Arch Microbiol (1991) 155:272–277
030289339100029W

Archives of
Microbiology

H_2: heterodisulfide oxidoreductase, a second energy-conserving system in the methanogenic strain Göl

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Received October 15, 1990/Accepted November 12, 1990

Abstract. Washed everted vesicles of the methanogenic bacterium strain Göl catalyzed an H_2-dependent reduction of the heterodisulfide of HS-CoM (2-mercaptoethanesulfonate) and HS-HTP (7-mercaptoheptanoylthreonine phosphate) (CoM-S-S-HTP). This process was independent of coenzyme F_{420} and was coupled to proton translocation across the cytoplasmic membrane into the lumen of the everted vesicles. The maximal H^+/CoM-S-S-HTP ratio was 2. The transmembrane electrochemical gradient thereby generated was shown to induce ATP synthesis from ADP + P_i, exhibiting a stoichiometry of 1 ATP synthesized per 2 CoM-S-S-HTP reduced (H^+/ATP = 4). ATP formation was inhibited by the uncoupler 3,5-di-*tert*-butyl-4-hydroxy-benzylidene-malononitrile (SF 6847) and by the ATP synthase inhibitor *N,N'*-dicyclohexylcarbodiimide (DCCD). This energy-conserving system showed a stringent coupling. The addition of HS-CoM and HS-HTP at 1 mM each decreased the heterodisulfide reductase activity to 50% of the control. Membranes from *Methanolobus tindarius* showed $F_{420}H_2$-dependent but no H_2-dependent heterodisulfide oxidoreductase activity. Neither of these activities was detectable in membranes of *Methanococcus thermolithotrophicus*.

Key words: Archaebacteria – Methanogenesis – Membranes – Proton translocation – ATP synthesis – Electrontransport phosphorylation – Hydrogenase – F_{420} – Heterodisulfide reduction

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Abbreviations: $\Delta\mu_{H^+}$, transmembrane electrochemical gradient of H^+; CoM-SH, 2-mercaptoethanesulfonate; F_{420}, (*N*-L-lactyl-γ-L-glutamyl)-L-glutamic acid phosphodiester of 7,8-didemethyl-8-hydroxy-5-deazariboflavin-5′-phosphate; $F_{420}H_2$, reduced F_{420}; HTP-SH, 7-mercaptoheptanoylthreonine phosphate; DCCD, *N,N'*-dicyclohexylcarbodiimide; SF 6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile; *Mb.*, *Methanobacterium*; *Ml.*, *Methanolobus*; *Mc.*, *Methanococcus*; MV, methylviologen; BV, benzylviologen; MTZ, metronidazole

Methanogenic bacteria utilize a number of simple substrates (methanol, methylamines, acetate, formate and H_2/CO_2) as carbon and energy source. The central intermediate in the methanogenic pathway is CH_3-S-CoM [2-(methylthio)ethanesulfonate] which is reductively demethylated by the methyl-CoM methylreductase system. Recently the structure of factor B (HS-HTP, 7-mercaptoheptanoylthreoninephosphate), an obligatory cofactor of the methylreductase, was unravelled, and the reduction of CH_3-S-CoM could be formulated as the sum of two reactions:

$$CH_3\text{-S-CoM} + \text{HTP-SH} \rightarrow CH_4 + \text{CoM-S-S-HTP} \quad (1)$$

$$\text{CoM-S-S-HTP} + 2[H] \rightarrow \text{CoM-SH} + \text{HTP-SH} \quad (2)$$

(Bobik et al. 1987; Ellermann et al. 1988; Hedderich and Thauer 1988).

Recently it was shown that crude everted vesicle preparations of the methanogenic bacterium strain Göl catalyze the H_2-dependent reduction of the heterodisulfide of HS-CoM (mercaptoethanesulfonate) and HS-HTP and that this reduction is coupled to ATP synthesis (Peinemann et al. 1990). In subsequent work with washed vesicle preparations a reduced F_{420}: heterodisulfide oxidoreductase was discovered (Deppenmeier et al. 1990a) which accomplishes proton translocation and ATP synthesis (Deppenmeier et al. 1990b). The question arose whether the heterodisulfide reduction with H_2 as electron donor proceeds via coenzyme F_{420} or whether there are two independent redox systems present in the membrane, a coenzyme F_{420}-dependent one and an H_2-dependent one functioning in the absence of F_{420}. A soluble H_2-dependent enzyme was discovered and characterized in *Methanobacterium thermoautotrophicum* (Hedderich and Thauer 1988).

