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Methyl-Coenzyme M Reductase of *Methanobacterium thermoautotrophicum* ΔH Catalyzes the Reductive Dechlorination of 1,2-Dichloroethane to Ethylene and Chloroethane

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Reductive dechlorination of 1,2-dichloroethane (1,2-DCA) to ethylene and chloroethane (CA) by crude cell extracts of *Methanobacterium thermoautotrophicum* ΔH with H_2 as the electron donor was stimulated by Mg-ATP. The heterodisulfide of coenzyme M (CoM) and 7-mercaptoheptanoylthreonine phosphate together with Mg-ATP partially inhibited ethylene production but stimulated CA production compared Mg-ATP alone. The pH optimum for the dechlorination was 6.8 (at 60°C). Michaelis-Menten kinetics for initial product formation rates with different 1,2-DCA concentrations indicated the enzymatic character of the dechlorination. Apparent K_ms for 1,2-DCA of 89 and 119 μM and V_{max}s of 34 and 20 pmol/min/mg of protein were estimated for ethylene and CA production, respectively. 3-Bromopropanesulfonate, a specific inhibitor for methyl-CoM reductase, completely inhibited dechlorination of 1,2-DCA. Purified methyl-CoM reductase, together with flavin adenine dinucleotide and a crude component A fraction which reduced the nickel of factor F_{430} in methyl-CoM reductase, converted 1,2-DCA to ethylene and CA with H_2 as the electron donor. In this system, methyl-CoM reductase was also able to transform its own inhibitor 2-bromoethanesulfonate to ethylene.

Hydrogenotrophic and acetoclastic methanogenic bacteria reductively dechlorinate 1,2-dichloroethane (1,2-DCA) to ethylene and chloroethane (CA) (5, 12, 26). Corrinoids or factor F_{430}, two cofactors present in high amounts in methanogens (10, 11, 20, 35), catalyzed the same transformations in buffer with Ti(III) citrate as the electron donor (27).

Corrinoids are found in the soluble as well as the membrane fraction of methanogens (10). Despite its high cobamide content, the role of this cofactor has not yet been fully established. Similar to already known functions of corrinoids in other organisms, cobamides in methanogens are thought to be involved mainly in methyl transfer reactions. This role was verified for the highly purified methanol:5-hydroxybenzimidazolyl-cobamide methyltransferase of *Methanosarcina barkeri*, an enzyme involved in methanogenesis from methanol (49–52). A possible function as a "redox protein" was proposed for a 33-kDa purified corrinoid-containing membrane protein of *Methanobacterium thermoautotrophicum* Marburg (44). A monospecific polyclonal antiserum against the 33-kDa corrinoid-containing membrane protein of strain Marburg cross-reacted with the 33- and 31-kDa subunits of the corrinoid-containing 5-methyl-tetrahydromethanopterin: 5-hydroxy-benzimidazolyl cobamide methyltransferase isolated from *M. thermoautotrophicum* ΔH (29, 47). On the basis of this result, a possible function as methyltransferase was suggested for the membrane protein (47). Other processes in which a corrinoid enzyme is involved are methanogenesis from acetate and CO_2 fixation by methanogens. Both reactions are performed via the acetyl-coenzyme A pathway (Wood pathway) in which a corrinoid-iron-sulfur protein and CO dehydrogenase are central enzymes (17, 36, 48, 55). The involvement of corrinoid enzymes in the methanogenesis from acetate was shown with cell extracts of *M. barkeri* (8, 53).

Factor F_{430}, a hydrocorphinoid nickel(II) complex (39) found only in methanogens, can exist in two forms, a protein-bound form and a free form (2, 24). The free form was present only under Ni-sufficient growth conditions (2). Factor F_{430} is the chromophore of the methyl-coenzyme M (CoM) reductase, which catalyzes the last step in methanogenesis and contains 2 mol of factor F_{430} per mol of enzyme (14–16, 24, 37). Similar to the model for corrinoid-catalyzed methyl transfer reactions (4, 49), it is suggested that methyl-CoM reductase is active only when the transition metal in the prosthetic group is present in the most reduced form [Ni(I) state] (1, 42). 7-Mercaptoheptanoylthreonine phosphate (H-S-HTP) (component B) was found to be the electron donor for the methyl-CoM (CH_3-S-CoM) reduction, and the products of the reaction are a heterodisulfide (CoM-S-S-HTP) and methane (6, 15). The exact reaction mechanism, however, remains to be elucidated.

Reductive activation is a prerequisite for both methyltransferases and the methyl-CoM reductase in in vitro incubations (21, 31, 41, 50, 54). The enzyme system (component A) responsible for the reductive activation of the methyl-CoM reductase (component C) was intensively studied with *M. thermoautotrophicum* ΔH. Component A was resolved into four different enzyme fractions (22, 38, 41, 42). The cofactors Mg-ATP, flavin adenine dinucleotide (FAD), and H-S-HTP were required for the reconstitution of an active enzyme system (38, 41), whereas coenzyme F_{420} and cobalamin stimulated methanogenic activity (38). Enzymatic re-

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ductive activation of methyltransferases was studied only with crude cell extracts. These activations are also dependent on Mg-ATP (31, 50). In addition to Mg-ATP, methyltetrahydromethanopterin:CoM methyltransferase activity can be stimulated by the heterodisulfide CoM-S-S-HTP (30).

In this study, we report the effects of Mg-ATP and CoM-S-S-HTP on the reductive dechlorination of 1,2-DCA by crude cell extracts of *M. thermoautotrophicum* ΔH with H_2 as the electron donor. Experiments with specific inhibitors for methyltransferase or methyl-CoM reductase activity showed that methyl-CoM reductase was responsible for the dechlorination. Purified component C, a crude component A fraction, and FAD were required for reconstitution of H_2-dependent reductive dechlorination of 1,2-DCA.

MATERIALS AND METHODS

Materials. All chemicals were at least analytical grade. Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) was purchased from Serva Feinbiochemica (Brunschwig Chemie, Amsterdam, The Netherlands), 1,2-DCA was purchased from Aldrich (Brussels, Belgium), and hydroxocob(III) alamin was purchased from Fluka (Perstorp Analytical, Oud-Beijerland, The Netherlands). ATP, GTP, FAD, and hexokinase were purchased from Boehringer Mannheim (Almere, The Netherlands), and formaldehyde, 2-mercaptoethanesulfonic acid (H-S-CoM), Ti(III) chloride, and Tris were purchased from E. Merck (Amsterdam, The Netherlands). 2-Bromoethanesulfonic acid (BrES) and dithiothreitol (DTT) were from Janssen Chimica (Tilburg, The Netherlands), and Q-Sepharose (fast flow) and Phenyl-Sepharose CL-4B were from Pharmacia Biosystems (Woerden, The Netherlands). Gases and CA were from Hoekloos (Schiedam, The Netherlands). The oxygen concentration in the anaerobic glove box was kept low with RO-20 palladium catalyst provided by BASF (Arnhem, The Netherlands). Ti(III) citrate was prepared from $TiCl_3$ and sodium citrate as previously described (27). CoM-S-S-HTP and H-S-HTP isolated from *M. thermoautotrophicum* ΔH and CH_3-S-CoM, prepared by methylation of H-S-CoM with dimethylsulfate (28), were generous gifts of J. T. Keltjens (University of Nijmegen, Nijmegen, The Netherlands). Coenzyme F_{420}, isolated from *M. thermoautotrophicum* ΔH, was a generous gift of B. Gruson and P. Debeire (Institut National de la Recherche Agronomique, Villeneuve d'Ascq, France). 3-Bromopropanesulfonate (BrPS), synthesized as described by Ellermann et al. (16), was a generous gift of R. K. Thauer (Marburg, Germany). Factor F_{430} was isolated from *M. thermoautotrophicum* ΔH as described previously (27).

