The F$_{420}$H$_2$ Dehydrogenase from *Methanosarcina mazei* Is a Redox-driven Proton Pump Closely Related to NADH Dehydrogenases*

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The F$_{420}$H$_2$ dehydrogenase is part of the energy conserving electron transport system of the methanogenic archaeon *Methanosarcina mazei* Gø1. Here it is shown that cofactor F$_{420}$H$_2$-dependent reduction of 2-hydroxyphenazine as catalyzed by the membrane-bound enzyme is coupled to proton translocation across the cytoplasmic membrane, exhibiting a stoichiometry of 0.9 H$^+$ translocated per two electrons transferred. The electrochemical proton gradient thereby generated was shown to drive ATP synthesis from ADP + P$_i$. The gene cluster encoding the F$_{420}$H$_2$ dehydrogenase of *M. mazei* Gø1 comprises 12 genes that are referred to as fpoA, B, C, D, H, I, J, K, L, M, N, and O. Analysis of the deduced amino acid sequences revealed that the enzyme is closely related to proton translocating NADH dehydrogenases of respiratory chains from bacteria (NDH-1) and eukarya (complex I). Like the NADH-dependent enzymes, the F$_{420}$H$_2$ dehydrogenase is composed of three subcomplexes. The gene products FpoA, H, J, K, L, M, and N are highly hydrophobic and are homologous to subunits that form the membrane integral module of NDH-1. FpoB, C, and D have their counterparts in the amphipathic membrane-associated module of NDH-1. Homologues to the hydrophilic NADH-oxidizing input modules are not present in *M. mazei* Gø1. Instead, the gene product FpoF may be responsible for F$_{420}$H$_2$ oxidation and may function as the electron input part. Thus, the F$_{420}$H$_2$ dehydrogenase from *M. mazei* Gø1 resembles eu- karyotic and bacterial proton translocating NADH dehydrogenases in many ways. The enzyme from the methanogenic archaeon functions as a NDH-1/complex I homologue and is equipped with an alternative electron input unit for the oxidation of reduced cofactor F$_{420}$ and a modified output module adopted to the reduction of methanophenazine.

*Methanosarcina mazei* strain Gø1 is a strictly anaerobic methanogenic archaeon that converts a limited number of simple substrates (H$_2$ + CO$_2$, methanol, methylamines, and acetate) to methane. 2-methylthioethanesulfonate is the central intermediate in all methanogenic pathways and is reductively demethylated to methane catalyzed by the 2-methylthioethanesulfonate reductase. The two electrons required for the reduction are derived from 7-mercaptoheptanoylthreonine phosphate, resulting in the formation of a heterodisulfide (CoB-S-S-CoM) of 2-mercaptoethanesulfonate (HS-CoM) and 7-mercaptoheptanoylthreonine phosphate (HS-CoB) (1). An energy-conserving step in the metabolism of methanogens is the reduction of CoB-S-S-CoM with either molecular hydrogen or reduced coenzyme F$_{420}$. In recent years, the membrane-bound electron transfer of *M. mazei* Gø1 has been analyzed in detail, resulting in the discovery of two proton translocating systems referred to as H$_2$:heterodisulfide oxidoreductase and F$_{420}$H$_2$: heterodisulfide oxidoreductase, respectively (2).

During growth on methylated substrates, part of the methyl groups of the substrates is oxidized to CO$_2$, and reducing equivalents are transferred to F$_{420}$. The reduced cofactor (F$_{420}$H$_2$) is reoxidized by the above-mentioned membrane-bound electron transport system consisting of an F$_{420}$H$_2$ dehydrogenase and a heterodisulfide reductase. The transfer of electrons between the enzymes is most likely mediated by methanophenazine, a hydrophobic cofactor that has been isolated from the cytoplasmic membrane of *M. mazei* Gø1. The overall process has been shown to be competent in driving proton translocation across the cytoplasmic membrane (3). The resulting electrochemical proton gradient is the driving force for ATP synthesis from ADP + P$_i$ catalyzed by an A$_3$A$_g$-type ATP synthase (2, 4).

The F$_{420}$H$_2$ dehydrogenase with a molecular mass of 115 kDa has been purified from *M. mazei* Gø1 and contains iron-sulfur clusters and FAD (5). The isolated enzyme is very similar to the corresponding protein from *Methanobacterium tindarius* (6) and is composed of five different subunits with molecular masses of 40, 37, 22, 20, and 17 kDa. A F$_{420}$H$_2$ dehydrogenase has also been purified from the sulfate-reducing archaeon *Archaeoglobus fulgidus* (7).

