O, Hippe H (1977) Lack of peptidoglycan in the cell walls of *Methanosarcina barkeri*. Arch Microbiol 113:57-60

35. Kandler O, König H (1978) Chemical composition of the peptidoglycan-free cell walls of methanogenic bacteria. Arch Microbiol 118:141-152

36. Keltjens JT, Van der Drift C (1986) Electron transfer reactions in methanogens. FEMS Microbiol Rev 39:259-303

37. Keltjens JT, Vogels GD (1993) Conversion of methanol and methylamines to methane and carbon dioxide. In: Ferry JG (ed) Methanogenesis. Chapman and Hall, New York London, pp 253-303

38. Kengen SWM, Daas PJH, Duits EFG, Keltjens JT, Van der Drift C, Vogels GD (1992) Isolation of a 5-hydroxybenzimidazolylcobamide-containing enzyme involved in the methyltetrahydromethanopterin:coenzyme M methyltransferase reaction in *Methanobacterium thermoautotrophicum*. Biochim Biophys Acta 1118:249-260

39. Kengen SWM, Daas PJH, Keltjens JT, Van der Drift C, Vogels GD (1990) 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. Arch Microbiol 154:156-161

40. Kengen SWM, Mosterd JJ, Nelissen RLH, Keltjens JT, Van der Drift C, Vogels GD (1988) Reductive activation of the methyl-tetrahydromethanopterin: coenzyme M methyltransferase from *Methanobacterium thermoautotrophicum* strain ΔH. Arch Microbiol 150:405-412

41. Kohler HPE (1988) Isolation of cobamides from *Methanothrix soehngenii*: 5-methylbenzimidazole as the α-ligand of the predominant cobamide. Arch Microbiol 150:219-223

42. Krzycki JA, Zeikus JG (1980) Quantification of corrinoids in methanogenic bacteria. Curr Microbiol 3:243-245

43. Lezius A, Barker HA (1965) Corrinoid compounds of *Methanobacillus omelianskii*. Fractionation of the corrinoid compounds and identification of factor III and factor III coenzyme. Biochemistry 4:510-517

44. Matthews RG, Banerjee RV, Ragsdale SW (1990) Cobamide-dependent methyltransferases. Biofactors 2:147-152

45. Michel C, Albracht SPJ, Buckel W (1992) Adenosylcobalamin and cob(II)alamin as prosthetic groups of 2-methyleneglutarate mutase from *Clostridium barkeri*. Eur J Biochem 205:767-773

46. Müller V, Blaut M, Gottschalk G (1993) Bioenergetics of methanogenesis. In: Ferry JG (ed) Methanogenesis. Chapman and Hall, New York London, pp. 360-406

47. Naumann E, Fahlbusch K, Gottschalk G (1984) Presence of a trimethylamine: HS-coenzyme M methyltransferase in *Methanosarcina barkeri*. Arch Microbiol 138:79-83

48. Ni S, Boone DR (1993) Catabolism of dimethylsulfide and methane thiol by methylotrophic methanogens. In: Oremland RS (ed) Biogeochemistry of global change. Chapman and Hall, New York London, pp. 796-810

49. Poirot CM, Kengen SWM, Valk E, Keltjens JT, Van der Drift C, Vogels GD (1987) Formation of methylcoenzyme M from formaldehyde by cell-free extracts of *Methanobacterium thermoautotrophicum*. Evidence for the involvement of a corrinoid-containing methyltransferase. FEMS Microbiol Lett 40:7-13

50. Pol A, Gage RA, Neis JM, Reijnen JWM, Van der Drift C, Vogels GD (1984) Corrinoids from *Methanosarcina barkeri*: the β-ligands. Biochim Biophys Acta 797:83-93

51. Pol A, Van der Drift C, Vogels GD (1982) Corrinoids from *Methanosarcina barkeri*: structure of the α-ligand. Biochem Biophys Res Commun 108:731-737