Growth of the organism and preparation of cell extracts. *M. thermoautotrophicum* ΔH (DSM 1053) was routinely subcultured at 65°C in 120-ml serum bottles with 20 ml of a defined phosphate-bicarbonate-buffered mineral medium (43) on a rotary shaker at 200 rpm. The gas phase was H_2-CO_2 (4:1, vol/vol; 2×10^5 Pa). The medium was reduced with 0.1 mM Ti(III) citrate and 1.5 mM cysteine-HCl, and 2 mM $Na_2S_2O_3$ was added as an additional sulfur source (33). *M. thermoautotrophicum* was mass cultured in a 10-liter glass-steel fermentor containing 8 liters of medium with the composition as described above. The medium was flushed with H_2-CO_2 (4:1) and vigorously stirred. Cells were harvested at the late log phase by anaerobic continuous centrifugation (Carl Padberg Zentrifugenbau GmbH, Lahr/Schwarzwald, Germany) at ambient temperature, washed in 50 mM PIPES-KOH (pH 7.0) containing 1 mM DTT (buffer A), and centrifuged for 30 min at 27,500 × *g* and 4°C. The pellet was resuspended in the same buffer containing 50 μg of DNase per ml in a ratio of 1:5 (wt/vol). Cells were disrupted by being passed twice through a French pressure cell at 135 MPa, and cell debris was removed by centrifugation at 27,500 × *g* and 4°C. The supernatant, containing 28 mg of protein per ml, is referred to as crude cell extract. It was stored under 100% H_2 at −20°C.

Preparation of enzyme fractions. Crude cell extract was first centrifuged anaerobically at 110,000 × *g* and 4°C before being fractionated at ambient temperature with a high-resolution fast protein liquid chromatography system (Pharmacia Biosystems) in an anaerobic glove box. The supernatant (20 to 40 ml) was applied (4 ml/min) to a Q-Sepharose (fast-flow) column (2.2 by 8 cm; 30 ml) equilibrated with 50 mM Tris-HCl (pH 7.6) plus 0.1 mM DTT (buffer B) containing 0.2 M NaCl. Proteins were eluted with the following linear NaCl gradient: 0.2 M (200 ml), 0.2 to 0.5 M (240 ml), and 0.5 M (80 ml). The effluent was monitored for protein at 280 nm. The first 200 ml was pooled and concentrated to 2 ml in an Amicon Diaflo ultrafiltration cell equipped with a PM 30 filter (Grace B.V., Rotterdam, The Netherlands). The concentrated protein fraction was diluted with 50 ml of buffer A and again concentrated to 3.2 ml. This protein solution served as the crude component A fraction as described by Hartzell et al. (23). Ethylene glycol was added (20%, vol/vol) (38), and the fraction was stored under H_2 at −20°C in the anaerobic glove box after being analyzed for component A activity. Methyl-CoM reductase, detected by its yellow color, eluted after 384 ml in a volume of approximately 48 ml. After the addition of potassium acetate (2 M), this fraction was applied (1 ml/min) to a Phenyl-Sepharose CL-4B column (2.2 by 6 cm) equilibrated with buffer B containing 2 M potassium acetate. Methyl-CoM reductase was eluted by a linear potassium acetate gradient: 2 M (96 ml), 2 to 1 M (30 ml), and 1 M (72 ml). The enzyme eluted after 120 ml in a total volume of approximately 30 ml. This fraction was diluted with 30 ml of buffer A, concentrated to 1 ml in an Amicon Diaflo ultrafiltration cell equipped with a PM 30 filter, diluted with 60 ml of buffer A, and concentrated again to 3 ml. Glycerol was added (40%, vol/vol) (15), and the solution was stored under H_2 at −20°C. The methyl-CoM reductase solution contained 9 to 15 mg of protein per ml.

Enzyme assays. The assays were performed with 9-ml serum-type vials sealed with butyl rubber stoppers or with viton stoppers, as for assaying dechlorination. All components necessary for the different assays were dissolved in anaerobic buffer and added to the reaction vial inside the anaerobic glove box. The specific reaction mixtures are given in the table footnotes and figure legends. The vials were put on ice, and the gas phase was changed. The gas phase was, in general, 100% H_2 (1.2×10^5 Pa) unless stated otherwise. The reaction was started by increasing the temperature from 0 to 60°C. At the times indicated, one vial was sacrificed for analysis.

(i) Methyltransferase assay. The methyl-H_4MPT:HS-CoM methyltransferase reaction mixture was assayed by measuring formaldehyde conversion to CH_3-S-CoM in the presence of excess H-S-CoM as described by Kengen et al. (31) with one modification, no BrES added.

(ii) Methyl-CoM reductase assay. Methyl-CoM reductase in crude extracts was assayed by using the reaction mixture used for the methyltransferase, except that formaldehyde and H-S-CoM were omitted and CH_3-S-CoM was added instead. The gas phase was analyzed for CH_4 produced. Assays with purified methyl-CoM reductase (component C) were carried out either with the reaction mixture described

by Ellermann et al. (15, 16) or by reconstitution of the terminal step (components A, B, and C) described by Wolfe et al. (22, 38, 41, 42). The first reaction mixture contained 50 mM potassium phosphate (pH 7.0), 1.25 mM Ti(III) citrate, 7.5 mM DTT, 0.1 mM hydroxocob(III)alamin, 0.7 mM H-S-HTP, 10 mM CH_3-S-CoM, and 20 μl of component C (0.25 mg of protein) in a volume of 500 μl. The gas phase was 100% N_2. The second reaction mixture contained 50 mM PIPES-KOH (pH 6.8) (at 60°C), 25 mM $MgCl_2$, 2.5 mM ATP, 0.5 mM CoM-S-S-HTP, 25 μM cofactor F_{420}, 25 μM FAD, 25 μM hydroxocob(III)alamin, 1.5 mM H-S-HTP, 10 mM CH_3-S-CoM, 40 μl of component A (0.11 mg of protein), and component C (0.25 mg of protein) in a volume of 200 μl. The gas phase was 100% H_2.

(iii) Dechlorination assay. For assaying dechlorination by crude cell extracts, formaldehyde, H-S-CoM, or CH_3-S-CoM was omitted from the reaction mixture and 1,2-DCA in isopropanol (100 mM, unless otherwise stated) was added by syringe shortly before the temperature was increased to 60°C. Dechlorination with purified methyl-CoM reductase was assayed with two different reaction mixtures. One reaction mixture contained 50 mM buffer (PIPES-KOH [pH 7.0] or Tris-HCl [pH 9.0]), 10 mM Ti(III) citrate, 7.5 mM DTT, 20 μl of component C (0.25 mg of protein), and 1 mM 1,2-DCA in a volume of 500 μl. The gas phase was 100% N_2. The second reaction mixture is described in the footnotes to Tables 2 and 3. H_2 served as the electron donor. Five microliters of 1,2-DCA in isopropanol (100 mM) was added to this reaction mixture after a preincubation period of 5 min at 60°C. In all assays, the gas phase was analyzed for ethylene or CA produced.

