JOURNAL OF BACTERIOLOGY, June 2011, p. 2868–2870 Vol. 193, No. 11
0021-9193/11/$12.00 doi:10.1128/JB.00162-11
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Membrane-Bound Electron Transport in *Methanosaeta thermophila*▽

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Received 4 February 2011/Accepted 25 March 2011

The obligate aceticlastic methanogen *Methanosaeta thermophila* uses a membrane-bound ferredoxin:heterodisulfide oxidoreductase system for energy conservation. We propose that the system is composed of a truncated form of the $F_{420}H_2$ dehydrogenase, methanophenazine, and the heterodisulfide reductase. Hence, the electron transport chain is distinct from those of well-studied *Methanosarcina* species.

Biogenic methane production is dominated by methanoarchaea of the genera *Methanosarcina* (*Ms*.) and *Methanosaeta* (*Mt*.) that grow on acetate (6). Interestingly, *Methanosaeta* species can use only acetate as a substrate and are therefore obligate aceticlastic methanogens. Members of this genus are of special importance for the productivity of biogas plants, especially for reactor performance and stability at low acetate concentrations. To optimize biomethanation, it is necessary to acquire a comprehensive understanding of the biochemistry of acetate-dependent methanogenesis. Energy conservation in *Methanosaeta* species is not well understood, and even the sequencing of the *Methanosaeta thermophila* genome (13) did not unravel its mechanism. Comparative genomics indicated that the core methanogenic pathway, the breakdown of acetyl-coenzyme A (CoA) to methane, is obviously well conserved in *Mt. thermophila*. It can be concluded that acetate is activated by acetyl-CoA synthetases and the resulting acetyl-CoA serves as a substrate for a CO dehydrogenase/acetyl-CoA synthase (CODH/ACS) that oxidizes the carbonyl group to CO_2 and reduces ferredoxin. The methyl group is first transferred to tetrahydromethanopterin and then to coenzyme M (CoM) (2-mercaptoethanesulfonate) by the action of a membrane-bound Na^+ translocating methyltransferase. Methyl-CoM is oxidatively coupled to coenzyme B (CoB) (*N*-7-mercaptoheptanoyl-L-threonine phosphate) with the heterodisulfide CoM-S-S-CoB and methane as end products (5, 15). In contrast, the composition of the *Mt. thermophila* respiratory chain and the mode of energy conservation have remained largely unknown. Evidence was found only for the presence of the reduced ferredoxin (Fd_{red}) forming CODH/ACS and the heterodisulfide reductase (1, 13, 14). In *Methanosarcina* species, a ferredoxin:heterodisulfide oxidoreductase is used for energy conservation in acetate metabolism. *Methanosarcina mazei* and *Methanosarcina barkeri* employ the Ech hydrogenase for H_2 production from Fd_{red} and the H_2 uptake hydrogenase (Vho) that finally reduces methanophenazine, the electron donor for the heterodisulfide reductase (HdrDE). In *Methanosarcina acetivorans*, Ech hydrogenase is absent, and instead the Rnf complex is proposed to be responsible for Fd_{red} oxidation (6). Surprisingly, the *Mt. thermophila* genome does not contain genes coding for either hydrogenases or an Rnf complex (13). If Fd_{red} serves as an electron donor for the respiratory chain, the presence of a novel oxidoreductase must be postulated.

To investigate the electron transport processes in *Mt. thermophila*, we isolated cytoplasmic membranes from *Mt. thermophila* DSM6194 as described previously (17) with cell disruption by French pressure treatment (1,000 lb/in^2). Enzyme assays were carried out at 55°C (optimal growth temperature) (3, 17). Benzyl viologen-dependent heterodisulfide reductase activity was high, with 878 ± 90 mU mg^{-1} membrane protein (Table 1), and was comparable to activities found in *Methanosarcina* species (3) (Fig. 1). Hence, it is tempting to speculate that a membrane-bound heterodisulfide oxidoreductase system is used for energy conservation in *Mt. thermophila*, with a so-far-unidentified enzyme system that channels electrons into the respiratory chain. Genes encoding the $F_{420}H_2$ dehydrogenase (Fpo) were identified in the genome of *Mt. thermophila*, and this protein is therefore a candidate for electron input into the respiratory chain. Fpo is usually involved in methylotrophic methanogenesis and oxidizes $F_{420}H_2$ that is formed in the methanogenic pathway of *Methanosarcina* species. The *Methanosarcina* core enzyme FpoA to -O is highly homologous to NADH dehydrogenase I from bacteria and eukarya. However, the reduced cofactor oxidizing subunits from $F_{420}H_2$ dehydrogenases and NADH dehydrogenases are not homologous. The corresponding module of the bacterial and eukaryotic enzymes is made from the subunits NuoEFG. In contrast, the oxidation of reduced cofactor F_{420} is catalyzed by subunit FpoF of the $F_{420}H_2$ dehydrogenase (4). Interestingly, the *Mt. thermophila* genome codes only for an incomplete $F_{420}H_2$ dehydrogenase (FpoA to -N) that lacks FpoF and thus should be unable to oxidize $F_{420}H_2$, as shown for the *Ms. mazei* Δ*fpoF* mutant (16). Nevertheless, substantial quantities of F_{420} can be found in *Mt. thermophila* cells (9), so the $F_{420}H_2$ oxidizing reactivity of the membranes was determined. As expected, we could not detect either $F_{420}H_2$:heterodisulfide oxidoreductase activity or $F_{420}H_2$ dehydrogenase activity (Table 1). These findings show