Materials and methods

Growth and harvest of cells

Strain Göl (DSM 3647) was grown in 20-l carboys on the medium described by Hippe et al. (1979), but additionally supplemented

with 1 g/l sodium acetate. For cultivation of *Methanolobus tindarius* (DSM 2278) in 2-l bottles the medium described by König and Stetter (1982) was used. *Methanococcus thermolithotrophicus* (DSM 2095) was grown autotrophically in a medium described for growth of *Methanococcus voltae* (Balch et al. 1979) in 2-l glass bottles filled with 500 ml medium with shaking at 37°C. 20-l mass cultures of strain Gö1 were anaerobically harvested by continuous centrifugation. Anaerobic harvest of *Ml. tindarius* and *Mc. thermolithotrophicus* was done in 400-ml air-tight centrifuge bottles at 24000 *g*.

Preparation of membranes and washed vesicles

Crude vesicles of strain Gö1 with 90% inside-out orientation (Mayer et al. 1987) were prepared as described previously (Deppenmeier et al. 1990b) with the exception that a 40 mM K-phosphate buffer, pH 7.2 was used containing 20 mM $MgSO_4$, 0.5 M sucrose, 1 mM dithiothreitol and 1 mg resazurin/l. Vesicles were concentrated by ultracentrifugation at 120000 *g* for 1 h at 4°C. The sedimented material was diluted in 9 ml K-phosphate buffer and centrifuged at 38000 *g* for 30 min. After resuspending the sediment this centrifugation step was repeated twice. The resulting pellet was suspended in the same buffer to a final protein concentration of 5–10 mg/ml. Membranes of *Ml. tindarius* and *Mc. thermolithotrophicus* were prepared by freezing and thawing of cell suspensions in a 25 mM Na-PIPES-buffer, pH 6.8 reduced with 0.2 mM Ti(III)-citrate. A few crystals of DNase were added to the resulting crude extracts which were centrifuged under identical conditions as described for strain Gö1. Cytoplasmic fraction of strain Gö1 was prepared as described previously (Deppenmeier et al. 1989).

Assay conditions

The experiments were performed at room temperature under an atmosphere of hydrogen in 2.7-ml glass vials. 600 µl 40 mM K-phosphate-buffer, pH 7.2 gassed with O_2-free N_2 and containing 20 mM $MgSO_4$, 0.5 M sucrose and 1 mg resazurin/l was reduced by stepwise addition of a few microliters Ti(III)-citrate until resazurin turned colourless. After addition of 10–20 µl washed vesicles (27 µg protein) the reaction was started with 0.6 mM CoM-S-S-HTP. To follow the reduction of CoM-S-S-HTP aliquots of 20 µl were withdrawn and analyzed for thiol groups with Ellman's reagent as described previously (Ellman 1958). The thiol content measured at zero time was subtracted. To determine the ATP concentration 2–5 µl aliquots were withdrawn by syringe and analyzed using the luciferin/luciferase assay (Kimmich et al. 1975). Additions were made as indicated. *N,N'*-dicyclohexylcarbodiimide (DCCD) and 3,5-di-*tert*-butyl-4-hydroxy-benzylidenemalononitrile (SF 6847) were added as ethanolic solutions. The controls received ethanol only. Proton translocation was followed as described by Deppenmeier et al. (1990b).

F_{420} nonreactive- and F_{420}-dependent hydrogenases were assayed in 1.7-ml glass cuvettes gassed with H_2 and filled with 1 ml anaerobic 100 mM Tricine buffer, pH 8.0 containing 5 mM dithiothreitol, 3 mM cysteine and 1 mg resazurin/l. 20 µM F_{420} (ε_{420} = 40 $mM^{-1} \times cm^{-1}$) or 5 mM methylviologen (ε = 13.9 $mM^{-1} \times cm^{-1}$) were added as electron acceptors. The benzylviologen-dependent heterodisulfide reductase was determined at room temperature in 1 ml 50 mM Tris/HCl buffer, pH 7.5, pregassed with N_2. After addition of 1 µl benzylviologen (1 M), 3 µl 50 mM Na-dithionite and 1–5 µl of the membrane preparation, the reaction was started by addition of CoM-S-S-HTP to a final concentration of 90 µM and followed at 575 nm ($\varepsilon_{benzylviologen}$ = 8.9 $mM^{-1} \times cm^{-1}$) (Hedderich et al. 1989). The $F_{420}H_2$ dehydrogenase and the $F_{420}H_2$:CoM-S-S-HTP oxidoreductase were followed in N_2-gassed 1 ml K-phosphate-buffer, pH 7.0 containing 20 mM $MgSO_4$, 0.5 M sucrose, 10 mM dithiothreitol and 1 mg resazurin/l. 20 µM $F_{420}H_2$ was added as electron donor for both enzymes. Methylviologen (MV) (0.3 mM) + metronidazol (MTZ) (0.5 mM) and CoM-S-S-HTP (90 µM) were used as electron acceptor for the $F_{420}H_2$-dehydrogenase and the $F_{420}H_2$:CoM-S-S-HTP-oxidoreductase, respectively. The production of F_{420} was followed at 420 nm (ε = 40 $mM^{-1} \times cm^{-1}$). Protein was quantified by the method of Bradford (1976).