Other determinations. The analyses of ethylene and CA were done as described previously (27). Methane was analyzed with the same gas chromatograph used for the analysis of ethylene. Protein was determined with Coomassie brilliant blue G250 by using the method of Bradford (7) with bovine serum albumin as the standard.

RESULTS

Effect of Mg-ATP or CoM-S-S-HTP on 1,2-DCA dechlorination. For the corrinoid-containing methyl-tetrahydromethanopterin:CoM methyltransferase and the factor F_{430}-containing methyl-CoM reductase of *M. thermoautotrophicum* ΔH, reductive Mg-ATP-dependent preactivation is required for activity in in vitro systems (22, 31, 41). In addition to Mg-ATP, the heterodisulfide CoM-S-S-HTP stimulated methyl-tetrahydromethanopterin:CoM methyltransferase activity in crude cell extracts (30). In order to get indications about the possible involvement of the methyl-tetrahydromethanopterin:CoM methyltransferase or the methyl-CoM reductase in the reductive dechlorination of 1,2-DCA by crude cell extracts of *M. thermoautotrophicum* ΔH, the effects of Mg-ATP and CoM-S-S-HTP on this reaction were investigated.

Mg-ATP alone indeed stimulated ethylene or CA production from 1,2-DCA (Fig. 1). However, dechlorination also occurred in the absence of Mg-ATP, though at lower rates. The effect of Mg-ATP was clearest within the first 30 min of incubation. If incubations proceeded, ethylene production remained significantly higher in the presence of Mg-ATP, whereas CA reached almost the same level in controls to which nothing was added. Mg-GTP gave the same stimulation as Mg-ATP (data not shown). The effect of Mg-ATP on formaldehyde conversion (methyltransferase activity) or methane production from CH_3-S-CoM (methyl-CoM reductase) was also tested with the same cell extracts. Just as in earlier reports (31), a decrease in formaldehyde concentration was found only in the presence of Mg-ATP (Fig. 1). Mg-ATP had no effect on methane production from CH_3-S-CoM (Fig. 1). This is probably due to active methyl-CoM reductase still present in these extracts (42).

CoM-S-S-HTP alone inhibited ethylene production but had no effect on CA production or methane formation (Fig. 1). Formaldehyde was not converted in the presence of CoM-S-S-HTP alone. When Mg-ATP was present in addition, ethylene production decreased and CA production increased compared with results with Mg-ATP alone (Fig. 1). There was no lag in formaldehyde conversion in the presence of CoM-S-S-HTP together with Mg-ATP. In the reaction mixture with Mg-ATP and CoM-S-S-HTP, all CH_3-S-CoM was converted to methane, whereas methane formation ceased after 32 min in all other assays and CH_3-S-CoM was not completely converted (data not shown).

A requirement for Mg-ATP was tested by two experiments. Preincubations of the reaction mixtures for 30 min with the ATP trap hexokinase-glucose at 37 or 60°C had no effect on dechlorination (data not shown). A second possible way of showing a requirement for Mg-ATP was to make use of the fact that methane production from CH_3-S-CoM ceased after 30 min of incubation. This result indicated exhaustion of a certain component, possibly ATP. Dechlorination still occurred after preincubations of 60 min with CH_3-S-CoM at 60°C. Hence, it was not possible to show an absolute requirement for MgATP in dechlorination by these two experiments.

For methyltransferase or methyl-CoM reductase, pH optima between 7.0 and 7.2 or 5.6 and 6.0, respectively, were reported (21, 31). Dechlorination rates were highest at pH 6.8 (at 60°C). It did not matter whether the reaction was followed with PIPES-KOH or Tris-HCl.

Kinetics of 1,2-DCA dechlorination. Initial ethylene or CA formation rates at different 1,2-DCA concentrations fitted Michaelis-Menten kinetics nicely (Fig. 2). With Lineweaver-Burk plots, apparent K_ms for 1,2-DCA of 89 μM for ethylene production and 119 μM for CA production were estimated for a reaction mixture with Mg-ATP but without CoM-S-S-HTP. Estimations of the V_{max} were 34 and 20 pmol/min/mg of protein for production of ethylene and CA, respectively.

Inhibition of 1,2-DCA dechlorination. The use of specific inhibitors for methyltransferase or methyl-CoM reductase should provide a possible method for distinguishing between the involvement of these two enzymes in the reductive dechlorination of 1,2-DCA. After an extensive screening of several compounds (N_2O, 20,000 ppm; NO_2^-, 1 mM; methyl viologen, 5 to 50 μM; cobalamin, 5 to 50 μM; Triton X-100, 4% [vol/vol]; bathophenanthroline disulfonate, 1 to 5 mM; BrES, 50 to 250 μM; BrPS, 100 μM), cobalamin was chosen as a specific inhibitor for methyltransferase activity and BrPS was chosen as a specific inhibitor for methyl-CoM reductase. All other compounds either inhibited both methyltransferase and methyl-CoM reductase activity or interfered with the measurements of dechlorination products. BrES, for example, a specific inhibitor of methyl-CoM reductase and routinely used to inhibit methanogenesis from CH_3-S-CoM, was also reductively transformed to ethylene by crude cell extracts.

Cobalamin totally inhibited formaldehyde conversion, but methane production from CH_3-S-CoM was slightly stimulated (Table 1). The effects of cobalamin on dechlorination were diverse. Ethylene formation was stimulated, whereas CA production was partially inhibited (Table 1). BrPS

