Another Unusual Type of Citric Acid Cycle Enzyme in *Helicobacter pylori*: the Malate:Quinone Oxidoreductase

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The only enzyme of the citric acid cycle for which no open reading frame (ORF) was found in the *Helicobacter pylori* genome is the NAD-dependent malate dehydrogenase. Here, it is shown that in this organism the oxidation of malate to oxaloacetate is catalyzed by a malate:quinone oxidoreductase (MQO). This flavin adenine dinucleotide-dependent membrane-associated enzyme donates electrons to quinones of the electron transfer chain. Similar to succinate dehydrogenase, it is part of both the electron transfer chain and the citric acid cycle. MQO activity was demonstrated in isolated membranes of *H. pylori*. The enzyme is encoded by the ORF HP0086, which is shown by the fact that expression of the HP0086 sequence from a plasmid induces high MQO activity in *mgo* deletion mutants of *Escherichia coli* or *Corynebacterium glutamicum*. Furthermore, this plasmid was able to complement the phenotype of the *C. glutamicum* *mgo* deletion mutant. Interestingly, the protein predicted to be encoded by this ORF is only distantly related to known or postulated MQO sequences from other bacteria. The presence of an MQO shown here and the previously demonstrated presence of a 2-ketogluutarate:ferredoxin oxidoreductase and a succinyl-coenzyme A (CoA):acetoacetyl-CoA transferase indicate that *H. pylori* possesses a complete citric acid cycle, but one which deviates from the standard textbook example in three steps.

A controversy with regard to the presence of malate dehydrogenase (MDH) in *Helicobacter pylori* became apparent when the genomic sequences of two strains of this organism were published (2, 29). Whereas biochemical measurements indicated that MDH activity (EC 1.1.1.37) was present in this organism, no open reading frame (ORF) for a possible MDH could be found in the genomic sequences (14, 17, 19, 24). In some organisms genes encoding MDH are more similar to those encoding a protein predicted to be encoded by this ORF is only distantly related to known or postulated MQO sequences from other bacteria. The presence of an MQO shown here and the previously demonstrated presence of a 2-ketogluutarate:ferredoxin oxidoreductase and a succinyl-coenzyme A (CoA):acetoacetyl-CoA transferase indicate that *H. pylori* possesses a complete citric acid cycle, but one which deviates from the standard textbook example in three steps.

A high level of SDH activity does exist (5, 7; see below). Thus, an ORF encoding an MDH is, in principle, the only omission in the list for a complete citric acid cycle (19).

We observed that the *H. pylori* genome contains an ORF encoding a protein with distant similarity to malate:quinone oxidoreductase (MQO), or the malate dehydrogenase (acceptor), EC 1.1.99.16, from *Corynebacterium glutamicum* (22). MQO is a citric acid cycle enzyme that, like MDH, converts malate to oxaloacetate but is a membrane-associated enzyme (or peripheral membrane enzyme) containing tightly bound flavin adenine dinucleotide (FAD) as a cofactor. In contrast to MDH, it donates the electrons from malate oxidation to quinones. The quinones are subsequently oxidized by the electron transfer chain. Cohn proved the existence of MQO in the 1950s in *Micrococcus lysodeikticus* ("Micrococcus luteus"), and the enzyme has since then been found in several gram-positive and gram-negative bacteria (8; see also references cited in reference 22). However, in the past decades MQO seems to have escaped the attention of most microbiologists. Recently, we were able to clone a gene from *C. glutamicum* encoding an MQO (22). Several homologues, with previously unknown functions, were observed in other bacteria. The protein hypothetically encoded by HP0086 from strain 26695 and its homologue in strain J99 are distantly related to these MQO sequences (Fig. 1). We wondered whether HP0086 might encode an MQO and thus complete the list of genes encoding citric acid cycle enzymes in *H. pylori*. Convincing experimental evidence for this hypothesis is presented here.