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▽ Published ahead of print on 8 April 2011.

TABLE 1. Activities of membrane-bound oxidoreductases in *Mt. thermophila*

Enzyme[a] (system)	Electron donor	Electron acceptor	Reduction rate of electron acceptor (nmol min^{-1} mg^{-1})
$F_{420}H_2$ dehydrogenase	$F_{420}H_2$	Metronidazole	<1
Hydrogenase	H_2	Methyl viologen	<1
Heterodisulfide reductase	Benzyl viologen	CoM-S-S-CoB	878 ± 90
$F_{420}H_2$: heterodisulfide oxidoreductase	$F_{420}H_2$	CoM-S-S-CoB	<1
H_2:heterodisulfide oxidoreductase	H_2	CoM-S-S-CoB	<1
Fd:heterodisulfide oxidoreductase	Fd_{red}	CoM-S-S-CoB	470 ± 44
NADH:heterodisulfide oxidoreductase	NADH	CoM-S-S-CoB	<1
NADPH:heterodisulfide oxidoreductase	NADPH	CoM-S-S-CoB	<1

[a] Assay conditions were as described elsewhere (3, 17).

that energy conservation is not dependent on F_{420}. Also, NAD(P)H did not serve as an electron donor for heterodisulfide reduction in *Mt. thermophila* (Table 1). Many methanogens rely on hydrogen as an electron donor and/or obligate intermediate in the oxidation of other reducing equivalents ($Fd_{red}/F_{420}H_2$). For this purpose, some methanogens make use of a cytoplasmic F_{420} reducing hydrogenase (Frh) that can oxidize $F_{420}H_2$ with concomitant H_2 production and then use the membrane-bound Vho hydrogenase:heterodisulfide oxidoreductase to conserve energy (10, 16). *Mt. thermophila* does not possess genes coding for Frh or Vho, and indeed there was no hydrogenase activity or hydrogen:heterodisulfide oxidoreductase activity in *Mt. thermophila* membranes (Table 1). Therefore, an involvement of hydrogen or a hydrogen cycling mechanism for energy conservation in *Mt. thermophila* can be excluded. For the investigation of the Fd:heterodisulfide oxidoreductase in *Mt. thermophila*, an *Ms. mazei* ferredoxin, MM1619, was employed. The gene *mm1619* was cloned into pPR-IBA1 using BsaI restriction sites (primers 5′-ATGGTA GGTCTCAAATGCCAGCAATAGTTAACGCAGATGA A-3′ and 5′-ATGGTAGGTCTCAGCGCTTTCCGTTACTT TAATTGCCTGGTTC-3′), and the recombinant protein was produced in *Escherichia coli* BL21(DE3) (8, 12) and purified anaerobically (16). When ferredoxin MM1619 was reduced with the thermophilic organism *Moorella thermoacetica* CODH/ACS and incubated with *Mt. thermophila* membranes, heterodisulfide reduction was observed. This reaction was strictly dependent on ferredoxin and proceeded with a velocity of 470 ± 44 mU mg^{-1} membrane protein (Table 1). In comparison to experiments performed with membranes isolated from acetate-grown *Ms. mazei* (unpublished results), this reaction was 2- to 3-fold faster in *Mt. thermophila* than in *Ms. mazei*. These experiments elucidate the identity of the electron donor to the *Methanosaeta* respiratory chain as ferredoxin (Fig. 1). Our current working hypothesis is that in *Mt. thermophila*, the energy-conserving system is an Fd:heterodisulfide oxidoreductase that comprises the Fpo complex (without FpoF) and the heterodisulfide reductase, both of which are probably able to translocate H^+ or Na^+ across the cytoplasmic membrane (2, 7). The "headless" Fpo complex does not interact with $F_{420}H_2$, but it is tempting to speculate that iron-sulfur clusters in the FpoB or FpoI subunits directly accept electrons from Fd_{red}. In addition, it is evident that the membrane-bound methyltransferase contributes to the maintenance of the electrochemical ion gradient. An A_1A_O ATP synthase finally takes advantage of the ion motive force and produces ATP from ADP + P_i (11).