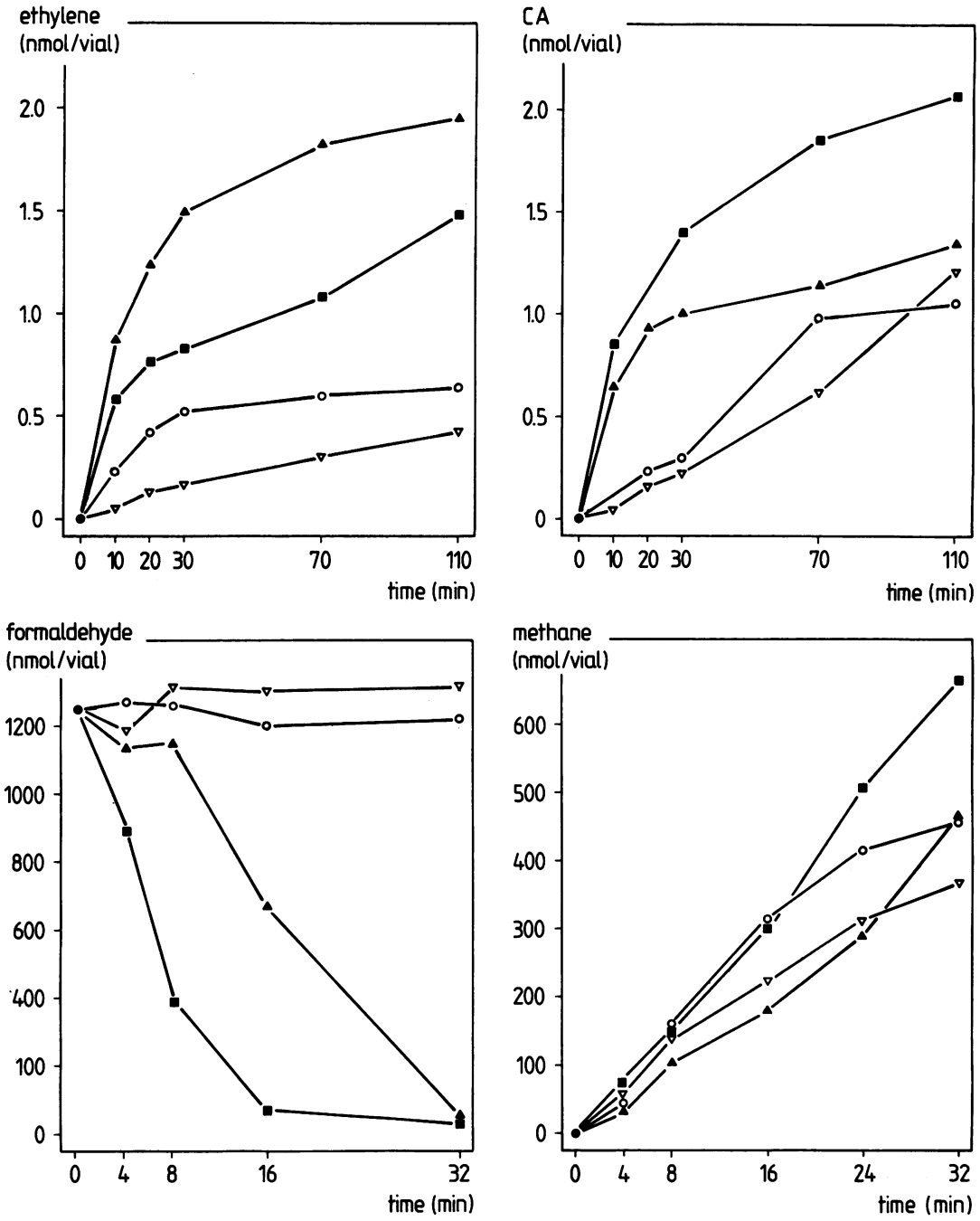

FIG. 1. Effects of Mg-ATP and CoM-S-S-HTP on the reductive dechlorination of 1,2-DCA by crude cell extracts of *M. thermoautotrophicum* ΔH. The reaction mixture contained 50 mM PIPES-KOH (pH 6.8) (at 60°C), 25 mM $MgCl_2$, 2.5 mM ATP, 0.5 mM CoM-S-S-HTP, and 100 μl of crude cell extract (2.8 mg of protein) in a volume of 500 μl. For formaldehyde conversion, the reaction mixture contained, in addition, 5 mM formaldehyde and 5 mM H-S-CoM, and for methane formation, it contained 5 mM CH_3-S-CoM. For dechlorination experiments, 5 μl of 100 mM 1,2-DCA in isopropanol was added by syringe. Assays were carried out with the complete reaction mixture (■) or with reaction mixtures in which either CoM-S-S-HTP (▲), $MgCl_2$ plus ATP (▽), or $MgCl_2$ plus ATP plus CoM-S-S-HTP (○) was omitted. The omission of ATP, $MgCl_2$ plus CoM-S-S-HTP, and ATP plus CoM-S-S-HTP produced results similar to those produced by a mixture to which $MgCl_2$ plus ATP plus CoM-S-S-HTP was not added.

showed a very clear inhibition pattern. Methyl-CoM reductase was completely inactivated, and methyltransferase activity was not affected at all (Table 1). Dechlorination did not occur in the presence of this inhibitor (Table 1), suggesting that methyl-CoM reductase is responsible for ethylene as well as CA production.

Dechlorination of 1,2-DCA by purified methyl-CoM reductase (component C). Methyl-CoM reductase was purified

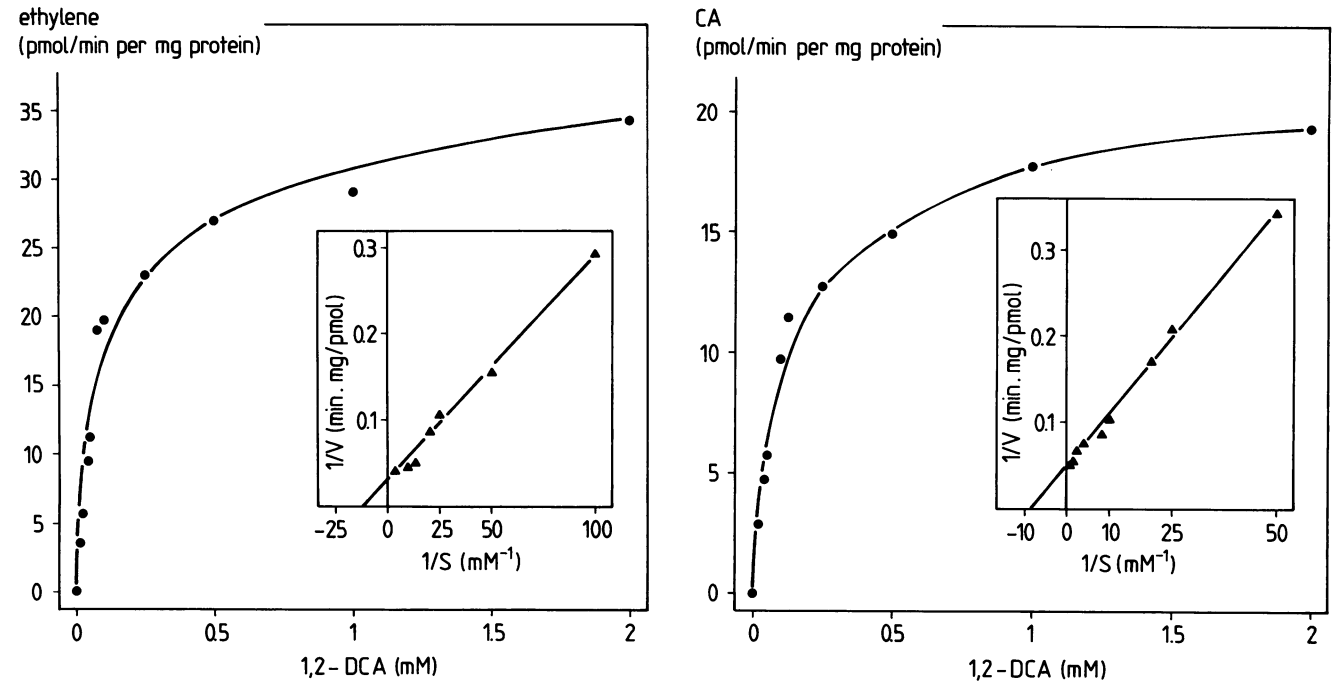

FIG. 2. Kinetics of the reductive dechlorination of 1,2-DCA by crude cell extracts of *M. thermoautotrophicum* ΔH. The reaction mixture contained 50 mM PIPES-KOH (pH 6.8) (at 60°C), 25 mM $MgCl_2$, 2.5 mM ATP, and 100 μl of crude cell extract (2.8 mg of protein) in a volume of 500 μl. The gas phase was 100% H_2. Five microliters of 1,2-DCA stock solution in isopropanol (100 times the initial concentration) was added by syringe shortly before the reaction was started by placing vials at 60°C. Initial rates were determined by analyzing vials after 5 and 10 min of incubation. Product formation was linear within this time frame. Insets are Lineweaver-Burk plots of the same data.

with a yield of about 40 to 50%. Purity was checked with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Only three major proteins, which represented the α, β, and γ subunits of methyl-CoM reductase with apparent M_rs of 70,000, 46,000, and 39,000, which are in close agreement with M_rs reported by others, were detected (14, 23, 24). In a reaction mixture with Ti(III) citrate as the electron donor, methane was produced from CH_3-S-CoM at a rate of 360 nmol/min/mg of component C. In an H_2-dependent enzyme system with a crude component A fraction and purified component C, methanogenic activities of 35 nmol/min/mg of component C were obtained. No methane was formed when component A or C was omitted.