**MATERIALS AND METHODS**

Bacterial strains, plasmids, growth, and medium compositions. The strains and plasmids used in this work are listed in Table 1. For isolation of membranes, *Escherichia coli* was routinely grown overnight on Luria-Bertani medium (25) at 37°C. When strains carried the kanamycin resistance marker, 50 μg of kanamycin ml⁻¹ was added to the medium. *C. glutamicum* was grown overnight on 2% tryptone-yeast extract medium (25) at 30°C with, in the case of the strains carrying the marker, 25 μg of kanamycin ml⁻¹.
Membrane fragments of \emph{Helicobacter pylori} were isolated from overnight cultures (22). An overnight culture was adjusted to an absorbance of 0.5 at 578 nm. Cells were harvested by centrifugation at 3,400 \( \times \) g for 15 min. The membrane fraction was collected at 200,000 \( \times \) g for 45 min and washed once with buffer A.

DNA manipulations and cloning of the gene encoding the MQO from \emph{H. pylori}. All common molecular biological techniques used have been described previously (25). Preparation of electrocompetent cells and electroporation of \emph{C. glutamicum} were performed as described previously (30). PCR was performed using chromosomal DNA from \emph{H. pylori} strain ATCC 49503 as the template, which was prepared by boiling cells for 5 min and removing debris by centrifugation. The oligonucleotides HpF1 (GATAGGGTGCTTGGAATG) and HpR1 (GCATGTAAAGGTGTATATA) were used to amplify a 1.6-kbp fragment containing the entire HP0086 ORF, starting from 125 bases upstream of the ORF to 113 bases downstream of it. The amplification was performed using Phusion polymerase. The fragment containing the HP0086 ORF was isolated from this plasmid using the restriction enzymes PstI and SfiI. The PstI-SfiI fragment was cloned into the \emph{E. coli} or \emph{C. glutamicum} shuttle vector pEKExi. This plasmid was called pHp-mqo. PCR and plasmid isolation were performed to confirm the presence of pHp-mqo in the transformants. Although in php-mqo ORF HP0086 is under the control of a lac promoter originating from pEKExi, this promoter is leaky. The level of MQO activity was very high even in the absence of inducer, both in \emph{E. coli} and \emph{C. glutamicum}. Addition of 1 mM IPTG was, for both organisms, deleterious for growth and, in the case of \emph{C. glutamicum}, for specific activity of MQO. Therefore, all experiments were performed with cells grown in the absence of inducer.

Measurement of MQO, SDH, and NADPH dehydrogenase activities. MQO, SDH, and NADPH dehydrogenase activities in membrane fragments were measured by absorbance changes of the electron acceptor 2,6-dichlorophenolindophenol (DCPIP) in the presence of a 1 mM concentration of either malate, succinate, or NADPH (22). The NADPH dehydrogenase measurements included correction for the high rate of chemical reduction of DCPIP by NADPH. The existence of a coupled reaction of oxaloacetate reduction with NADH oxidation in isolated membranes of \emph{C. glutamicum} was also catalyzed by oxaloacetate reduction. Membranes were isolated from \emph{C. glutamicum} cell pellets or at 37°C in the case of \emph{E. coli}. Membranes were isolated by centrifugation for 45 min at 200,000 \( \times \) g. Membranes were resuspended in 100 mM sodium phosphate buffer, pH 6.0, titrated with NaOH to pH 7.5 (buffer A). The pellet was resuspended in the same amount of buffer A and centrifuged again. The supernatant was centrifuged for 30 min at 75,000 \( \times \) g and 4°C. The membrane pellet was resuspended in the same amount of buffer A and centrifuged again. The pellet was then resuspended in a small volume of buffer A, 100 to 200 \( \mu \)l for every 10 ml of the original extract. The final protein concentration was usually between 4 and 12 mg ml\(^{-1}\).

For the preparation of \emph{H. pylori} membranes, cells grown for 48 h were harvested by centrifugation at 3,400 \( \times \) g for 15 min. After being washed with buffer A, cells were resuspended in buffer A to an optical density at 578 nm of approximately 20 and sonicated four times for 1 min on ice (Branson cell disrupter B 15; output control, 5; 50% pulsed) under an N\(_2\) atmosphere, with the sonications adapted from one provided elsewhere (27).

Protein determination. Protein was determined with bicinchoninic acid in the presence of 0.5% (mass/vol) sodium dodecyl sulfate, according to a protocol adapted from one provided elsewhere (27).

