Cloning and Characterization of Helicobacter pylori Succinyl CoA:Acetoacetate CoA-transferase, a Novel Prokaryotic Member of the CoA-transferase Family*

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Sequencing of a fragment of Helicobacter pylori genome led to the identification of two open reading frames showing striking homology with Coenzyme A (CoA) transferases, enzymes catalyzing the reversible transfer of CoA from one carboxylic acid to another. The genes were present in all H. pylori strains tested by polymerase chain reaction or slot blotting but not in Campylobacter jejuni. Genes for the putative A and B subunits of H. pylori CoA-transferase were introduced into the bacterial expression vector pKK223-3 and expressed in Escherichia coli JM105 cells. Amino acid sequence comparisons, combined with measurements of enzyme activities using different CoA donors and acceptors, identified the H. pylori CoA-transferase as a succinyl CoA:acetoacetate CoA-transferase. This activity was consistently observed in different H. pylori strains. Antibodies raised against either recombinant A or B subunits recognized two distinct subunits of Mr ~28,000 and 24,000 that are both necessary for H. pylori CoA-transferase function. The lack of α-ketoglutarate dehydrogenase and of succinyl CoA synthetase activities indicates that the generation of succinyl CoA is not mediated by the tricarboxylic acid cycle in H. pylori. We postulate the existence of an alternative pathway where the CoA-transferase is essential for energy metabolism.

Helicobacter pylori is a Gram-negative, microaerophilic bacterium that establishes long term chronic infections in the human stomach. It has been shown to lead to gastritis, peptic ulcers, and cancer (1–4). However, despite the importance of H. pylori as a pathogen, little is known about its metabolic and biosynthetic pathways. Information about the metabolism and substrate utilization by H. pylori is essential to understand bacterial colonization and survival and to design new drugs to treat the infection it causes.

Reversible transfer of CoA from one carboxylic acid to another. They have been identified in many prokaryotes (5–12) and in mammalian tissues (13–18). Although the CoA-transferases appear to be mechanistically and functionally very similar (5, 6, 10), their substrate ranges and activities differ.

The best characterized CoA-transferases are the β-ketoacyl-CoA synthase of Pseudomonas putida and Acinetobacter calcoaceticus, the butyrate-acetoacetate CoA-transferase from Clostridium acetoacetilicum, and the succinyl CoA-3-oxoadic-CoA transferase found in mammalian mitochondria (12, 18–22). The β-ketoacyl-CoA transferase (β-ketoacyl-CoA transferase; succinyl CoA transferase, EC 2.8.3.6) carries out the penultimate step in the conversion of benzoate and 4-hydroxybenzoate to tricarboxylic acid cycle intermediates in bacteria utilizing the β-ketoacyl pathway.

The acetate (butyrate)-acetoacetate CoA-transferase (EC 2.8.3.9) found among Clostridia acts mainly to detoxify the medium by removing the acetate and butyrate excreted earlier in the fermentation. This enzyme therefore has a role fundamentally different from other CoA-transferases, usually involved in the uptake of substrates for energy and structural use (6).

The succinyl CoA-3-oxoadic-CoA transferase (SCOT, EC 2.8.3.5) is responsible for the formation of acetoacetyl CoA by transfer of a CoA moiety from succinyl CoA to a 3-oxoadic, usually acetoacetate (23). This enzyme has been found to have the highest activity in heart and kidney of various mammals (18, 24). In the mitochondrion of these tissues, acetoacetate is converted to acetoacetyl CoA, which is further broken down to two acetyl CoA molecules capable of entering the tricarboxylic acid cycle. Therefore, this enzyme plays a crucial role in ketone body metabolism (18) as exemplified by the inborn error in humans (25).

In this study, we report the cloning and characterization of the two subunits of a CoA-transferase from H. pylori. A high degree of homology was found by comparing the deduced amino acid sequences from the two H. pylori genes with the pig and human CoA-transferases as well as with different bacterial CoA-transferases. These comparisons combined with measurements of enzyme activities identify H. pylori CoA-transferase as a succinyl CoA-acetoacetate CoA-transferase. Our data show that the bacterial enzyme is a dimeric protein in contrast to the monomeric eukaryotic homologues and indicate that it is rather a unique feature of H. pylori to harbor such an enzy-
mactic activity, which is not present in Escherichia coli or Campylobacter jejuni.