The dechlorinating activity of component C was first assayed with Ti(III) citrate as the electron donor. In the presence of component C, dechlorinating activity was two times the background activity of Ti(III) citrate alone (data not shown). These initial results confirmed results obtained by inhibition experiments and showed the involvement of component C in the reductive dechlorination of 1,2-DCA.

A second possible way of assaying dechlorinating activity was to use the enzyme system (component A) responsible for the activation of component C. 1,2-DCA was indeed dechlorinated in reaction mixtures in which both components A and C were present (Table 2). When component A or C was omitted, only small amounts of ethylene were formed, and CA remained below the detection limit. The omission of ATP, CoM-S-S-HTP, or both had no effect on dechlorination. When boiled cell extract was omitted, slow or no dechlorination was observed. The omission of component C already indicated that component A was not able to reduce free corrinoids and factor F_{430} present in boiled cell extract, which could also act as catalysts for dechlorination (27). Controls in which additional cobalamin or factor F_{430} was

TABLE 1. Inhibition of the reductive dechlorination of 1,2-DCA by crude cell extracts of *M. thermoautotrophicum* ΔH compared with inhibition of formaldehyde conversion or methane production from CH_3-S-CoM[a]

Inhibitor	Formaldehyde converted (nmol/vial)	Methane formed (nmol/vial)	Ethylene formed (pmol/vial)	CA formed (pmol/vial)
None	1,069 ± 40	218 ± 40	1,102 ± 35	799 ± 37
Cobalamin[b]	<DL[c]	293 ± 14	1,777 ± 29	267 ± 23
BrPS[d]	1,084 ± 25	<DL	25 ± 2	<DL

[a] The reaction mixture contained 50 mM PIPES-KOH (pH 6.8) (at 60°C), 25 mM $MgCl_2$, 2.5 mM ATP, and 50 μl of crude cell extract (1.4 mg of protein) in a volume of 250 μl. For formaldehyde conversion, the reaction mixture contained, in addition, 5 mM formaldehyde and 5 mM H-S-CoM, and for methane formation it contained, in addition, 5 mM CH_3-S-CoM. For dechlorination experiments, 3 μl of 100 mM 1,2-DCA in isopropanol was added by syringe. The values given in the table are the amounts converted or formed within 30 min of incubation and are the means of duplicate experiments.

[b] 50 μM hydroxocob(III)alamin added.

[c] <DL, below detection limit.

[d] 100 μM added.

TABLE 2. Reductive dechlorination of 1,2-DCA by methyl-CoM reductase (component C) of *M. thermoautotrophicum* ΔH with boiled cell extract as the cofactor fraction

Reaction mixture[a]	Product formed from 1,2-DCA (pmol/vial)[b]	
	Ethylene	CA
Alone	450	656
Minus ATP	534	295
Minus CoM-S-S-HTP	531	455
Minus BCE	194	329
Minus ATP/CoM-S-S-HTP	575	621
Minus ATP/BCE	19	<DL[c]
Minus CoM-S-S-HTP/BCE	12	<DL
Minus ATP/CoM-S-S-HTP/BCE	59	<DL
Minus component A	12	<DL
Minus component C	22	<DL
Plus cobalamin[d]	1,062	382
Minus component C and plus cobalamin	50	<DL
Plus factor F_{430}[e]	537	334
Minus component C and plus factor F_{430}	25	<DL
Plus CH_3-S-CoM[f]	250	414

[a] The complete reaction mixture contained 50 mM PIPES-KOH (pH 6.8) (at 60°C), 25 mM $MgCl_2$, 2.5 mM ATP, 0.25 mM CoM-S-S-HTP, 50 μl of protein-free boiled cell extract (BCE) (28 mg of protein · ml^{-1} before boiling), 200 μl of component A (0.57 mg of protein), and 100 μl of component C (1.24 mg of protein) in a volume of 500 μl.

[b] The values given are the amounts formed within the 120 min of incubation.

[c] <DL, below detection limit.

[d] 50 μM hydoxocob(III)alamin.

[e] 50 μM 12,13-di-epi-F_{430}.

[f] 5 mM CH_3-S-CoM was added; 128 nmol of CH_4 was formed in the presence of 1,2-DCA, and 286 nmol was formed in the absence of 1,2-DCA.

added in the absence of component C showed no dechlorination at all. It was interesting that cobalamin had the same stimulating effect on ethylene production in the presence of component C (Table 2) as that observed with crude cell extracts (Table 1). Partial inhibitions of dechlorination by CH_3-S-CoM and of methane formation by 1,2-DCA were found (Table 2).

Cofactor requirement for 1,2-DCA dechlorination by component C. The experiments with boiled cell extract as the crude cofactor fraction revealed that a component present in boiled cell extract must be needed for dechlorinating activity. According to the data in Table 3, FAD was the cofactor required to reconstitute a dechlorinating enzyme system out of component A and purified component C. Mg-ATP or factor F_{420} had a stimulatory effect (Table 3).

Ethylene production from BrES catalyzed by cobalamin, factor F_{430}, or methyl-CoM reductase. BrES was transformed to ethylene by using crude cell extracts of *M. thermoautotrophicum* ΔH, as was observed in the inhibition studies described above. BrES is structurally similar to 1,2-DCA, a C2 compound with two leaving groups, one on each carbon. BrES was rapidly transformed to ethylene in buffer reduced with Ti(III) citrate and cobalamin or factor F_{430} as the catalyst (Fig. 3). In contrast to results obtained with 1,2-DCA, in which dechlorination by cobalamin and factor F_{430} took place at different rates (27), the formation of ethylene from BrES occurred at about the same rate (500 pmol/min/nmol of catalyst) with either catalyst. Purified component C was also able to transform its own inhibitor BrES to ethylene (Table 3).

TABLE 3. Cofactor requirements for the reductive dechlorination of 1,2-DCA by methyl-CoM reductase (component C) of *M. thermoautotrophicum* ΔH

Reaction mixture[a]	Product formed (pmol/vial)[b]	
	Ethylene	CA
Alone	1,797	1,282
Minus ATP	962	905
Minus FAD	28	<DL[c]
Minus factor F_{420}	787	867
Minus component A	40	<DL
Minus component C	34	<DL
Minus H_2	0	<DL
Minus 1,2-DCA and plus BrES[d]	1,199	—[e]
Minus 1,2-DCA-component C and plus BrES	0	—

[a] The reaction mixture contained 50 mM PIPES-KOH (pH 6.8) (at 60°C), 25 mM $MgCl_2$, 2.5 mM ATP, 25 μM FAD, 25 μM factor F_{420}, 150 μl of component A (0.43 mg of protein), and 150 μl of component C (2.16 mg of protein) in a volume of 500 μl. The gas phase was 100% H_2, unless stated otherwise.

[b] The values given are amounts formed within the 120 min of incubation.

[c] <DL, below detection limit.

[d] 2 mM BrES added by syringe from a 200 mM stock in 50 mM PIPES-KOH (pH 6.8) (at 60°C).

[e] Not formed in this reaction mixture.