**Table 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strains or plasmid</th>
<th>Characteristics</th>
<th>Source or reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\emph{C. glutamicum} ATCC 13032</td>
<td>Wild type</td>
<td>1</td>
</tr>
<tr>
<td>\emph{C. glutamicum} ( \Delta )mqo</td>
<td>\emph{mqo} deletion derivative of ATCC 13032</td>
<td>1 M. E. van der Rest, unpublished data</td>
</tr>
<tr>
<td>\emph{H. pylori} ATCC 49503 \emph{E. coli}</td>
<td>Wild type</td>
<td>12</td>
</tr>
<tr>
<td>\emph{DH5}(\alpha) (\Delta )mqo</td>
<td>(\sup{E4}\ lac{U169 (\text{p80dlacZ2}\Delta M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1 } ) \emph{mqo} (yojH) deletion derivative of MC4100</td>
<td>25 4 M. E. van der Rest, unpublished data</td>
</tr>
<tr>
<td>Plasmids \emph{pBluescript II SK}</td>
<td>\emph{Amp}(\beta) \emph{lacZ}</td>
<td>26</td>
</tr>
<tr>
<td>\emph{pEKExi}</td>
<td>\emph{Kan}(\beta) \emph{lacZ} \emph{tac}</td>
<td>15</td>
</tr>
<tr>
<td>\emph{pHP-mqo}</td>
<td>\emph{pEKExi} with \emph{H. pylori} \emph{mqo} gene (ORF HP0086)</td>
<td>This paper</td>
</tr>
</tbody>
</table>
Swissprot databases or with the BLAST service at the National Center for Biotechnology Information. Protein sequence alignment and clustering analysis were performed at the Multalin server of the Institut National de la Recherche Agronomique (Toulouse, France), using the blosum62 comparison matrix with the penalties for gap opening and gap extension set to 10 and 0, respectively (10).

For the sources for the MQO sequences were the Genbank, EMBL, and Swissprot databases (sequence accession numbers are as follows: C. glutamicum, O69282; Pseudomonas fluorescens, AF176206; H. pylori, AE000530; Bacillus halodurans, AB013369; E. coli, P33946; Mycobacterium tuberculosis, O69807; Campylobacter jejuni, AL111680), the Neisseria meningitidis Sequencing Group at the Sanger Centre (N. meningitidis, ORF NM0333, preliminary sequence data at the group's website [ftp://ftp.sanger.ac.uk/pub/pathogens/nm]), the Institute for Genomic Research (Neisseria meningitidis, preliminary sequence data at the institute's website [http://www.tigr.org]), and the Pseudomonas Genomic Project (Pseudomonas aeruginosa, preliminary sequence data at the project website [http://www.pseudomonas.com]).

RESULTS

Comparison of putative and experimentally established MQO sequences. Two experimentally established MQO sequences from C. glutamicum and E. coli are known (22; M. E. van der Rest, C. Lange, and D. Molenaar, unpublished data). Furthermore, a number of putative MQO sequences, all of eubacterial origin, can be found in databases. The derived protein sequences, although originating from both gram-positive and gram-negative organisms, form a coherent cluster in an alignment analysis, with 42% or higher identity and 61% or higher similarity (Fig. 1). A distinct feature in these sequences is a putative nucleotide-binding fold close to the N terminus, where FAD is thought to bind (22). The hypothetical protein HP0086 from H. pylori strain J99 and its homologue in strain Cj0393c are only distantly related to this cluster of MQO sequences, with identity ranging from 15 to 18% and similarity from 31 to 34%. However, this similarity is concentrated in a number of regions distributed over the whole protein. In searches with the position-specific iterated BLAST algorithm (3) the HP0086 sequence of H. pylori was also found to be similar to glycerol-3-phosphate dehydrogenase (GPD) flavoprotein subunits of bacterial origin; for example, it was found to be similar to the GPD subunit B of Haemophilus influenzae (Swissprot accession no. P43800). Close examination of the alignment of HP0086 with these GPD’s revealed, however, that the similarity is mainly concentrated in the N-terminal region, which is the putative cofactor-binding region. A gene (Cf0393c) in the genome of Campylobacter jejuni encoding a hypothetical protein with 48% identity to HP0086 is also distantly related to the cluster of MQO sequences.