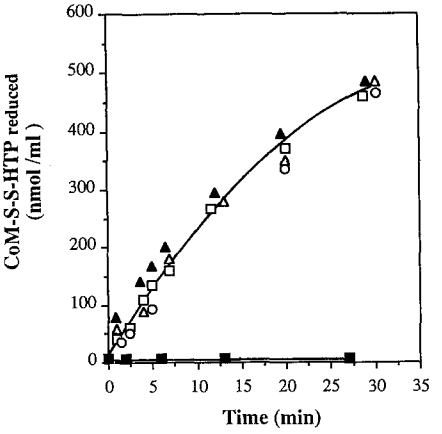

Fig. 1. Effect of oxidized F_{420} on H_2-dependent CoM-S-S-HTP reduction. Washed vesicles (80 µg protein) were incubated with 10 nmol SF 6847/mg protein in 2.7-ml glass vials containing 0.6 ml K-phosphate buffer, pH 7.2 (described in Materials and methods) at 25°C under H_2. The reaction was started by addition of 360 nmol CoM-S-S-HTP. ▲ F_{420} omitted; △ + 3.2 µM F_{420}; □ + 9.5 µM F_{420}; ○ + 32 µM F_{420}; ■ no protein added

Results

1) H_2-dependent CoM-S-S-HTP reduction independent of coenzyme F_{420}

Washed everted vesicles were tested for their ability to catalyze H_2-dependent CoM-S-S-HTP reduction in the presence or absence of added F_{420}. The experiments were performed in the presence of the uncoupler SF 6847 in order to compare maximal electron transport rates; SF 6847 has been shown to stimulate the heterodisulfide reduction in this system twofold. As is evident from Fig. 1 the reduction of CoM-S-S-HTP as determined from thiol formation proceeded at an initial rate of 240 nmol/min · mg protein. The F_{420} content of washed everted vesicles was 0.11 nmol/mg protein corresponding to a F_{420} concentration of 20–30 nM in the reaction mixture after addition of washed vesicles (0.17–0.26 mg protein/ml assay). The K_m values of F_{420}-dependent enzymes for F_{420} are in the order of 10–20 µM (Yamazaki and Tsai 1986; Livingston et al. 1987; Ma and Thauer 1990). The H_2-dependent CoM-S-S-HTP reduction was not stimulated by the addition of coenzyme F_{420} at concentrations of 3.2 to 32 µM. This showed that the H_2-dependent system was independent of coenzyme F_{420}. The reduction of CoM-S-S-HTP was not observed in the cytoplasmic fraction or when washed vesicles were omitted.

2) Proton translocation

Washed everted vesicles of the methanogenic bacterium strain Gö1 transfer protons across the cytoplasmic mem-