In this report the gene locus encoding the F$_{420}$H$_2$ dehydrogenase on the *M. mazei* genome is described. Furthermore, it is shown that the corresponding enzyme is a novel proton pump.
It was shown that electron transport from F420H2 to CoM-S-CoB from M. mazei Gö1 was performed according to Abken et al. (8). After gassing with N2, the vessel was filled with 3 ml of 40 mM KSCN solution containing 0.5 mg/ml resazurin, 10 mM dithioerythritol, and 200 nmol F420H2, followed by the addition of 50–80 µl of washed vesicles (1–1.4 mg protein/assay). The assay was continuously stirred, and the pH was adjusted to 6.8–6.9. The reaction was started by the addition of 20 nmol of 2-OH-phenazine (20 mM stock solution in ethanol). After finishing the experiments the pH changes were calibrated with a NaOH standard solution. SF 6847 was added to a final concentration of 15 mM/mg protein where indicated.

contributing to the generation of the electrochemical proton gradient in the methanogenic organism.

**EXPERIMENTAL PROCEDURES**

**Assay Conditions**—Washed inverted vesicles of M. mazei Gö1 (DSM 3647) were prepared according to Ide et al. (8). Proton translocation, electron transport, and ATP synthesis were monitored as described previously (8). The isolation and reduction of F420 were as well as the synthesis of 2-OH-phenazine was performed according to Abken et al. (9).

**Determination of N-terminal Amino Acid Sequences**—The F420H2 dehydrogenase was purified as described previously (5). The subunits were separated on SDS-polyacrylamide gel electrophoresis and electroblotted onto polyvinylidene difluoride membranes (Pall GmbH, Dreieich, Germany). N-terminal sequences were determined by Dr. B. Schmidt (Zentrum Biochemie, University of Göttingen) on an Applied Biosystems Procise Sequencer.

**Cloning and Sequencing of the fpo Gene**—The complete genomic sequence of M. mazei Gö1 was determined by a whole genome shotgun approach. More than 18,000 clones carrying inserts of approximately 2.5 kilobases in length from small insert libraries representative of the whole genome were sequenced from both ends using LICOR IL 4200 and ABI PRISM 377 DNA sequencers. The generated sequence readings were assembled into contigs with the Prap software implemented in the STADEN software package.

**Computer Analysis**—Protein sequence analysis was performed with the following internet servers: PredictProtein server; SignalP V1.1 World Wide Web Prediction Server, Center for Biological Sequence Analysis; and PSORT Prediction server.

**RESULTS**

**Proton Translocation Activity of the F420H2 Dehydrogenase**—It was shown that electron transport from F420H2 to CoM-S-CoB as catalyzed by washed inverted vesicles from M. mazei Gö1 is coupled to proton translocation across the cytoplasmic membrane (3). With the identification of methanophenazine as an electron carrier in the membrane the redox-driven proton translocation could be analyzed in more detail. It became evident that the key enzymes of the membrane-bound electron transport systems were able to interact with 2-OH-phenazine, which is a water-soluble homologue of methanophenazine (9).

Reducing equivalents from F420H2 were transferred to 2-OH-phenazine by the membrane-bound F420H2 dehydrogenase. Furthermore, the heterodisulfide reductase present in the cytoplasmic membrane was able to use reduced 2-OH-phenazine as electron donor for the reduction of CoB-S-S-CoM (10).

Taking advantage of 2-OH-phenazine as electron acceptor, it became evident that the F420H2 dehydrogenase is directly involved in the generation of an electrochemical proton gradient (Fig. 1). Concentrated vesicles were diluted with a sucrose/thiocyanate solution containing 200 nmol of F420H2 and were pulsed with 2-OH-phenazine under an atmosphere of molecular nitrogen. In the course of electron transport from F420H2 to 2-OH-phenazine a rapid alkalinization of the medium occurred that was due to proton movement into the lumen of the inverted vesicles. Thiocyanate was used as a permeant charge-compensating cation required to exchange for the ejected protons, thus maintaining the electroneutrality across the membrane. After consumption of F420H2, the energy conserving electron transport stopped leading to a decay of the generated ∆pH by passive diffusion of protons from the lumen of the inverted vesicles to the medium. This is indicated in the second reaction phase where a reacidification took place until the base line was reached again. After calibration of the instrument response with NaOH as an internal standard the extent of reversible alkalinization was used to calculate the H+2e− ratio. As evident from Fig. 1 (inset), the extent of H+ ejection was dependent on the amount of 2-OH-phenazine added. From the linear part of the reaction curve (0–7 nmol of 2-OH-phenazine/assay) a stoichiometry of 0.9±0.2 H+2e− was determined. Proton translocation was not observed when 2-OH-phenazine was replaced by ethanol or when F420 instead of F420H2 was added, indicating that proton transfer was specifically coupled to the F420H2-dependent 2-OH-phenazine reduction. In the presence of the protonophore SF 6847 the generation of a pH gradient was abolished, and a reversible alkalinization was prevented (Fig. 1).