Cloning, expression, and complementation studies of HP0086. To demonstrate that the hypothetical protein encoded by ORF HP0086 from H. pylori is an MQO, the ORF was expressed from a plasmid in E. coli Δmqo and C. glutamicum Δmqo strains, which lack MQO activity. This plasmid, called pHP-mqo, was constructed by amplifying ORF HP0086 from H. pylori DNA by PCR and inserting the product in the E. coli or C. glutamicum shuttle vector pEKE1. In this plasmid the ORF is under the control of a lac promoter. However, because of the leakiness of this promoter, no inducer had to be added to obtain very high expression (see Materials and Methods).

Malate oxidation by MQO in isolated membranes can be measured either by monitoring oxygen consumption or by observing reduction of the artificial acceptor dye DCPIP upon addition of malate. The DCPIP-reducing activity of the MQO from H. pylori in the hosts C. glutamicum and E. coli (Table 2) was very high compared to rates of the endogenous MQO’s in E. coli or C. glutamicum wild types (0 to 50 or 100 to 400 nmol min⁻¹ mg of protein⁻¹, respectively [D. Molenaar et al., unpublished data; van der Rest et al., unpublished data]). Also, the oxygen consumption rate measured in membranes of E. coli was very high (Table 2). From the perspective of electron transfer the oxygen reduction rate would be equivalent to a DCPIP reduction rate of 1,820 (2 × 910) nmol min⁻¹ mg of protein⁻¹, which is similar to the measured DCPIP reduction rate. It shows, furthermore, that the MQO from H. pylori is fully coupled to the electron transfer chain of E. coli. d-Malate oxidation (8% of l-malate oxidation) was also observed in membranes from E. coli Δmqo/pHP-mqo but was not measurable in membranes from C. glutamicum Δmqo/pHP-mqo (data not shown).

To verify that the MQO from H. pylori is also active in vivo, its ability to complement the phenotype of an mqo deletion strain of C. glutamicum was tested. C. glutamicum Δmqo in general grows slowly on several carbon substrates, but most distinctly it is unable to grow on a minimal medium designed for optimal growth of the wild type (D. Molenaar et al., unpublished data). Figure 2 shows this phenotype of C. glutamicum Δmqo and the fact that it can be complemented by expression of ORF HP0086 from plasmid pHP-mqo.

**Table 2.** MQO activities measured by DCPIP reduction or oxygen consumption in E. coli and C. glutamicum Δmqo strains expressing ORF HP0086 from a plasmid.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Activity (nmol min⁻¹ mg of protein⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCPIP reduction</td>
</tr>
<tr>
<td></td>
<td>No plasmid</td>
</tr>
<tr>
<td>E. coli Δmqo</td>
<td>&lt;5</td>
</tr>
<tr>
<td>C. glutamicum Δmqo</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

*a ND, not determined.

![Fig. 2](http://jb.asm.org/Downloaded.png)