EXPERIMENTAL PROCEDURES

Bacterial Strains—The H. pylori strains are strain 69A (Department of Medical Microbiology, Amsterdam University, The Netherlands) isolated from a patient with nonulcer dyspepsia, strain 888–0 (Department of Medical Microbiology and Immunology, Hamburg University, Germany) isolated from a patient with a duodenal ulcer, strain NCTC 11637, and strains Ly2, Ly3, and Ly13 (Division of Gastroenterology, CHUV Lausanne) isolated from patients with a family history of gastric cancer.

The E. coli strains are XL-1 blue and SORL (Stratagene, JM105 (Pharmacia Biotech Inc.), M15 (Qiagen), and K12 (Institute of Microbiology, CHUV, Lausanne). Other strains include C. jejuni (Institute of Microbiology, CHUV, Lausanne) and H. felis strain ATCC 49179.

Media and Cell Culture Conditions—H. pylori strains were grown in solid medium containing 3.6% GC agar base (Life Technologies, Inc.) supplemented with 1% Isovitalex (Baltimore Biological Laboratories) and 10% donor horse serum (Biological Industries, Kibbutz Beth Haemek, Israel) and maintained in a microaerophilic atmosphere (85% N2/10% CO2/5% O2) at 37 °C for 2 days. C. jejuni and H. felis were cultured in liquid media and maintained in the same conditions.

E. coli strains were grown in Luria-Bertani (LB) medium at 37 or 30 °C. Solid medium was prepared by addition of 1.5% bacto-agar (Difco). The antibiotics used were: 50 μg/ml ampicillin, 12.5 μg/ml tetracyclin, and 40 μg/ml kanamycin (Sigma).

Genomic DNAIsolation—Bacterial DNA was prepared according to the method described by Hua et al. (26).

Amplification of H. pylori DNA by the Polymerase Chain Reaction—Southern blotting and screening of H. pylori DNA library (see below) were performed with oligonucleotides ICT 14 (5'-GATAAAACCCG-GCACC-3') and ICT 20 (5'GCGGGGCCGCTGTT-3'). Optimal PCR conditions were established using the PCR optimization kit (Boehringer Mannheim) to yield a 1000-bp DNA fragment. PCR was carried out in 50 μl containing 500 ng of H. pylori genomic DNA, 50 pmol of each primer, 200 μM dNTPs (Boehringer Mannheim), and a 2:1 mixture of TaqStart antibody (CLONTECH) + Taq DNA polymerase (Boehringer Mannheim) (final concentrations, 56 and 2 μM, respectively), in 10 mM Tris-HCl, pH 9.2, 50 mM KCl, and 1.5 mM MgCl2. The cycling program was one cycle consisting of denaturation at 94 °C for 3 min; hybridization at 50 °C for 2 min; extension at 72 °C for 3 min followed by 35 cycles at 94 °C for 30 s; 50 °C for 30 s; 72 °C for 1 min and one cycle of 94 °C for 20 s; 50 °C for 20 s; 72 °C for 5 min in the microprocessor controlled incubation system Crocidole III from Appli-
geneces. For subcloning in the XbaI and CiaI sites of pBluescript KS+ (Stratagene), the oligonucleotides used were ICT 21 (5'-GCCTCGTAG- CGATAAACCACCCGAC-3') and ICT 22 (5'-CCATCTGATG-GGCGGGCCGCTGTT-3').

To confirm the sequences, the putative open reading frames (ORFs) of the H. pylori CoA-transferase subunits were subcloned in the XbaI and CiaI sites of pBluescript KS+ using as primers the oligonucleo-
etides as recommended by the manufacturer (Pharmacia).

DNA Slot Blotting—Prior to application to nitrocellulose membranes (Bio-Rad), 2 μg of genomic DNA was denatured in 0.4 M NaOH, 10 mM EDTA, pH 8.0, then heated at 100 °C for 10 min, and neutralized by addition of an equal volume of cold 2 M ammonium acetate, pH 7.0. Treatment of the membranes and vacuum filtration of denatured DNA samples were performed according to the manufacturer's instructions (Bio-Dot® SF Microfiltration, Bio-Rad). After filtration, DNA was cross-linked to the membranes (UV Crosslinker; Hoefer Scientific Instruments).

Probes La beling, Hybridization, and Chemoluminescence (ECL) De-
tection Conditions—The different PCR fragment s used as probes were labeled with fluorescein-dUTP using the random prime labeling system (New England Biolabs) and then hybridized overnight with the membrane. The hybridized filters were washed at different stringency conditions (twice in 2× SSC, 0.1% SDS for 5 min at 21 °C and twice in 0.1× SSC, 0.1% SDS for 15 min at the indicated temperature) with constant agitation. Blocking of the membrane background and ECL detection were performed according to the manufacturer's instructions (ECL detection system, version II, Amersham Corp.). Exposure times of all membranes were 10 s (F, KODAK) and then chosen to visually optimize the chemiluminescent signals.