**Coupling of Electron Transport and ATP Synthesis**—When the electron transfer from F420H2 to 2-OH-phenazine was analyzed the initial velocity of substrate conversion was 0.42±0.03 µmol/min×mg protein−1 resulting in an ATP/2e− stoichiometry of about 0.26 within the first minute of the reaction. In the absence of ADP, 2-OH-phenazine reduction slowed down to 0.3±0.02 unit/mg protein (Table I), and ATP synthesis was not possible (Fig. 2). The ATP synthase inhibitor DCCD led to a strong inhibition of phosphorylation of ADP and resulted in a slight decrease of the electron transport rate (0.32±0.04 unit/mg protein).

The conditions were the same as those described in the legend to Fig. 2. Each value represents an average of at least 10 determinations.

### Table I

| Additions            | Electron transport rateα (µmol/min×mg protein−1) |
|----------------------|-----------------------------------------------|
| ADP                  | 0.42±0.03                                      |
| No addition          | 0.30±0.02                                      |
| SF 6847              | 0.47±0.02                                      |
| SF 6847 + ADP        | 0.44±0.05                                      |
| DCCD + ADP           | 0.32±0.04                                      |
| DCCD + SF 6847 + ADP | 0.42±0.03                                      |

α 1 µmol of F420H2 oxidized or 1 µmol 2-OH-phenazine reduced per min and mg protein.
F420H2 dehydrogenase (Fig. 3). The proposed name for the gene codes for the 40-, 22-, 20-, and 16-kDa subunits of the purified enzyme. Finally, one DNA fragment was identified that amino acid sequences obtained from five subunits of the purified F420H2 dehydrogenase were currently sequenced in the Genomic Laboratory Goettingen.

The low control ratio is explained by the fact that about 50% of the vesicles were uncoupled and catalyzed electron transfer without generating an electrochemical proton gradient (2).

Structure of the fpo Operon Coding for the F420H2 Dehydrogenase from M. mazei Go1—The entire genome of M. mazei Go1 is currently sequenced in the Genomic Laboratory Goettingen. In the course of the sequencing process the data were checked for the presence of regions coding for the known N-terminal amino acid sequences obtained from five subunits of the purified enzyme. Finally, one DNA fragment was identified that codes for the 40-, 22-, 20-, and 16-kDa subunits of the purified F420H2 dehydrogenase (Fig. 3). The proposed name for the gene locus is fpo for F420H2:phenazine oxidoreductase. The fragment comprises 12 genes that were designated fpoA, B, C, D, H, J, K, L, M, N, and O. Each gene is preceded by at least one putative ribosome binding site starting with the initiation codon ATG or CTG (fpoL) and is terminated by the stop codons TAA or TGA. Other putative open reading frames could not be identified in the direct neighborhood of the flanking genes fpoA and fpoO, respectively. Upstream of fpoA is an AT-rich region, which contains potential archaeal consensus promoter sequences (Fig. 4A). At the opposite boundary a stem loop was found downstream of fpoO (Fig. 4B), followed by a T-rich region. The RNA duplex stability is ~51 kJ/mol (11). Furthermore, two different repeats of a 9-bp sequence and a 10-bp sequence (TAAAAGTTGGCT and CTTTATTTTT) were identified in this region (Fig. 4B). These structures are similar to transcriptional termination sites of polypeptide-encoding genes from other archaea (12). All 12 structural genes are organized so compactly in the cluster that there is almost no intergenic space for promoter or terminator-like sequences. Therefore, the genes fpoA–O may represent one operon. Evidence for this assumption came from Northern blot analysis. A single 11-kilobase signal was obtained when RNA from methanol-grown cells was hybridized with a specific probe (not shown). The size of the transcript is in full accordance with the length of the predicted fpo genes.