**FIG. 2.** Complementation of the phenotype of an mqo deletion mutant of C. glutamicum by ORF HP0086 expressed from a plasmid. The C. glutamicum wild-type (Wt), Δmqo, and Δmqo/pHP-mqo strains were plated on minimal medium containing 1% (wt/vol) glucose. In the case of strain Δmqo/pHP-mqo the plate also contained 25 μg of kanamycin ml⁻¹. The plates were incubated for 60 h at 30°C.
involved in the reductive branch of a branched citric acid cycle. An important factor in reversibility would be the nature of the quinone acceptor. The standard redox potential of ubiquinone redox couples would in general be too high \((E^\circ_v = +113 \text{ mV})\) (28) to reduce oxaloacetate to malate \((E^\circ_v = -172 \text{ mV})\). On the other hand, menaquinones, having a much lower redox potential \((E^\circ_v = -74 \text{ mV})\), may be able to reduce oxaloacetate. As \(H. pylori\) contains only menaquinones (20), reversibility of the MQO reaction could not be excluded beforehand. The MQO activities of membranes from \(H. pylori\) were expected to be too low to be able to detect malate formation. Therefore, it was decided to study this reaction with membranes from \(C. glutamicum\) \(\Delta\text{mqo}\) in which the MQO of \(H. pylori\) was expressed. \(C. glutamicum\) seems to be the ideal host, since it also possesses only menaquinones (9). The experiments were designed to induce the net reduction of oxaloacetate by NADH through the subsequent action of NADH:quinone oxidoreductase and MQO. The competing oxidation of NADH by oxygen was prevented by inhibiting the electron transfer chain with stigmatellin downstream of the dehydrogenases at the level of tase and MQO. The competing oxidation of NADH by oxygen through the subsequent action of NADH:quinone oxidoreductase and MQO activities of membranes from \(H. pylori\) could not be excluded beforehand. The MQO activity in \(H. pylori\) membranes is in general low compared to, e.g., rates of oxygen consumption \((7.5 \text{ mmol min}^{-1} \text{ mg protein}^{-1})\) by membranes of \(E. coli\) (batch 6 in Table 3). In an experiment with batch 1 an oxygen consumption rate of 4.3 mmol min\(^{-1}\) mg of protein\(^{-1}\) was determined. If, as in the \(E. coli\) membranes, the rates of transfer of electrons from MQO into the electron transfer chain had been comparable to DCPIP reduction rates, an oxygen consumption rate of approximately 50 to 70 or 10 to 15 mmol min\(^{-1}\) mg of protein\(^{-1}\) might have been expected with batch 6 or 1, respectively. Since respiration is dependent on the integrity not only of the MQO but also of the subsequent redox enzymes, a possible explanation for the low rate of oxygen consumption may be that other components of the electron transfer chain are inactivated during membrane isolation.

### Discussion

It has been suggested before by others that \(H. pylori\) might possess MQO activity (13, 20) (MQO was referred to as dye-linked malate dehydrogenase). However, the experimental details underlying this assertion were not published. The results presented in this paper clearly show that \(H. pylori\) does possess MQO and that it is encoded by the HP0086 ORF. This also implies that an MQO previously detected in \(C. jejuni\) is probably encoded by the gene \(Cj0393c\) (16). It is apparent from Fig. 1 and the alignment analysis that the \(H. pylori\) and \(C. jejuni\) MQOs form a separate group of MQOs. Like all other MQO sequences, \(H. pylori\) and \(C. jejuni\) MQOs contain a conserved hydrophobic sequence at the N terminus in the proximity of the \(\alpha\) motif of a putative Rossmann fold involved in binding the ADP moiety of the FAD cofactor (22, 31). Most MQOs are positively correlated. With Spearman’s rank correlation test the absence of correlation was rejected when \(P\) was <0.01. The SDH activity equals 21% ± 4.5% (mean ± standard deviation of the sample) of the MQO activity. This correlation might be expected if both enzymes operate in the citric acid cycle. The NADPH dehydrogenase activity is not correlated with MQO or SDH activities. NADH dehydrogenase activity was not measurable with DCPIP as the acceptor. This is in accordance with the low NADH dehydrogenase activities determined by oxygen consumption rates (5).

### Table 3. Dehydrogenase activities in membrane fragments isolated from different batches of \(H. pylori\) cells

<table>
<thead>
<tr>
<th>Batch</th>
<th>OD at harvest</th>
<th>DCPIP-reducing activity (nmol min(^{-1}) mg protein(^{-1}))</th>
<th>MQO</th>
<th>NADPH dehydrogenase</th>
<th>SDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.33</td>
<td>33.7</td>
<td>14.0 (42)</td>
<td>8.4 (25)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.33</td>
<td>45.1</td>
<td>46.0 (102)</td>
<td>8.9 (20)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td>55.8</td>
<td>20.1 (36)</td>
<td>13.0 (23)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.65</td>
<td>67.0</td>
<td>6.1 (9)</td>
<td>11.5 (17)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.54</td>
<td>69.6</td>
<td>0.0 (0)</td>
<td>19.2 (28)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>106.6</td>
<td>84.6 (79)</td>
<td>15.9 (15)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>136.9</td>
<td>43.6 (32)</td>
<td>25.9 (19)</td>
<td></td>
</tr>
</tbody>
</table>