Preparation of Antibodies against H. pylori Gene Products—The two H. pylori putative ORFs were subcloned separately into pGEX-11 (Qiagen) with primers ICT 45 (5' and 3' primer sequences, see below) and ICT 46 (5'-GGATCCTGTCGACACCTATGAACTGG-3') for the A subunit and ICT 47 (5'-GGATCCTGTCGACACCTATGAACTGG-3') for the B subunit. The PCR amplification of the subcloned DNA fragments was performed with the primers ICT 22 and ICT 33 were used as a probe for slot blotting.

To perform the CoA-transferase activity assays (see below), the putative ORF1 (705 bp), ORF2 (624 bp), and ORF1 and ORF2 (1322 bp) subcloned under the P1BAD promoter were transformed in E. coli, cellular lysates (see below) prepared in the presence or the absence of 100 μM phenylmethylsulfonyl fluoride and 100 μM EDTA were run on 15% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes by electroblotting. After blocking overnight in 5% nonfat dry milk in Tris-buffered saline at pH 7.4, filters were incubated 60 min with rabbit serum directed against H. pylori sub-His tag or H. pylori subB-His tag and washed twice in Tris-buffered saline, pH 7.4, and twice in Tris-buffered saline containing 0.05% Nonidet P-40 (Sigma). Filters were then incubated for 60 min with goat anti-rabbit IgG antibodies coupled to horse-
radial peroxidase (Amersham Corp.), washed as above, and developed using the chemiluminescence detection kit (ECL) from Amersham Corp.

Preparation of Cellular Lysates—E. coli JM105 cells (Pharmacia) were transformed with the plasmid pFR222-3 containing the 1322-bp fragment encoding the complete putative H. pylori CoA-transferase (pCoAT), the 705 bp and the 624 bp corresponding to the H. pylori CoA-transferase ORF1 (pCoATA) and ORF2 (pCoATB), respectively, or the H. pylori Urease A gene (pUrea) (28). Cells were grown overnight at 37 °C in 25 ml of LB/50 µg/ml ampicillin, transferred to 200 ml of fresh medium, and grown up to A600 = 0.25. Cells were stimulated with 100 μM isopropyl-D-thiogalactopyranoside (Stratagene) for 1 h and harvested by centrifugation at 3000 × g for 10 min.

For β-ketoacid CoA and succinyl CoA:acetocetate CoA-transferase assays, bacteria were washed once with M9 salts minimal medium and harvested by centrifugation at 3000 × g for 10 min, and pellets were stored at −20 °C until just prior to disruption. Thawed bacterial pellets were reuspended in 1 ml of 50 mM phosphate buffer, pH 6.8, 1 mM dithiothreitol (Merek). Bacterial suspensions were disrupted by sonication, and debris was removed by centrifugation. For acetate-acetocetate CoA-transferase assay, the buffer used for bacterial wash and pellets resuspension was 50 mM MOPS, pH 7.0, 0.5 mM (NH4)2SO4, 20% (v/v) glycerol, 1 mM EDTA. For measuring the generation of succinyl CoA, bacterial pellets were reuspended in 50 mM Tris buffer, pH 7.4. For the determination of α-ketoglutarate dehydrogenase activity, bacterial pellets were reuspended in phosphate-buffered saline, pH 7.4, and disrupted by mild sonication. Protein concentration in cellular lysates was determined using the Bio-Rad protein assay with γ globulin as standard according to the supplier’s instructions.

β-Ketoacid CoA-Transferase Activity Assay—The β-ketoacid CoA-transferase assay was performed as described previously (9). Briefly, the assay mix contained 10 mM β-ketoacid (Sigma), 400 mM succinyl CoA (Fluka), and 40 mM MgCl2 in 200 mM Tris-HCl buffer, pH 8.0. After addition of cell lysate, the increase in A340, corresponding to the formation of the β-ketoacidyl CoA-Mg2⁺ complex, was measured.

Acetate Acetocetate CoA-Transferase Activity Assay—Activity was measured by monitoring the decrease in A310, due to the disappearance of acetocetate CoA as described previously (6, 21). The assay was performed in 100 mM Tris-HCl, pH 7.5, containing 5% (v/v) glycerol, 40 mM MgCl2, 50 μl of cellular lysate, 100 mM acetocetyl CoA, and 150 mM potassium acetate as carbonyl acid source.