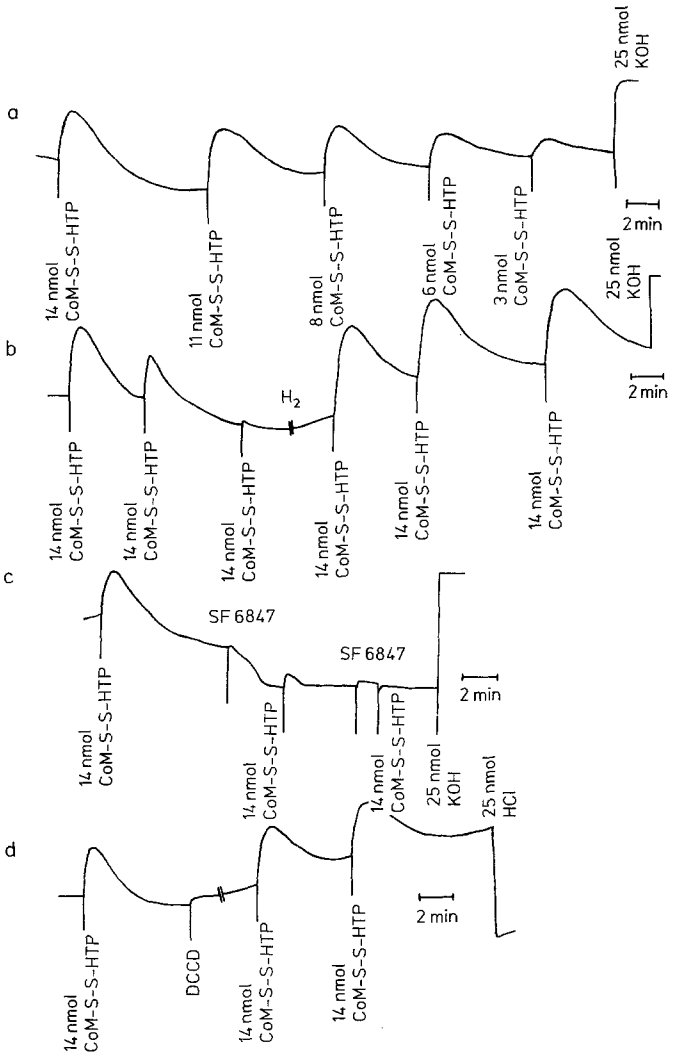

Fig. 2a–d. Proton uptake coupled to H_2-dependent reduction of CoM-S-S-HTP as catalyzed by washed vesicles of strain Gö1. Proton translocation was followed at room temperature in a glass vessel (11 ml) filled with 2.6 ml 40 mM KSCN, 0.5 M sucrose, 1 mg/l resazurin and 10 mM dithiothreitol. A pH electrode (model 8103 Ross, Orion research, Küsnacht, Switzerland), connected with a pH meter and a chart recorder, was inserted into the vessel from the top through a rubber stopper. After gassing with H_2 (in **b** with N_2 instead of H_2) the medium was reduced with 1 µl Ti(III)-citrate (100 mM) followed by the addition of washed vesicles (0.67 mg protein). The pH was adjusted to 6.8–6.9. The reaction was started by pulses of CoM-S-S-HTP. Where indicated the following agents were added: DCCD (150 nmol/mg protein); SF 6847 (2.4 nmol/mg protein). The amount of H^+ translocated was calculated from the difference between the maximum of alkalinization and the final baseline after reacidification. The pH changes were calibrated with standard solutions of HCl or KOH

brane in response to the $F_{420}H_2$-dependent CoM-S-S-HTP reduction (Deppenmeier et al. 1990b). The question arose whether such a proton translocation could also be observed with the substrate combination H_2 + CoM-S-S-HTP. Washed everted vesicles incubated under H_2 were pulsed with CoM-S-S-HTP and the resulting medium pH was monitored (Fig. 2). Each pulse was accompanied by a short period of alkalinization followed by a longer period of reacidification until a stable baseline was reached. These effects were due to proton uptake by the everted vesicles and a subsequent decay of the generated $\Delta\mu H^+$ after consumption of the CoM-S-S-HTP. It is apparent from Fig. 2a that the extent of alkalinization was dependent on the amount of CoM-S-S-HTP added. A maximal H^+/CoM-S-S-HTP ratio of 2 was determined. If H_2 was replaced by N_2 only the first CoM-S-S-HTP pulse led to a maximal alkalinization; proton translocation decreased largely after repeated addition of CoM-S-S-HTP (Fig. 2b). This was probably due to a consumption of reducing equivalents derived from traces of Ti(III)-citrate in the reaction mixture or from low potential electrons stored in membrane components. After gassing with H_2 the addition of CoM-S-S-HTP led to a reproducible maximal alkalinization. Proton translocation was completely inhibited by the addition of the protonophor SF 6847 (4.8 nmol/mg protein; Fig. 2c). Since the ATP synthase inhibitor DCCD had only a slight effect on H^+ uptake by everted vesicles (Fig. 2d), this enzyme could not be responsible for H^+ translocation via ATP hydrolysis. The absence of ATP or ADP in the reaction mixture also argues against H^+ translocation induced by ATP hydrolysis via an ATPase.