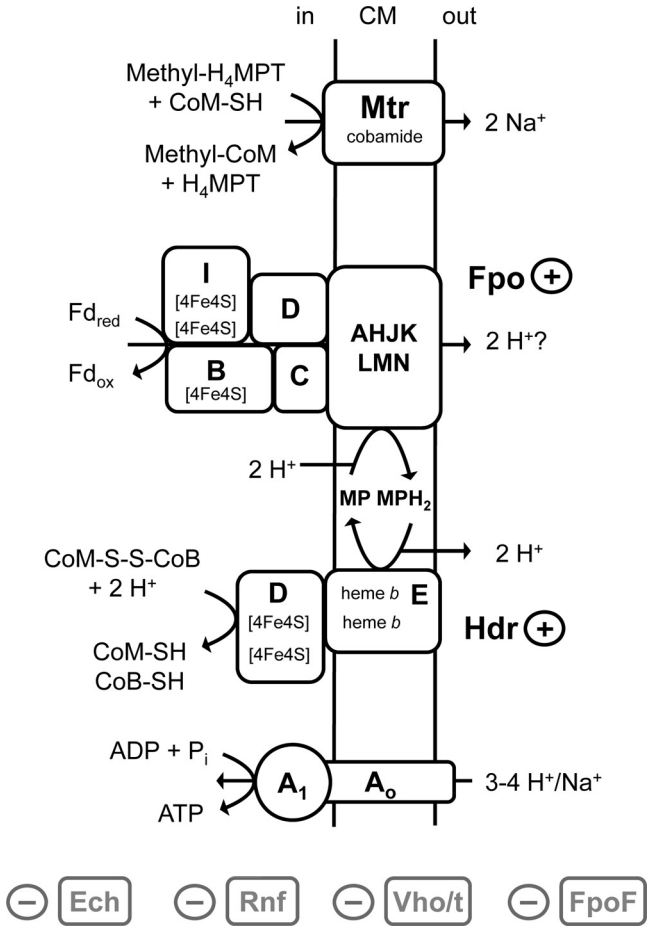

FIG. 1. Putative model of energy-conserving electron transfer reactions in *Mt. thermophila*. A_1A_O, A_1A_O ATP synthase; CM, cytoplasmic membrane; Ech, Ech hydrogenase; Fd_{red}, reduced ferredoxin; Fd_{ox}, oxidized ferredoxin; FpoABCDHIJKLMN, subunits A to N of the $F_{420}H_2$ dehydrogenase; FpoF, F subunit of the $F_{420}H_2$ dehydrogenase; H_4MPT, tetrahydromethanopterin; HdrDE, heterodisulfide reductase subunits D and E; Mtr, methyltransferase; MP, methanophenazine; MPH_2, reduced methanophenazine; Rnf, Rnf complex; Vho/t, viologen-reducing hydrogenase one/two; (+), present in *Mt. thermophila*; (−), not present in *Mt. thermophila*.

In light of the discussion about energy-conserving systems in aceticlastic methanogens, it is important to note that an Fd:heterodisulfide oxidoreductase activity was also found in the

membrane fraction of an *Ms. mazei* Δ*ech* mutant (17). The organism also contains the Fpo complex, and it was shown that subunit FpoF is in part located in the cytoplasm, indicating that the Fpo complex is not always completely covered by FpoF. This situation resembles the electron transport system of *Mt. thermophila*, and it is tempting to speculate that the Fpo complex (without FpoF) of *Ms. mazei* is also able to catalyze the oxidation of Fd_{red}, thereby channelling electrons directly into the respiratory chain.

We thank Elisabeth Schwab for technical assistance and Paul Schweiger for critical reading of the manuscript. Many thanks also go to Gunes Bender and Steve Ragsdale, Department of Biological Chemistry, University of Michigan Medical School, for providing the CODH/ACS from *Moorella thermoacetica*.

This work was supported by the Deutsche Forschungsgemeinschaft (grant De488/9-1).

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