DISCUSSION

The reductive dechlorinating activity of methyl-CoM reductase substantiates the hypothesis that factor F_{430} is involved in reductive dechlorination reactions catalyzed by whole cells of methanogens (27). The first evidence for this hypothesis was obtained by using model systems with purified factor F_{430} and Ti(III) citrate as the electron donor (19, 27, 34). Because factor F_{430} is present in protein-bound and free forms in cells of methanogens when cultivated in a nickel-sufficient medium, the question was whether free or protein-bound factor F_{430} or both are responsible for the dechlorination. Stimulation of dechlorination by Mg-ATP

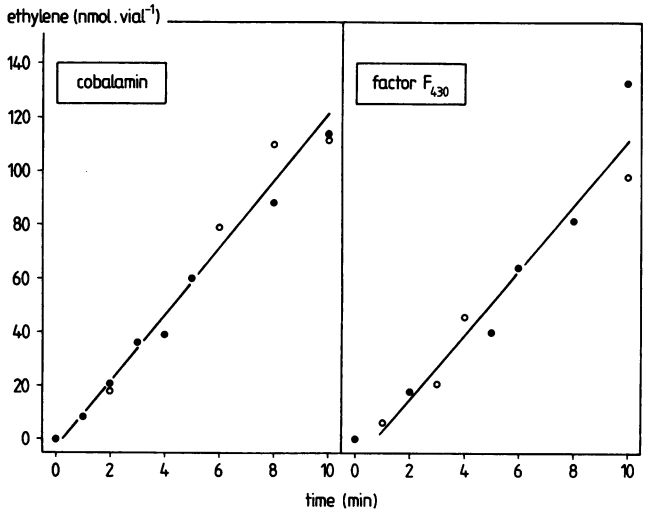

FIG. 3. Ethylene production from BrES in buffer reduced with Ti(III) citrate and with cobalamin or factor F_{430} as the catalyst. The reaction mixture contained 790 mM Tris-HCl (pH 9), 20 mM Ti(III) citrate, 50 μM hydroxo-cob(III)alamin or 12,13-di-epi-F_{430}, and 2 mM BrES in a volume of 500 μl. The gas phase was 100% N_2. ○ and ●, data of two independent experiments.

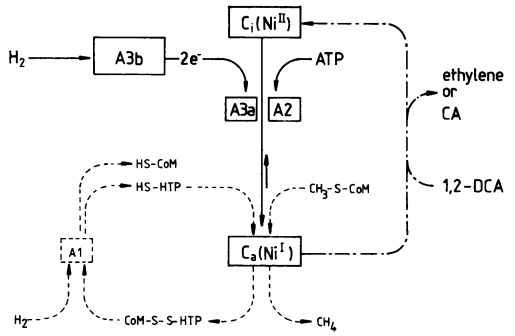

FIG. 4. Model for the reductive dechlorination of 1,2-DCA by methyl-CoM reductase adapted from the model for the functioning of the methylreductase system presented by Rouvière and Wolfe (22). ——, reductive activation of inactive component C; – – – –, methanogenesis from CH_3-S-CoM and H-S-HTP catalyzed by active component C; — — — — — —, oxidation of activated component C by reductive dechlorination of 1,2-DCA to ethylene or CA.

and the Michaelis-Menten-type kinetics in crude cell extracts, as shown in Fig. 1 and 2, indicated that enzymatic catalyzed reactions were responsible for dechlorination by crude cell extracts with hydrogen as the electron source. The complete inhibition of dechlorination by BrPS and the dependence of dechlorinating activity on purified component C unequivocally proved protein-bound factor F_{430} to be the catalyst. These results excluded not only free factor F_{430} as the in vivo catalyst, but also the involvement of corrinoid enzymes. This was surprising, since the reductive dechlorinating activity of other anaerobes (9, 13, 18), which do not contain factor F_{430}, demonstrates that other enzymes are also able to catalyze these reactions. The possible involvement of a corrinoid-iron-sulfur enzyme of the acetyl-coenzyme A pathway present in some of these organisms remains to be proven (13). Reductive dechlorination by a corrinoid enzyme has been shown for N^5-methyl tetrahydrofolate-homocysteine transmethylase (56).

The reductive dechlorination of 1,2-DCA by methyl-CoM reductase of *M. thermoautotrophicum* ΔH could be incorporated in the model of Rouvière and Wolfe for the methyl-CoM reductase system (42), as shown in Fig. 4. In this model, inactive component C, with the nickel in factor F_{430} in the Ni(II) state, is reduced to the active Ni(I) form by the actions of components A3a and A2. Mg-ATP is required as the cofactor. The electrons are derived from H_2 by component A3b and transferred by an unknown electron carrier. When 1,2-DCA is added to the assay instead of CH_3-S-CoM and HS-HTP or CoM-S-S-HTP, the reduced nickel in active component C is most probably oxidized and ethylene or CA is formed. Nonenzymatic dechlorination by free factor F_{430} with Ti(III) citrate as the electron donor (27) supports such a mechanism. Since the dihalo-elimination to ethylene and the hydrogenolysis to CA require a two-electron reduction, one may envisage that both molecules of factor F_{430}, present in one component C molecule, take part in this reaction. The oxidation of Ni(I) to Ni(II) short-circuits the activation process, and component C has to be reduced again.

According to the model, the reductive activation of component C would require Mg-ATP as an activator. However, the results obtained with Mg-ATP are completely contradictory to those of the mechanism suggested above. Mg-ATP was not required for the reconstitution of an H_2-dependent dechlorinating system, whereas it was shown that methanogenesis did not proceed in the absence of Mg-ATP (41). Possibly, in our study, enough Mg-ATP was still present in the crude component A fraction. There is only one study which showed Mg-ATP-independent methane formation from CH_3-S-CoM by purified methyl-CoM reductase of *M. thermoautotrophicum* ΔH (40). However, component A enzymes also were not needed in these experiments. This methanogenic activity could have been the consequence of the isolation of already reduced component C which does not require Mg-ATP-dependent reductive activation (42). It is puzzling, however, that in crude cell extracts an absolute requirement for Mg-ATP in the dechlorination of 1,2-DCA also could not be shown. This is in contrast with results found for methyltransferase as well as methyl-CoM reductase activity in crude systems in which the hexokinase-glucose system worked perfectly as an ATP trap (31, 50, 54).

The absolute requirement for FAD for dechlorination and the stimulatory effect of cobalamin and factor F_{420} are in agreement with results obtained for methane production from CH_3-S-CoM by this enzyme system (38). The role of FAD in the methyl-CoM reducing enzyme system has not yet been fully established. Whereas FAD was specifically required for methanogenesis in a study with protein components A1, A2, A3, and C (38), it had no effect in a later study using a reaction mixture with protein components A1, A2, A3a, A3b, and C (42). FAD is found in the heterodisulfide reductase of *M. thermoautotrophicum* Marburg, in which it could be involved in the disulfide reduction (25). The FAD-dependent dechlorination suggests that this cofactor could also be involved in the electron transfer from component A3b to A3a.

An interesting observation is the transformation of BrES to ethylene by crude cell extracts or purified methyl-CoM reductase. Cultures of methanogens amended with BrES are known to recover from BrES inhibition (5, 46). These observations might have been the result of degradation of BrES to ethylene by its target enzyme, component C. However, results obtained with BrES-resistant cells indicated that spontaneous mutagenesis of the transport system for H-S-CoM (3) rather than the degradation of BrES was responsible for resistance (32, 46). Characterization of BrES-resistant strains of *M. barkeri* 227 supported the evidence of a resistance due to an altered cell permeability, since component C of BrES-resistant mutants was as sensitive to BrES as the wild-type methyl-CoM reductase was (45).