3) Coupling of heterodisulfide reduction and ATP synthesis

It is evident from Fig. 3 that the H_2-dependent reduction of CoM-S-S-HTP induced ATP synthesis. The initial rate of ATP synthesis was 105 nmol/min · mg protein while the initial rate of CoM-S-S-HTP reduction was 198 nmol/min · mg protein corresponding to a ATP/$2e^-$ ratio of 0.5 (determined within the first 4 min). Absolutely no ATP was formed when ADP was omitted. Furthermore, in the absence of ADP the CoM-S-S-HTP reductase activity decreased to 60% of the control (ADP added). Preincubation with the uncoupler SF 6847 enhanced this activity to 360 nmol/min · mg protein. Addition of SF 6847 to vesicles preincubated with ADP or ADP + DCCD similarly stimulated the heterodisulfide reduction 1.75-fold and 2.9-fold, respectively, in comparison to the controls without the uncoupler. This effect resembles the phenomenon of respiratory control observed in mitochondria. Control experiments under N_2 revealed a largely decreased rate of CoM-S-S-HTP reduction (8.3 nmol/min · mg protein), the electrons being probably derived from reducing agents in the reaction mixture. The rate of ATP synthesis decreased to 1.4 nmol/min · mg protein under N_2 or when the uncoupler SF 6847 or the ATP synthase inhibitor DCCD was present. This low rate of ADP phosphorylation is due to an adenylate kinase activity found in washed vesicles of strain Gö1 (Deppenmeier et al. 1990b). The activity explains the relatively high ATP level at zero time in all assays which had been preincubated with ADP 15 min prior to zero time (e.g. Fig. 3a).

4) Feed back inhibition

It is apparent from Figs. 1 and 3 that the activity of the H_2-dependent heterodisulfide reductase slowed down in

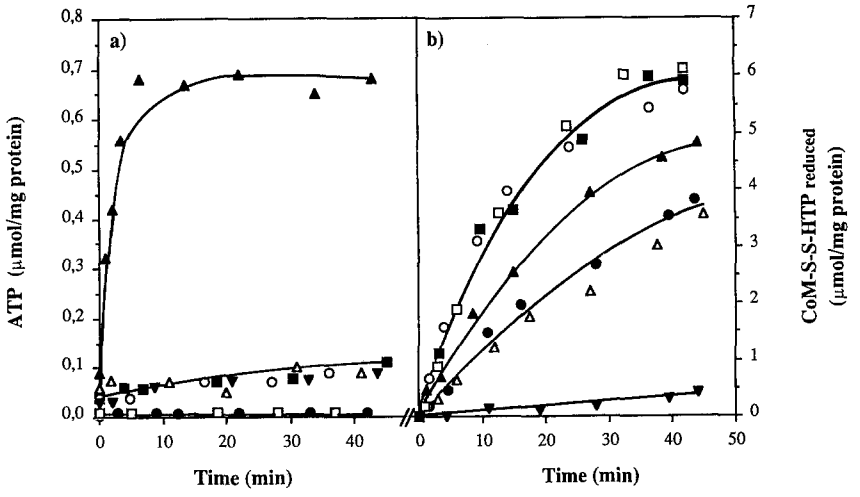

Fig. 3a, b. ATP synthesis and CoM-S-S-HTP reduction as catalyzed by washed vesicles. In parallel experiments washed vesicles (50 μg protein/assay) were preincubated for 15 min under H_2 in a final volume of 0.6 ml K-phosphate buffer, pH 7.2 at 25° C. The reaction was started by addition of 360 nmol CoM-S-S-HTP. The formation of ATP or thiol groups was determined as described under Materials and methods: **a** ATP synthesis, **b** CoM-S-S-HTP reduction. The following additions were made: ▲ ADP; △ ADP + DCCD; ● ADP omitted; ■ SF 6847 + ADP; □ SF 6847, ADP omitted; ○ DCCD + SF 6847 + ADP; ▼ ADP under N_2. The concentrations of ADP, DCCD and SF 6847 were 50 μM, 160 nmol/mg protein and 10 nmol/mg protein, respectively

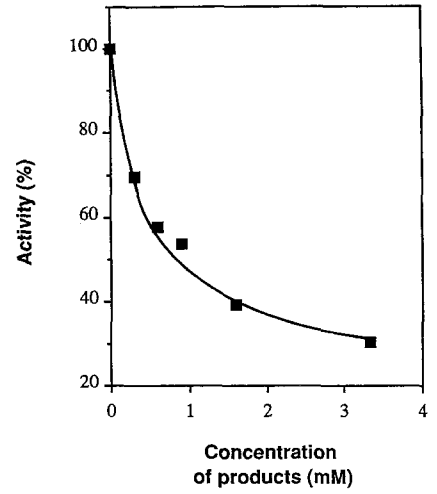

Fig. 4. Product inhibition of the benzylviologen-dependent heterodisulfide reductase. Washed vesicles of strain Gö1 (15 μg protein) were preincubated for 10 min with HS-CoM and HS-HTP at the indicated concentrations in a 1.7-ml glass cuvette containing 1 ml 50 mM Tris/HCl pH 7.5, 1 mM benzylviologen and 0.15 mM Na dithionite under N_2 at room temperature. After addition of 180 nmol CoM-S-S-HTP the oxidation of reduced benzylviologen was followed at 575 nm. In control experiments the solution of HS-CoM and HS-HTP was replaced by the same volume of water. 100% activity corresponded to 1.45 μmol CoM-S-S-HTP reduced/min · mg protein

the course of the reaction. Using reduced benzylviologen instead of H_2 as electron donor the heterodisulfide reductase activity was determined in the presence of the reaction products HS-CoM and HS-HTP. Increasing concentrations of the end products lowered the rate of CoM-S-S-HTP reduction (Fig. 4). Fifty percent inhibition was observed if both, HS-CoM and HS-HTP were present at 1 mM.