\*Rows are arranged in order of ascending MQO activity. The numbers in parentheses are percentages showing the level of activity relative to that of MQO OD, optical density at 578 nm.
FIG. 3. Tentative scheme for the citric acid cycle of *H. pylori* based on genome sequence data and biochemical data. Unusual enzymes are labeled with an asterisk. Pyr, pyruvate; AcCoA, acetyl-CoA; Cit, citrate; Icit, isocitrate; Kg, 2-ketoglutarate; Suc-CoA, succinyl-CoA; Suc, succinate; Fum, fumarate; Mal, malate; Oaa, oxaloacetate; Fdox and Fd red: oxidized and reduced ferredoxin, respectively; MQ and MQH 2, oxidized and reduced menaquinone, respectively. Enzymes: 1, pyruvate:ferredoxin oxidoreductase; 2, citrate synthase; 3, aconitase; 4, isocitrate dehydrogenase; 5, α-ketoglutarate:ferredoxin oxidoreductase; 6, succinyl-CoA acetoacetyl-CoA transferase; 7, fumarate reductase (SDH); 8, fumarase; 9, malate:quinone oxidoreductase. EC numbers and corresponding enzymes: 1, 1.8.1.3; 2, 1.2.7.1; 3, 1.2.4.1; 4, 1.2.4.2; 5, 1.3.5.1; 6, 1.3.5.1; 7, 1.6.99.1; 8, 1.3.3.2; 9, 1.6.99.2.

MOQ, a possibly new type of NAD-dependent MDH can only be answered clearly by purification of such an enzyme. Raw cell extracts contain metabolites and also contain many enzymes that use NAD as a cofactor. Thus, one runs the risk of observing artifacts instead of the supposed enzyme activities.

One of the reasons for organisms to use an MQO for malate oxidation might be that the oxidation of malate by an NAD-dependent MDH has a very unfavorable standard free energy difference (\(\Delta G^\circ = +28.5 \text{ kJ mol}^{-1}\)). In contrast, the oxidation of malate by MQO has a very favorable standard free energy difference (\(\Delta G^\circ = -18.5 \text{ kJ mol}^{-1}\) with menaquinone as the electron acceptor) (22). This difference should allow MQO to oxidize malate under circumstances where an MDH may not be able to do so, for example, when cytoplasmic [oxaloacetate]/[malate] or [NADH]/[NAD] ratios are high. Most MDHs are, when assayed under the right circumstances, capable of both malate oxidation and oxaloacetate reduction. However, as was shown above, MQO from *H. pylori* does not catalyze the reduction of oxaloacetate. This implies that MQO can only operate in the oxidative direction, which consequently strongly suggests that the citric acid cycle of *H. pylori* should, at least under some circumstances, operate oxidatively. It is an argument against a purely reductive function of the left branch (MDH or MQO, fumarase, and fumarate reductase or succinate dehydrogenase) (Fig. 3) of the citric acid cycle, as was asserted by others (24). If MQO were the only malate dehydrogenase in *H. pylori*, which at present seems uncertain, it would even be incompatible with such a function of the left branch.

Figure 3 shows a scheme of the proposed citric acid cycle of *H. pylori*. For all enzymes displayed, corresponding genes are found in the chromosome of *H. pylori*. In this scheme the conversion of succinyl-CoA to succinate by succinyl-CoA:acetoacetyl-CoA transferase is dependent on the continuous supply of acetoacetate and degradation of acetoacetyl-CoA (11). Alternatively, a continuous regeneration of acetoacetate from acetoacetyl-CoA may take place, possibly in reactions generating metabolic energy. It is also possible that a succinyl-CoA hydrolase (EC 3.1.2.3), for which no gene is presently known, catalyzes the hydrolysis of succinyl-CoA. As can be seen, the generation of NADH is avoided in central metabolism of *H. pylori*. For example, the pyruvate and α-ketoglutarate dehydrogenase of *H. pylori* are flavodoxin and ferredoxin dependent instead of NADH dependent (18). Consequently, the main pyridine nucleotide dehydrogenase activity of *H. pylori* is an NADPH dehydrogenase instead of an NADH dehydrogenase. The presence of an MQO instead of an NAD-dependent MDH would be in accordance with this fact, whereas an NAD-dependent MDH with a role in oxidative phosphorylation would constitute an exception to this scheme.

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B.K. and K.S. contributed equally to the paper.

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