All studies of the methyl-CoM reductase activating enzyme system were assayed by methane formation from CH_3-S-CoM (21, 38, 41, 42). However, interpretation of the data was complicated by uncertainty about whether a component was indeed directly involved in the activation or, rather, was involved in the catalytic process. If the dechlorination of 1,2-DCA by component C really functions as indicated in the model, it could provide an instrument to assay the activation of this enzyme directly. Possible additional effects of a certain compound on other catalytic functions in the multicomponent enzyme system (e.g., reduction of the heterodisulfide CoM-S-S-HTP) would then be excluded from this assay.

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REFERENCES

1. **Albracht, S. P. J., D. Ankel-Fuchs, R. Böcher, J. Ellermann, J. Moll, J. W. van der Zwaan, and R. K. Thauer.** 1988. Five new EPR signals assigned to nickel in methyl-coenzyme M reductase from *Methanobacterium thermoautotrophicum*, strain Marburg. Biochim. Biophys. Acta **955:**86–102.
2. **Ankel-Fuchs, D., R. Jaenchen, N. A. Gebhardt, and R. K. Thauer.** 1984. Functional relationship between protein-bound and free factor F430 in *Methanobacterium*. Arch. Microbiol. **139:**332–337.
3. **Balch, W. E., and R. S. Wolfe.** 1979. Transport of coenzyme M (2-mercaptoethanesulfonic acid) in *Methanobacterium ruminantium*. J. Bacteriol. **137:**264–273.
4. **Banerjee, R. V., S. R. Harder, S. W. Ragsdale, and R. G. Matthews.** 1990. Mechanism of reductive activation of cobalamin-dependent methionine synthase: an electron paramagnetic resonance spectroelectrochemical study. Biochemistry **29:** 1129–1135.
5. **Belay, N., and L. Daniels.** 1987. Production of ethane, ethylene, and acetylene from halogenated hydrocarbons by methanogenic bacteria. Appl. Environ. Microbiol. **53:**1604–1610.
6. **Bobik, T. A., K. D. Olson, K. M. Noll, and R. S. Wolfe.** 1987. Evidence that the heterodisulfide of coenzyme M and 7-mercaptoheptanoylthreonine phosphate is a product of the methylreductase reaction in *Methanobacterium thermoautotrophicum*. Biochem. Biophys. Res. Commun. **149:**455–460.
7. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
8. **Cao, X., and J. A. Krzycki.** 1991. Acetate-dependent methylation of two corrinoid proteins in extracts of *Methanosarcina barkeri*. J. Bacteriol. **173:**5439–5448.
9. **Criddle, C. S., J. T. DeWitt, and P. L. McCarty.** 1990. Reductive dechlorination of carbon tetrachloride by *Escherichia coli* K-12. Appl. Environ. Microbiol. **56:**3247–3254.
10. **Dangel, W., H. Schulz, G. Diekert, H. König, and G. Fuchs.** 1987. Occurrence of corrinoid-containing membrane proteins in anaerobic bacteria. Arch. Microbiol. **148:**52–56.
11. **Diekert, G., U. Konheiser, K. Piechulla, and R. K. Thauer.** 1981. Nickel requirement and factor F_{430} content of methanogenic bacteria. J. Bacteriol. **148:**459–464.
12. **Egli, C., R. Scholtz, A. M. Cook, and T. Leisinger.** 1987. Anaerobic dechlorination of tetrachloromethane and 1,2-dichloroethane to degradable products by pure cultures of *Desulfobacterium* sp. and *Methanobacterium* sp. FEMS Microbiol. Lett. **43:**257–261.
13. **Egli, C., T. Tschan, R. Scholtz, A. M. Cook, and T. Leisinger.** 1988. Transformation of tetrachloromethane to dichloromethane and carbon dioxide by *Acetobacterium woodii*. Appl. Environ. Microbiol. **54:**2819–2824.
14. **Ellefson, W. L., and R. S. Wolfe.** 1981. Component C of the methylreductase system of *Methanobacterium*. J. Biol. Chem. **256:**4259–4262.
15. **Ellermann, J., R. Hedderich, R. Böcher, and R. K. Thauer.** 1988. The final step in methane formation. Investigations with highly purified methyl-CoM reductase (component C) from *Methanobacterium thermoautotrophicum* (strain Marburg). Eur. J. Biochem. **172:**669–677.
16. **Ellermann, J., S. Rospert, R. K. Thauer, M. Bokranz, A. Klein, M. Voges, and A. Berkessel.** 1989. Methyl-coenzyme-M reductase from *Methanobacterium thermoautotrophicum* (strain Marburg). Purity, activity and novel inhibitors. Eur. J. Biochem. **184:**63–68.
17. **Fuchs, G.** 1986. CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol. Rev. **39:**181–213.
18. **Gälli, R., and P. L. McCarty.** 1989. Biotransformation of 1,1,1-trichloroethane, trichloromethane, and tetrachloromethane by a *Clostridium* sp. Appl. Environ. Microbiol. **55:**837–844.
19. **Gantzer, C. J., and L. P. Wackett.** 1991. Reductive dechlorination catalyzed by bacterial transition-metal coenzymes. Environ. Sci. Technol. **25:**715–722.
20. **Gorris, L. G. M., and C. van der Drift.** 1986. Methanogenic cofactors in pure cultures of methanogens in relation to substrate utilization, p. 144–150. *In* H. C. Dubourgier, G. Albagnac, J. Montreuil, C. Romond, P. Sautiere, and J. Guillaume (ed.), Biology of anaerobic bacteria, progress in biotechnology, vol 2. Elsevier Science Publisher B.V., Amsterdam.
21. **Gunsalus, R. P., and R. S. Wolfe.** 1978. ATP activation and properties of the methyl coenzyme M reductase system in *Methanobacterium thermoautotrophicum*. J. Bacteriol. **135:** 851–857.
22. **Gunsalus, R. P., and R. S. Wolfe.** 1980. Methyl coenzyme M reductase from *Methanobacterium thermoautotrophicum*. Resolution and properties of the components. J. Biol. Chem. **255:**1891–1895.
23. **Hartzell, P. L., and R. S. Wolfe.** 1986. Comparative studies of component C from the methylreductase system of different methanogens. Syst. Appl. Microbiol. **7:**376–382.
24. **Hausinger, R. P., W. H. Orme-Johnson, and C. Walsh.** 1984. Nickel tetrapyrrole cofactor F_{430}: comparison of the forms bound to methyl coenzyme M reductase and protein free in cells of *Methanobacterium thermoautotrophicum* ΔH. Biochemistry **23:**801–804.
25. **Hedderich, R., A. Berkessel, and R. K. Thauer.** 1990. Purification and properties of heterodisulfide reductase from *Methanobacterium thermoautotrophicum* (strain Marburg). Eur. J. Biochem. **193:**255–261.
26. **Holliger, C., G. Schraa, A. J. M. Stams, and A. J. B. Zehnder.** 1990. Reductive dechlorination of 1,2-dichloroethane and chloroethane by cell suspensions of methanogenic bacteria. Biodegradation **1:**253–261.
27. **Holliger, C., G. Schraa, E. Stupperich, A. J. M. Stams, and A. J. B. Zehnder.** 1992. Evidence for the involvement of corrinoids and factor F_{430} in the reductive dechlorination of 1,2-dichloroethane by *Methanosarcina barkeri*. J. Bacteriol. **174:**4427–4434.
28. **Keltjens, J. T., R. van Erp, R. J. Mooijaart, C. van der Drift, and G. D. Vogels.** 1988. Inorganic pyrophosphate synthesis during methanogenesis from methylcoenzyme M by cell-free extracts of *Methanobacterium thermoautotrophicum* (strain ΔH). Eur. J. Biochem. **172:**471–476.
29. **Kengen, S. W. M., P. J. H. Daas, E. F. G. Duits, J. T. Keltjens, C. van der Drift, and G. D. Vogels.** 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.
30. **Kengen, S. W. M., P. J. H. Daas, J. T. Keltjens, C. van der Drift, and G. D. Vogels.** 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.
31. **Kengen, S. W. M., J. J. Mosterd, R. L. H. Nelissen, J. T. Keltjens, C. van der Drift, and G. D. Vogels.** 1988. Reductive activation of the methyltetrahydromethanopterin:coenzyme M methyltransferase from *Methanobacterium thermoautotrophicum* ΔH. Arch. Microbiol. **150:**405–412.
32. **Kiener, A., C. Holliger, and T. Leisinger.** 1984. Analogue-resistant and auxotrophic mutants of *Methanobacterium thermoautotrophicum*. Arch. Microbiol. **139:**87–90.
33. **Korteland, J., L. G. M. Gorris, G. J. C. M. de Boer, and C. van der Drift.** 1989. Simultaneous utilization of cysteine and thiosulfate for growth of *Methanobacterium thermoautotrophicum* strain ΔH. Biotechnol. Lett. **11:**595–596.
34. **Krone, U. E., K. Laufer, R. K. Thauer, and H. P. C. Hogenkamp.** 1989. Coenzyme F_{430} as a possible catalyst for the reductive dehalogenation of chlorinated C_1 hydrocarbons in methanogenic bacteria. Biochemistry **28:**10061–10065.
35. **Krzycki, J., and J. G. Zeikus.** 1980. Quantification of corrinoids