52. Ragsdale SW (1991) Enzymology of the acetyl-CoA pathway of CO_2 fixation. Crit Rev Biochem Mol Biol 26:261-300

53. Rospert S, Böcher R, Albracht SPJ, Thauer RK (1991) Methyl-coenzyme M reductase preparations with high specific activity from H_2-preincubated cells of *Methanobacterium thermoautotrophicum*. FEBS Lett 291:371-375

54. Schnellen CGTP (1947) Onderzoekingen over de methaangisting. Ph.D. thesis, Delft university of Technology

55. Shapiro S (1982) Do corrinoids function in the methanogenic dissimilation of methanol by *Methanosarcina barkeri*? Can J Microbiol 28:629-635

56. Shapiro S (1982) Hysteric activation of methanol-coenzyme M methyltransferase by ATP. Can J Microbiol 28:1409-1411

57. Shapiro S, Wolfe RS (1980) Methyl-coenzyme M, an intermediate in methanogenic dissimilation of C_1 compounds by *Methanosarcina barkeri*. J Bacteriol 141:728-734

58. Sprott GD, Beveridge TJ (1993) Microscopy. In: Ferry JG (ed) Methanogenesis. Chapman and Hall, New York London, pp 81-127

59. Stupperich E (1993) Recent advances in elucidation of biological corrinoid functions. FEMS Microbiol Rev 12:349-366

60. Stupperich E, Aulkemeyer P, Eckerskorn C (1992) Purification and characterization of a methanol-induced cobamide-containing protein from *Sporomusa ovata*. Arch Microbiol 158:370-373

61. Stupperich E, Eisinger HJ, Albracht SPJ (1990) Evidence for a super-reduced cobamide as the major corrinoid fraction *in vivo* and a histidine residue as a cobalt ligand of the *p*-cresolyl cobamide in the acetogenic bacterium *Sporomusa ovata*. Eur J Biochem 193:105-109

62. Stupperich E, Juza A, Eckerskorn C, Edelmann L (1990) An immunological study of corrinoid proteins from bacteria revealed homologous antigenic determinants of a soluble corrinoid-dependent methyltransferase and corrinoid-containing membrane proteins of *Methanobacterium* species. Arch Microbiol 155:28-34

63. Stupperich E, Juza A, Hoppert M, Mayer F (1993) Cloning, sequencing and immunological characterization of the corrinoid-containing subunit of the N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase from *Methanobacterium thermoautotrophicum*. Eur J Biochem 217:115-121

64. Stupperich E, Konle R (1993) Corrinoid-dependent methyl transfer reactions are involved in methanol and 3,4-dimethoxy-benzoate metabolism by *Sporomusa ovata*. Appl Environ Microbiol 59:3110-3116

65. Stupperich E, Krautler B (1988) Pseudo vitamin B_{12} or 5-hydroxybenzimidazolylcobamide are the corrinoids found in methanogenic bacteria. Arch Microbiol 149:268-271

66. Stupperich E, Steiner I, Eisinger HJ (1987) Substitution of Coα-(5-hydroxybenzimidazolyl)cobamide (factor III) by vitamin B_{12} in *Methanobacterium thermoautotrophicum*. J Bacteriol 169:3076-3081

67. Tanaka K (1994) Anaerobic degradation of tetramethylammonium by a newly isolated marine methanogen. J Ferment Bioeng 78:386-388

68. Te Brömmelstroet BW (1984) Methanol omzetting in *Methanosarcina barkeri*. Stageverslag 455, Department of Microbiology, University of Nijmegen

69. Thauer RK (1990) Energy metabolism of methanogenic bacteria. Biochim Biophys Acta 1018:256-259

70. Thauer RK, Hedderich R, Fischer R (1993) Methanogenesis from CO_2 and H_2. In: Ferry JG (ed) Methanogenesis. Chapman and Hall, New York London, pp. 209-252

71. Van Beelen PJ, Labro FA, Keltjens JT, Geerts WJ, Vogels GD, Laarhoven WH, Guijt W, Haasnoot CAG (1984) Derivatives of methanopterin, a coenzyme involved in methanogenesis. Eur J Biochem 139:359-365