The deduced N-terminal amino acid sequences from fpoB, C, D, and I were identical to the N termini of four subunits of the purified F420H2 dehydrogenase. The fifth subunit is encoded by the fpoF gene, which will be described below. Because the gene products of the remaining fpo genes were not found in the homogeneous protein preparation, it is most likely that only a subcomplex of the F420H2 dehydrogenase was purified and that the other subunits were lost during purification. However, the core enzyme composed of FpoB, C, D, F, and I showed catalytic activity with F420H2 as electron donor and several artificial dyes as electron acceptors (10).

The deduced primary sequences of all predicted F420H2 dehydrogenase subunits from M. mazei Go1 were compared with those of other organisms. Eleven polypeptides showed significant homologies to NADH:plastoquinone oxidoreductases from cyanobacteria or chloroplasts and to NADH:UQ oxidoreductases from mitochondria and bacteria (complex I, NDH-1). Alignments of the fpo gene products A–N indicated similarities of 42–71% and identities of 37–60% to the corresponding subunits of the above-mentioned enzyme complexes (Table II). With the exception of nqo6 from Thermus thermophilus (13) and of nua1 from Pyrococcus abyssi (14), highest scores were obtained for gene products of higher plants and algae. On the other hand the fpo genes are arranged in the same order as bacterial NDH-1 genes. This fact prompted us to number the genes according to the nomenclature of the nua operon from Escherichia coli (Fig. 3).

Hydropathy plots revealed that the deduced polypeptides from fpoA, H, J, K, L, M, and N are membrane-integral components. The largest subunits are predicted to contain 14 (FpoM, N) to 16 (FpoL) transmembrane helices, and the smaller peptides FpoJ and FpoI are predicted to contain 2 and 8 membrane-spanning helices. Computer programs (PSORT Prediction/SignalP-Server) revealed that FpoA and FpoK may contain N-terminal signal peptides with cleavage sites at amino acid positions 38 and 23, respectively. If this prediction is correct, each of the processed polypeptides would comprise two transmembrane helices. In summary, the membrane integral part of the F420H2 dehydrogenase complex showed high similarities to the corresponding module of bacterial NDH-1 with respect to composition and homology of the amino acid sequences. In the bacterial nua/nqo operons known so far the genes encoding hydrophobic subunits are clustered at the 3’ end of the operon with the exception of nuaA/nqo7, which is located at the very beginning of the operon. The same organization is given in the fpo gene cluster from M. mazei Go1. It is important to note that the hydrophobic subunits of bacterial NDH-1 and of the F420H2 dehydrogenase have their counterparts in mitochondrially encoded complex I subunits from Eukarya (15).

Secondary structure prediction classifies the gene products FpoB, FpoC, FpoD, and FpoI as nonmembrane proteins. This is in agreement with the cellular localization of the homologous polypeptides from bacterial NDH-1 of Nuo BCDI from E. coli (16) and eukaryotic complex I of PSST, 30k, 49k, TYKV from bovine heart (17). These subunits comprise a module that connects the membrane-integral subcomplex to the NADH-oxidizing device (Fig. 3). It is most likely that FpoB, C, D, and I have a similar function in the F420H2 dehydrogenase. In subunit FpoB and FpoI, binding motifs for up to three tetranuclear iron-sulfur centers are present that are invariably conserved in the bacterial and eukaryotic equivalents (Table III). It has been suggested that these prosthetic groups mediate electron transport between the subcomplexes and play an important role in energy conversion of NDH-1 and complex I (18).

The amino acid sequence derived from the open reading...
was purified to homogeneity. The subunit contained nonheme iron, acid-labile sulfur, and FAD and was able to oxidize F_{420}H_{2} when the artificial electron acceptor methylviologen was added. Because AF1833 and FpoF are structurally equivalent and FpoF is part of the purified F_{420}H_{2} dehydrogenase from *M. mazei* Gö1, it may function as electron input device of the enzyme.