in methanogenic bacteria. Curr. Microbiol. **3**:243–245.
36. **Ljungdahl, L. G.** 1986. The autotrophic pathway of acetate synthesis in acetogenic bacteria. Annu. Rev. Microbiol. **40**:415–450.
37. **Moura, I., J. J. G. Moura, H. Santos, A. V. Xavier, G. Burch, H. D. Peck, Jr., and J. LeGall.** 1983. Proteins containing the factor F_{430} from *Methanosarcina barkeri* and *Methanobacterium thermoautotrophicum*. Biochim. Biophys. Acta **742**:84–90.
38. **Nagle, D. P., Jr., and R. S. Wolfe.** 1983. Component A of the methyl coenzyme M methylreductase system of *Methanobacterium*: resolution into four components. Proc. Natl. Acad. Sci. USA **80**:2151–2155.
39. **Pfaltz, A., B. Jaun, A. Fässler, A. Eschenmoser, R. Jaenchen, H. H. Gilles, G. Diekert, and R. K. Thauer.** 1982. 81. Zur Kenntnis des Faktors F430 aus methanogenen Bakterien: Struktur des porphinoiden Ligandsystems. Helv. Chim. Acta **65**:828–865.
40. **Rospert, S., D. Linder, J. Ellermann, and R. K. Thauer.** 1990. Two genetically distinct methyl-coenzyme M reductases in *Methanobacterium thermoautotrophicum* strain Marburg and ΔH. Eur. J. Biochem. **194**:871–877.
41. **Rouvière, P. E., T. A. Bobik, and R. S. Wolfe.** 1988. Reductive activation of the methyl coenzyme M methylreductase system of *Methanobacterium thermoautotrophicum* ΔH. J. Bacteriol. **170**:3946–3952.
42. **Rouvière, P. E., and R. S. Wolfe.** 1989. Component A3 of the methylcoenzyme M methylreductase system of *Methanobacterium thermoautotrophicum* ΔH: resolution into two components. J. Bacteriol. **171**:4556–4562.
43. **Schönheit, P., J. Moll, and R. K. Thauer.** 1979. Nickel, cobalt, and molybdenum requirement for growth of *Methanobacterium thermoautotrophicum*. Arch. Microbiol. **123**:105–107.
44. **Schulz, H., S. P. J. Albracht, J. M. C. C. Coremans, and G. Fuchs.** 1988. Purification and some properties of the corrinoid-containing membrane protein from *Methanobacterium thermoautotrophicum*. Eur. J. Biochem. **171**:589–597.
45. **Smith, M. R.** 1983. Reversal of 2-bromoethanesulfonate inhibition of methanogenesis in *Methanosarcina* sp. J. Bacteriol. **156**:516–523.
46. **Smith, M. R., and R. A. Mah.** 1981. 2-Bromoethanesulfonate: a selective agent for isolating resistant *Methanosarcina* mutants. Curr. Microbiol. **6**:321–326.
47. **Stupperich, E., A. Juza, C. Eckerskorn, and L. Edelmann.** 1990. An immunological study of corrinoid proteins from bacteria revealed homologous antigenic determinants of a soluble corrinoid-dependent methyltransferase and corrinoid-containing membrane proteins from *Methanobacterium* species. Arch. Microbiol. **155**:28–34.
48. **Thauer, R. K., D. Möller-Zinkhan, and A. M. Spormann.** 1989. Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. Annu. Rev. Microbiol. **43**:43–67.
49. **Van der Meijden, P., H. J. Heythuysen, A. Pouwels, F. P. Houwen, C. van der Drift, and G. D. Vogel.** 1983. Methyltransferases involved in methanol conversion by *Methanosarcina barkeri*. Arch. Microbiol. **134**:238–242.
50. **Van der Meijden, P., H. J. Heythuysen, H. T. Sliepenbeek, F. P. Houwen, C. van der Drift, and G. D. Vogel.** 1983. Activation and inactivation of methanol:2-mercaptoethanesulfonic acid methyltransferase from *Methanosarcina barkeri*. J. Bacteriol. **153**:6–11.
51. **Van der Meijden, P., L. P. J. M. Jansen, C. van der Drift, and G. D. Vogel.** 1983. Involvement of corrinoids in the methylation of coenzyme M (2-mercaptoethanesulfonic acid) by methanol and enzymes from *Methanosarcina barkeri*. FEMS Microbiol. Lett. **19**:247–251.
52. **Van der Meijden, P., B. W. te Brömmelstroet, C. M. Poirot, C. van der Drift, and G. D. Vogels.** 1984. Purification and properties of methanol:5-hydroxybenzimidazolylcobamide methyltransferase from *Methanosarcina barkeri*. J. Bacteriol. **160**:629–635.
53. **Van de Wijngaard, W. M. H., C. van der Drift, and G. D. Vogels.** 1988. Involvement of a corrinoid enzyme in methanogenesis from acetate in *Methanosarcina barkeri*. FEMS Microbiol. Lett. **52**:165–172.
54. **Whitman, W. B., and R. S. Wolfe.** 1983. Activation of the methylreductase system from *Methanobacterium bryantii* by ATP. J. Bacteriol. **154**:640–649.
55. **Wood, H. G., S. W. Ragsdale, and E. Pezacka.** 1986. The acetyl-CoA pathway of autotrophic growth. FEMS Microbiol. Rev. **39**:345–362.
56. **Wood, J. M., F. S. Kennedy, and R. S. Wolfe.** 1968. The reaction of multihalogenated hydrocarbons with free and bound reduced vitamin B_{12}. Biochemistry **7**:1707–1713.