5) Comparison of membrane-bound enzymes involved in electron transport

Two energy conserving system – 1. the H_2:CoM-S-S-HTP oxidoreductase and 2. the $F_{420}H_2$:CoM-S-S-HTP oxidoreductase – have been detected in washed vesicles of strain Gö1. In order to analyze the distribution of the two systems in other methanogenic bacteria the membranes from two additional methanogenic species were tested for the presence of activities related to these systems. It is evident from Table 1 that the membrane-bound F_{420} dependent hydrogenase activity in strain Gö1 was 25-fold lower than in the hydrogenotrophic *Methanococcus (Mc.) thermolithotrophicus*. It was not detectable at all in washed membranes of the obligate methylotrophic organism *Methanolobus (Ml.) tindarius*. High hydrogenase activities were found in both, washed vesicles of strain Gö1 and membranes of *Mc. thermolithotrophicus* (in the former organism due to the F_{420}-nonreactive enzyme, in the latter organism probably due to the F_{420}-dependent enzyme), whereas the activity of F_{420}-nonreactive hydrogenase in membranes of *Ml. tindarius* was only 0.02 U/mg. Accordingly, there was no

Table 1. Comparison of membrane-bound enzymes involved in electron transport

Enzyme [U/mg protein]	Washed vesicles strain Gö1	Washed membranes	
		Ml. tindarius	*Mc. thermolithotrophicus*
F_{420}-dependent hydrogenase	0.03	< 0.01	0.77
F_{420}-nonreactive hydrogenase	11.6	0.02	37.8[c]
Benzylviologen-dependent heterodisulfide reductase	1.40	2.53	0.05
H_2:heterodisulfide oxidoreductase	0.49[a]	< 0.01	< 0.01
$F_{420}H_2$ dehydrogenase	0.52	0.38	0.23[b]
$F_{420}H_2$:heterodisulfide oxidoreductase	0.19[a]	0.21	< 0.01

[a] Uncoupled by SF 6847
[b] May be catalyzed by F_{420}-dependent hydrogenase
[c] Represents the sum of the F_{420}-dependent and the F_{420}-nonreactive hydrogenases

H_2:CoM-S-S-HTP oxidoreductase detectable in membranes of *Ml. tindarius*. But the activities of the benzylviologen-dependent heterodisulfide reductase (2.53 U/mg) and of the $F_{420}H_2$ dehydrogenase (0.38 U/mg) corresponded well with the $F_{420}H_2$:CoM-S-S-HTP oxidoreductase activity (0.21 U/mg protein). These values are in accordance with activities found in washed vesicles of strain Göl (Table 1).

In *Mc. thermolithotrophicus*, the benzylviologen-dependent heterodisulfide reductase was almost completely recovered from the cytoplasmic fraction after ultracentrifugation (about 90%). Therefore, the membrane-bound specific activity of this enzyme was very low (0.05 U/mg) compared to membranes from the methylotrophic methanogens. A similar situation is also encountered in *Mb. thermoautotrophicum* (Hedderich and Thauer 1988). The low membrane-bound heterodisulfide reductase activity in *Mc. thermolithotrophicus* explains the lack of a membrane-bound electron transfer system in this organism (H_2:CoM-S-S-HTP oxidoreductase and $F_{420}H_2$:CoM-S-S-HTP oxidoreductase < 0.01 U/mg).

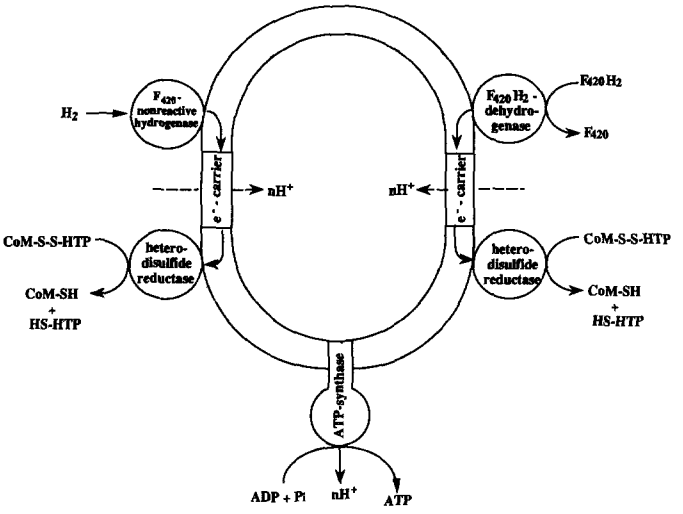

Fig. 5. Scheme of electron transfer coupled to proton translocation and ATP synthesis in washed vesicles of the methanogenic strain Göl