**DISCUSSION**

**Energetics of the F_{420}H_{2} Dehydrogenase**—F_{420} is the central cytoplasmic electron carrier in the methanogen *M. mazei* Gö1. The cofactor is involved in the process of methanogenesis from H_{2} + CO_{2} and from methylated compounds such as methanol and methylamines. In the methylotrophic pathway of methane formation (21) three out of four methyl-groups are reduced to methane. The remaining methyl-moieties are oxidized to CO_{2}, and the resulting reducing equivalents are transferred to F_{420}. The membrane-bound F_{420}H_{2} dehydrogenase catalyzes the reoxidation of F_{420}H_{2} and most likely transfers the electrons to the novel membrane-integral cofactor methanophenazine, which is a linear sesterterpenic ether of 2-OH-phenazine (2). Previous studies indicate that the protein is part of the membrane-bound F_{420}H_{2}:heterodisulfide oxidoreductase, which is one of the major energy conserving systems of *M. mazei* Gö1 (3). It was found that the electron transport from F_{420}H_{2} to the heterodisulfide (CoM-S-S-CoB) is coupled to the transfer of 3–4 protons across the cytoplasmic membrane. Using 2-OH-phenazine as a water-soluble precursor of methanophenazine, it became evident that the overall electron transfer can be divided into two partial reactions catalyzed by the F_{420}H_{2} dehydrogenase and the heterodisulfide reductase, respectively.

\[
F_{420}H_{2} + 2-OH-phenazine \rightarrow F_{420} + \text{dihydro-2-OH-phenazine}(\Delta \Gamma') = -20.2 \text{ kJ/mol}
\]

\[
\text{dihydro-2-OH-phenazine} + \text{CoM-S-S-CoB} \rightarrow 2-OH-phenazine + \text{HS-CoM} + \text{HS-CoB}(\Delta \Gamma'' = -10.6 \text{ kJ/mol})
\]

**REACTIONS 1 AND 2**

Very recently, it was shown that the dihydrophenazine-dependent heterodisulfide reduction (Reaction 2) is coupled to proton translocation exhibiting a stoichiometry of 2H^{+}/2e^{-} (8). The experiments in this publication show that in the first partial reaction catalyzed by the F_{420}H_{2} dehydrogenase, a maximum of 0.9 ± 0.2 protons/two electrons were translocated. Taking into account that about 50% of the vesicles are uncoupled (2), a H^{+}/2e^{-} ratio of about 2.0 could be considered. Then the H^{+}/2e^{-} stoichiometries of the partial reactions would add up to 4 and support the value of 3–4 H^{+}/2e^{-} translocated in the overall electron transport from F_{420}H_{2} to heterodisulfide (3). In summary, the data clearly indicate that the F_{420}H_{2} dehydro-
TABLE II
Homologies of deduced amino acid sequences of fpo genes from M. mazei Go1 to corresponding gene products of NADH dehydrogenases from other organisms

| Deduced amino acid sequence from M. mazei Gø1 | Highest homology to deduced amino acid sequence from | Identity | Similarity |
|---------------------------------------------|----------------------------------------------------|----------|-----------|
| **kDa** | **%** | **%** |
| fpoA | 14.1 | ndhC (Synechocystis PCC 6803) | 43 | 56 |
| fpoB | 20.7 | ndq6 (T. thermophilus) | 53 | 64 |
| fpoC | 18.3 | ndhJ (Nicotiana tabacum) | 38 | 42 |
| fpoD | 42.5 | ndhH (Secale cereale) | 48 | 63 |
| fpoH | 38.0 | ndhI (Plectonema boryanum) | 43 | 55 |
| fpoI | 15.2 | nufI (P. abyssi) | 40 | 53 |
| fpoJ | 18.5 | ndhE (P. boryanum) | 39 | 51 |
| fpoK | 11.3 | ndhE (Synechocystis PCC 6803) | 52 | 67 |
| fpoL | 72.3 | ndh7 (Pisum sativum) | 60 | 71 |
| fpoM | 53.9 | ndhH (Artemisia salina) | 40 | 47 |
| fpoN | 52.7 | ndhB (Synechocystis PCC 6803) | 37 | 50 |
| fpoO | 15.0 | frhB (M. jannaschii) | 38 | 48 |

* Part of the F420-reducing hydrogenase.

TABLE III
Binding motifs in the deduced amino acid sequences of the fpo genes

| Subunit | Positions of conserved cysteine residues | Putative type |
|---------|----------------------------------------|--------------|
| FpoB   | C109,C111,XX...C125,XX...C155,P         | [Fe2-S2]     |
| FpoI   | C112,C124,XX...C128,XX...C159,P         | [Fe4-S4]     |
| FpoO   | C129,XX...C131,XX...C136,XX...C148,P    | [Fe4-S4]     |
| FpoF   | C132,XX...C136,XX...C140,XX...C154,P    | [Fe4-S4]     |

FIG. 5. Tentative models of the F420H2 dehydrogenase and the NADH dehydrogenase 1 from E. coli. Proton translocating activity is indicated by broken arrows. UQ, ubiquinone 10; MPphen, methanophenazine. Functionally homologous subcomplexes are indicated by equal shading: I, membrane-integral module; II, membrane-associated module; III, input module; IV, unknown function.

