Membrane-bound electron transport in *Methanosaeta thermophila*

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Short title: Respiratory chain of *Methanosaeta thermophila*

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Abstract

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 \( \mathrm{F}_{420} \)-\( \mathrm{H}_2 \) dehydrogenase, methanophenazine and the heterodisulfide reductase. Hence, the electron transport chain is distinct from those of well studied *Methanosarcina* species.

Main text

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 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* sp. is not well understood, and even the sequencing of the *Mt. thermophila* genome (13) did not unravel its mechanism. Comparative genomics indicated that the core methanogenic pathway, the breakdown of acetyl-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 substrate for a CO dehydrogenase/acetyl-CoA synthase (CODH/ACS) that oxidizes the carbonyl group to \(\text{CO}_2\) 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 \(\text{Na}^+\) 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 remained largely unknown. Evidence was only found for the presence of the reduced ferredoxin (Fd\(_{\text{red}}\)) forming CODH/ACS and the heterodisulfide reductase (1, 13, 14). In *Methanosarcina* sp. a ferredoxin: heterodisulfide oxidoreductase is used for energy conservation in acetate metabolism. *Ms. mazei* and *Ms. barkeri* employ the Ech hydrogenase for \(\text{H}_2\) production from Fd\(_{\text{red}}\) and the \(\text{H}_2\)-uptake hydrogenase (Vho) that finally reduces methanophenazine, the electron donor for the heterodisulfide reductase (HdrDE). In *Ms. acetivorans*, Ech hydrogenase is absent, and instead the Rnf complex is proposed to be responsible for Fd\(_{\text{red}}\) oxidation (6). Surprisingly, the *Mt. thermophila* genome does not contain genes coding for either hydrogenases or an Rnf complex (13). If Fd\(_{\text{red}}\) serves as 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 (17) with cell disruption by French pressure treatment (1000 psi). Enzyme assays were carried out at 55° C (optimal growth temperature) (3, 17). Benzyl viologen-dependent heterodisulfide reductase...
activity was high with $878 \pm 90$ mU mg$^{-1}$ membrane protein (Table 1) and was comparable to activities found in *Methanosarcina* sp. (3) (Figure 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* sp. The *Methanosarcina* core enzyme FpoA-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 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 only codes for an incomplete F$_{420}$H$_2$ dehydrogenase (FpoA-N) that lacks FpoF and thus should be unable to oxidize F$_{420}$H$_2$ as shown for the *Ms. mazei* ΔfpoF 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 neither detect F$_{420}$H$_2$-heterodisulfide oxidoreductase activity nor F$_{420}$H$_2$ dehydrogenase activity (Table 1). These findings show that energy conservation is not dependent on F$_{420}$. Also NAD(P)H did not serve as electron donor for heterodisulfide reduction in *Mt. thermophila* (Table 1). Many methanogens rely on hydrogen as electron donor and/or obligate intermediate in the oxidation of other reducing equivalents (F$_{red}$/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). So 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*, a *Ms. mazei* ferredoxin – MM1619 – was
employed. The gene *mm1619* was cloned into pPR-IBA1 using *Bsa*I restriction sites (primers
5'-ATGGTAGGTCTCAATGCAGCAATAGTTAACGAGATGAA-3' and 5'-ATGGTAGGTCTCAGCGCTTTCCGTTACTTTAATTGCCTGGTTC-3') and the recombinant
protein produced in *Escherichia coli* BL21 (DE3) (8, 12) and purified anaerobically (16).
When ferredoxin MM1619 was reduced with the thermophilic *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 two- to three-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 (Figure 1). Our current working hypothesis is that in *Mt. thermophila* the energy
conserving system is a Fd: heterodisulfide oxidoreductase that comprises the Fpo complex
(without FpoF) and the heterodisulfide reductase, both of which are probably able to
translocated H⁺ or Na⁺ across the cytoplasmic membrane (2, 7). The “head-less” Fpo
complex does not interact with F₄₃₀H₂ but it is tempting to speculate that iron-sulfur clusters in
the FpoB or Fpol 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 A₁Aₐ ATP synthase finally takes advantage of the ion
motive force and produces ATP from ADP + Pᵢ (11).

In the light of the discussion about energy conserving systems in aceticlastic
methanogens, it is important to note that a Fd: heterodisulfide oxidoreductase activity was
also found in the membrane fraction of a *Ms. mazei* ∆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 reduction of Fd_{red} thereby channelling electron directly into the respiratory chain.

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

<table>
<thead>
<tr>
<th>Enzyme 1) (system)</th>
<th>electron donor</th>
<th>electron acceptor</th>
<th>reduction rate of electron acceptor (nmol min$^{-1}$ mg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_{420}$H$_2$ dehydrogenase</td>
<td>F$_{420}$H$_2$</td>
<td>metronidazole</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Hydrogenase</td>
<td>H$_2$</td>
<td>methyl viologen</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Heterodisulfide reductase</td>
<td>benzyl viologen</td>
<td>CoM-S-S-CoB</td>
<td>878 ± 90</td>
</tr>
<tr>
<td>F$_{420}$H$_2$: heterodisulfide oxidoreductase</td>
<td>F$_{420}$H$_2$</td>
<td>CoM-S-S-CoB</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>H$_2$: heterodisulfide oxidoreductase</td>
<td>H$_2$</td>
<td>CoM-S-S-CoB</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Fd: heterodisulfide oxidoreductase</td>
<td>F$_{\text{red}}$</td>
<td>CoM-S-S-CoB</td>
<td>470 ± 44</td>
</tr>
<tr>
<td>NADH: heterodisulfide oxidoreductase</td>
<td>NADH</td>
<td>CoM-S-S-CoB</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>NADPH: heterodisulfide oxidoreductase</td>
<td>NADPH</td>
<td>CoM-S-S-CoB</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

1) Assay conditions were according to (3, 17).

Figure 1: Putative model of energy conserving electron transfer reactions in *Mt. thermophila*. A$_1$A$_0$, A$_1$A$_0$ ATP synthase; CM, cytoplasmic membrane; Ech, Ech hydrogenase; Fd$_{\text{red}}$, reduced ferredoxin; Fd$_{\text{ox}}$, oxidized ferredoxin; FpoABCDHIJKLMNO, subunits A-O of the F$_{420}$H$_2$ dehydrogenase; FpoF, F-subunit of the F$_{420}$H$_2$ dehydrogenase; H$_2$MPT, 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*. 

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