72. Van der Meijden P (1984) Methanol conversion by methanogenic and acetogenic bacteria. Ph.D. thesis, University of Nijmegen

73. Van der Meijden P, Heythuysen HJ, Pouwels A, Houwen F, Van der Drift C, Vogels GD (1983) Methyltransferases involved in methanol conversion by *Methanosarcina barkeri*. Arch Microbiol 134:238-242

74. Van der Meijden P, Heythuysen HJ, Sliepenbeek HT, Houwen FP, Van der Drift C, Vogels GD (1983) Activation and inactivation of methanol: 2-mercaptoethanesulfonic acid methyltransferase from *Methanosarcina barkeri*. J Bacteriol 153:6-11

75. Van der Meijden P, Jansen LPJM, Van der Drift C, Vogels GD (1983) Involvement of corrinoids in the methylation of coenzyme M (2-mercapto-ethanesulfonic acid) by methanol and enzymes from *Methanosarcina barkeri*. FEMS Microbiol Lett 19:247-251

76. Van der Meijden P, Te Brömmelstroet BW, Poirot CM, Van der Drift C, Vogels GD (1984) Purification and properties of methanol: 5-hydroxybenzimidazolyl-cobamide methyltransferase from *Methanosarcina barkeri*. J Bacteriol 160:629-635

77. Van der Meijden P, Van der Lest C, Van der Drift C, Vogels GD (1984) Reductive activation of methanol: 5-hydroxybenzimidazolylcobamide methyl-transferase of *Methanosarcina barkeri*. Biochem Biophys Res Commun 118:760-766

78. Van de Wijngaard WMH (1990) Corrinoid-dependent methyl group transfer reactions in methylotrophic methanogens. Ph.D. thesis, University of Nijmegen

79. Van de Wijngaard WMH, Lugtigheid RL, Van der Drift C (1991) Reductive activation of the corrinoid-containing enzyme involved in methyl group transfer between methyl-tetrahydromethanopterin and coenzyme M in *Methanosarcina barkeri*. Antonie van Leeuwenhoek 60:1-6

80. Van de Wijngaard WMH, Van der Drift C, Vogels GD (1988) Involvement of a corrinoid enzyme in methanogenesis from acetate in *Methanosarcina barkeri*. FEMS Microbiol Lett 52:165-172

81. Vogels GD, Keltjens JT, Van der Drift C (1988) Biochemistry of methane formation. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. John Wiley and Sons, New York, pp. 707-770

82. Weiss DS, Thauer RK (1993) Methanogenesis and the unity of biochemistry. Cell 72:819-822

83. Widdel F, Rouvière PE, Wolfe RS (1988) Classification of secondary alcohol-utilizing methanogens including a new thermophilic isolate. Arch Microbiol 150:477-481

84. Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221-271

85. Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576-4579

86. Wood JM, Moura I, Moura JJG, Santos MH, Xavier AV, LeGall J, Scandellari M (1982) Role of vitamin B_{12} in methyl transfer for methane biosynthesis by *Methanosarcina barkeri*. Science 216:303-305

87. Yeliseev A, Gärtner P, Harms U, Linder D, Thauer RK (1993) Function of methylcobalamin: coenzyme M methyltransferase isoenzyme II in *Methanosarcina barkeri*. Arch Microbiol 159:53-536

88. Zillig W, Stetter KO, Schnabel R, Thomm M (1985) DNA-dependent RNA polymerases of the archaebacteria. In: Woese CR, Wolfe RS (eds.) The bacteria, vol 8, Archaebacteria. Academic Press Inc, New York, pp 499-524

89. Zinder SH (1993) Physiological ecology of methanogens. In: Ferry JG (ed) Methanogenesis. Chapman and Hall, New York London, pp 128-206

90. IUPAC-IUB Commission on Biochemical Nomenclature (1974) The nomenclature of corrinoids, recommendations (1973). Eur J Biochem 45:7-12