Discussion

The reduction of the heterodisulfide of HS-HTP and HS-CoM (CoM-S-S-HTP) which is the product of the actual methyl-CoM reductase was considered to be associated with energy conservation in methanogenic bacteria (Ellermann et al. 1988). First evidence for this assumption came from experiments with crude vesicle preparations of strain Göl (Peinemann et al. 1990). Here we report on an H_2-dependent reduction of CoM-S-S-HTP catalyzed by washed everted vesicles of this organism. This reaction was coupled with a proton transfer across the cytoplasmic membrane exhibiting a H^+/e^--ratio of 1. The $\Delta\mu H^+$ thereby generated served to drive ATP synthesis. This is evident from the inhibition of ATP formation by the protonophor SF 6847 and the ATP synthase inhibitor DCCD. The coupling between CoM-S-S-HTP reduction and ATP synthesis was stringent as revealed by the following results:

1) Addition of SF 6847 stimulated the reduction of CoM-S-S-HTP by H_2 and reversed the DCCD-induced inhibition of this reaction.
2) ADP stimulated the reductive cleavage of CoM-S-S-HTP.
3) 1 mol ATP was synthesized per 2 mol CoM-S-S-HTP reduced. From the data given a H^+/ATP ratio of 4.0 was calculated.

Similar results were obtained with $F_{420}H_2$ as electron donor (Deppenmeier et al. 1990b). In this case the H^+/e^- ratio was 0.95 and the H^+/ATP ratio was 4.6. The phenomenon of respiratory control was observed in both systems.

A tentative scheme of electron transfer involved in energy conservation in the methanogenic strain Göl is given in Fig. 5. Two membrane-bound enzymes – a F_{420}-nonreactive hydrogenase and a $F_{420}H_2$ dehydrogenase channel reducing equivalents from H_2 and $F_{420}H_2$, respectively, via unknown carriers to the heterodisulfide reductase. This process is accompanied by proton uptake into the everted vesicles.

The question may be asked why strain Göl contains two proton-translocating systems with the heterodisulfide as the terminal electron acceptor. It can be speculated that methanogenic bacteria growing at the expense of CO_2 reduction rely on the H_2-dependent system, whereas those growing on methanol or on methylamines take advantage of the $F_{420}H_2$-coupled system. In the latter case $F_{420}H_2$ is generated by methyl group oxidation and provided for the terminal reduction step. Accordingly a $F_{420}H_2$:heterodisulfide oxidoreductase is found in *Ml. tindarius* which does not utilize H_2 and contains only low hydrogenase activities. Strain Göl is methylotrophic but also able to grow with $H_2 + CO_2$ and, therefore, contains the two systems. The same can be expected for *Ms. barkeri*. There exists an interesting analogy: Those methanogens able to oxidize methyl groups contain cytochromes (Kühn et al. 1983; Jussofie and Gottschalk 1986) and it can be speculated that these electron carriers are involved in the $F_{420}H_2$:heterodisulfide oxidoreductase. Chemolithotrophic methanogens such as *Mc. thermolithotrophicus* do not contain cytochromes and may just be able to operate an H_2:heterodisulfide oxidoreductase. Unfortunately, there is no experimental evidence for a membrane-bound nature of this system in chemolithotrophic methanogens, neither in *Mb. thermoautotrophicum* (Hedderich and Thauer 1988; Hedderich et al. 1990) nor in *Mc. thermolithotrophicus* (this study). The distribution of hydrogenase is also interesting in this context. Two types, a F_{420}-reactive and a F_{420}-nonreactive one, have been detected in many methanogenic bacteria. On the basis of the above discussion, the latter may be involved in energy conservation and the former in the provision of $F_{420}H_2$ for the initial CO_2 reduction steps. The different function of $F_{420}H_2$ is then apparent: in chemolithotrophic methanogens it is generated from H_2 for CO_2 reduction to the level of

methyl-CoM, in methylotrophic methanogens it is generated by methyl group oxidation to serve as electron donor in energy conservation.

Acknowledgements. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (Bonn-Bad Godesberg).