The detailed reaction mechanism of the enzyme is unknown. Several intrinsic redox components were detected that are involved in NADH-dependent quinone reduction (18). The NuoE, F, and G module catalyzes the oxidation of NADH. NuoA, H, J, K, L, M, and N form the membrane-integral module and are involved in quinone reduction and proton-translocation. NuoB, C, D, and I connect the above-mentioned subunits and catalyze electron transfer from module 1 to module 3 (29).

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NuoB, C, D, and I. It is still a matter of debate whether this cluster is bound to NuoB or NuoI. However, it is well established that reduced N2 successively injects single electrons into the membrane subcomplex, thereby activating a serial array of quinones that are directly involved in H+ translocation (30).

The native structure of the \( \text{F}_{420}\text{H}_2 \) dehydrogenase is still unknown. However, the primary sequence informations, operon structure, and the homology to bacterial NDH-1 allow composition of a tentative model (Fig. 5). The gene product FpoF forms the input module that oxidizes \( \text{F}_{420}\text{H}_2 \) by hydride transfer. FAD present in this subunit catalyzes a two-electron/one-electron switch to reduce the [Fe4-S4] clusters. It is still an open question whether the gene product of \( fpoO \) is also part of the input module. This polypeptide is predicted to be hydrophilic and probably contains [Fe2-S2]-clusters. On the other hand this polypeptide was not copurified with the core enzyme, indicating that it is not essential for catalytic activity. From the \( \text{F}_{420}\text{H}_2 \)-oxidizing device the electrons are then channeled to the amphipathic connecting fragment composed of FpoB, C, D, and I, which is highly homologous to the corresponding module of NDH-1. Because all iron-sulfur signatures are conserved in FpoB and FpoI, it is reasonable to assume that a FeS-cluster comparable with N2 is present in one of these subunits. In analogy to NDH-1 and complex I cluster N2 should transfer electrons to the membrane integral module composed of FpoA, H, J, K, L, M, and N. In spite of the fact that the composition of the membrane part of the \( \text{F}_{420}\text{H}_2 \) dehydrogenase and NDH-1 is identical, the further electron transport pathway of the \( \text{F}_{420}\text{H}_2 \)-dependent enzyme is difficult to predict because methanogenic archaea do not contain quinones. Therefore, the reaction mechanism of the \( \text{F}_{420}\text{H}_2 \) dehydrogenase must be different at this point and must involve the electron carrier methanophenazine. The mid-point potential of 2-OH -phenazine, which is a potential precursor of methanophenazine, was determined to be \(-255 \text{ mV} \) (23). With the assumption that the redox potential of methanophenazine is similar, the change of free energy (\( \Delta G^\circ \)) coupled to the \( \text{F}_{420}\text{H}_2 \)-dependent methanophenazine reduction is only \(-20.2 \text{ kJ/mol} \) compared with a \( \Delta G^\circ \) of \(-89.9 \text{ kJ/mol} \) for the NADH-dependent reduction of ubiquinone. These thermodynamic facts are reflected by the coupling efficiencies of the enzymes because the maximal \( H^+ / 2e^- \) ratio of the \( \text{F}_{420}\text{H}_2 \) dehydrogenase is 1.8, in contrast to NDH-I/complex I, which translocates four or even more protons across the membrane per reaction cycle (30).

Despite the aforementioned differences, the \( \text{F}_{420}\text{H}_2 \) dehydrogenase represents a NDH-I homologue in the methanogenic archaea \( M. \text{maezi} \) Go1, which is equipped with an alternative input device and a modified proton translocating machinery. Further analysis of the enzyme may contribute to the understanding also of the reaction mechanism of NADH dehydrogenases.

Interestingly, \( fpo \)-like gene clusters were not detected in the methanogenic archaea \( M. \text{jannaschii} \) (31) and \( M. \text{thermoautotrophicum} \) (32). indicating that the \( \text{F}_{420}\text{H}_2 \) dehydrogenase is absent in these organisms. This fact is in accordance with the finding that the electron transport chains from obligate hydrogenotrophic methanogens of the order Methanobacteriales and Methanococcales are different from those of methylothetic methanogens belonging to the order Methanosarcinales. (2)
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