References

Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS (1979) Methanogens: reevaluation of a unique biological group. Microbiol Rev 43:260–296

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bobik TA, Olson KD, Noll KM, Wolfe RS (1987) Evidence that the heterodisulfide of coenzyme M and 7-mercaptoheptanoylthreoninephosphate is a product of the methylreductase reaction in *Methanobacterium*. Biochem Biophys Res Commun 149:455–460

Deppenmeier U, Blaut M, Gottschalk G (1989) Dependence on membrane components of methanogenesis from methyl-CoM with formaldehyde or molecular hydrogen as electron donors. Eur J Biochem 186:317–323

Deppenmeier U, Blaut M, Mahlmann A, Gottschalk G (1990a) Membrane-bound $F_{420}H_2$-dependent heterodisulfide reductase in methanogenic bacterium strain Gö1 and *Methanolobus tindarius*. FEBS Lett 261:199–203

Deppenmeier U, Blaut M, Mahlmann A, Gottschalk G (1990b) Reduced coenzyme F_{420}:heterodisulfide oxidoreductase, a novel proton-translocating redox system in methanogenic bacteria. Proc Natl Acad Sci USA 87:9449–9453

Ellermann J, Hedderich R, Böcher R, Thauer RK (1988) The final step in methane formation-investigations with highly purified methyl-CoM reductase (component C) from *Methanobacterium thermoautotrophicum* (strain Marburg). Eur J Biochem 171:669–677

Ellman GL (1958) A colorimetric method for determining low concentrations of mercaptans. Arch Biochem Biophys 74:443–450

Hedderich R, Thauer RK (1988) *Methanobacterium thermoautotrophicum* contains a soluble enzyme system that specifically catalyzes the reduction of the heterodisulfide of coenzyme M and 7-mercaptoheptanoylthreonine phosphate with H_2. FEBS Lett 234:223–227

Hedderich R, Berkessel A, Thauer RK (1989) Catalytic properties of the heterodisulfide reductase involved in the final step of methanogenesis. FEBS Lett 255:67–71

Hedderich R, Berkessel A, Thauer RK (1990) Purification of heterodisulfide reductase from *Methanobacterium thermoautotrophicum* (strain Marburg). Eur J Biochem 193:255–261

Hippe H, Caspari D, Fiebig K, Gottschalk G (1979) Utilization of trimethylamine and other methyl compounds and methane formation by *Methanosarcina barkeri*. Proc Natl Acad Sci USA 76:494–498

Jussofie A, Gottschalk G (1986) Further studies on the distribution of cytochromes in methanogenic bacteria. FEMS Microbiol Lett 22:15–18

Kimmich GA, Randles J, Brand JS (1975) Assay of picomole amounts of ATP, ADP and AMP using the luciferase enzyme system. Anal Biochem 69:187–206

König H, Stetter KO (1982) Isolation and characterization of *Methanolobus tindarius*, sp. nov., a coccoid methanogen growing only on methanol and methylamines. Zbl Bakteriol Hyg I. Abt Orig C 3:478–490

Kühn W, Fiebig K, Hippe H, Mah RA, Huser BA, Gottschalk G (1983) Distribution of cytochromes in methanogenic bacteria. FEMS Microbiol Lett 20:407–410

Livingston DJ, Fox JA, Orme-Johnson WH, Walsh CT (1987) 8-Hydroxy-5-deazaflavin-reducing hydrogenase from *Methanobacterium thermoautotrophicum*. 2. Kinetic and hydrogen-transfer studies. Biochemistry 26:4228–4237

Ma K, Thauer RK (1990) Purification and properties of *N*-5,*N*-10-methylenetetrahydromethanopterin reductase from *Methanobacterium thermoautotrophicum* (strain Marburg). Eur J Biochem 191:187–193

Mayer F, Jussofie A, Salzmann M, Lübben M, Rhode M, Gottschalk G (1987) Immunoelectron microscopic demonstration of ATPase on the cytoplasmic membrane of the methanogenic bacterium strain Gö1. J Bacteriol 169:2307–2309

Peinemann S, Hedderich R, Blaut M, Thauer RK, Gottschalk G (1990) ATP synthesis coupled to electron transfer from H_2 to the heterodisulfide of 2-mercaptoethanesulfonate and 7-mercaptoheptanoylthreonine phosphate in vesicle preparations of the methanogenic bacterium strain Gö1. FEBS Lett 263:57–60

Yamazaki S, Tsai L (1986) Purification and properties of 8-hydroxy-5-deazaflavin-dependent $NADP^+$ reductase from *Methanococcus vannielii*. J Biol Chem 255:6462–6465