Succinyl CoA:Acetocetate CoA-Transferase Activity Assay—Activity was measured by monitoring the increase in A310, corresponding to the formation of acetocetyl CoA as described previously (29). The assay contained 67 mM lithium-acetocetate, 300 mM succinyl CoA, and 15 mM MgCl2 in 50 mM Tris-HCl, pH 9.1.

All enzymatic activities were monitored every minute for 4 min. One unit of enzyme activity is defined as the amount of enzyme required to convert 1 mol of substrate to product in 1 min under the assay conditions used. The OD of the first minute was used to estimate specific activities, which were expressed as milliunits/mg of total protein in the cellular lysates.

Other Enzymatic Activity Assays—The activity of α-ketoglutarate dehydrogenase was assayed by determining the reduction of the oxidized form of NADH as described (30) except that the reaction was performed in the presence of 1 mM ADP and 10 mM α-ketoglutarate. Measure of succinyl CoA synthesis was performed in the presence of excess hydroxylamine by complexing the succinohydroxamic acid formed to ferric salts (31) or by measuring the activity of succinyl CoA synthetase in presence of succinate, CoA, and ATP according to Bridger et al. (32).

RESULTS

Amplification of H. pylori DNA by the Polymerase Chain Reaction and Screening of H. pylori lambda ZAP II Library—H. pylori 69A chromosomal DNA was amplified by PCR using primers ICT 14 and ICT 20 initially designed to select P-ATPase genes; the resultant PCR product of 1000 bp was partially sequenced. Sequence analysis revealed a striking homology with part of the genes encoding the CoA-transferase family (EC 2.8.3).

To verify the specificity of the PCR fragment as a probe, chromosomal DNA from H. pylori strains 69A and NCTC 11637 was cleaved with the restriction enzyme HaeIII and hybridized with the fluorescein-dUTP-labeled PCR product. Strong positive signals were observed with both strain DNAs (Fig. 1).

The probe was then used for the screening of an H. pylori Zap II library. Phages were amplified in E. coli XL-1 Blue cells, transfected by nylon membranes, and hybridized with the labeled PCR product. Fourteen positive clones were isolated after the third screening. Following in vivo excision, one plasmid named pGB1 was selected for further analysis.

Cloning and Analysis of the Nucleotide Sequence—Sequencing of pGB1 led to the identification of a 1395-bp fragment of H. pylori genomic DNA. Computer analysis of DNA sequence revealed two adjacent open reading frames designated ORF1 and ORF2 (Fig. 2). The largest open reading frame, ORF1, begins at nucleotide 73, terminates at nucleotide 771, and potentially encodes 233 amino acids. The second one, ORF2, located downstream, extends from nucleotide 771 to nucleotide 1391 and potentially encodes 207 amino acids. Both ORFs are oriented in the same direction. The position of the putative ribosome binding sites (Shine Dalgarno sequences) and the first methionine codons are underlined and written in bold type, respectively.

Sequence Comparison with Known Proteins—The predicted amino acid sequences were used to search through data bases by using the Blast programs from the University of Wisconsin Genetics Computer Group package. Significant homologies were found with five amino acid sequences, corresponding to CoA-transferases from very diverse organisms. The sequences of the different CoA-transferases were subsequently compared with each other using the Bestfit program. The protein most closely related to H. pylori ORFs is a B. subtilis protein. This protein of unknown function, listed as a probable β-ketoacid CoA-transferase, exhibits 75% and 87% similarity with ORF1 and ORF2, respectively. The next most closely related to H. pylori ORFs is the succinyl CoA:3-oxoadipate CoA-transferase from pig heart mitochondria (22) with 69 and 74% similarity.

The human counterpart was recently cloned, and the amino acid sequence was published (18); the homology between the ORFs is the succinyl CoA:3-oxoadipate CoA-transferase of P. putida (12) and A. calcoaceticus (33) and the acetate-acetoacetate CoA-transferase from C. acetobutylicum (21).

Amino acid sequence alignments prepared by using the Pileup program (University of Wisconsin Genetics Computer Group) confirmed the phylogeny of the different CoA-trans-
ferases and showed that *H. pylori* ORF1 and ORF2 align with the N and C termini, respectively, of the putative *Bacillus subtilis*, the pig and the human proteins (Fig. 3). Although the three latter enzymes are monomeric, all the other cloned CoA-transferases are made of two different subunits, named A and B.

**Detection of CoA-transferase Genes in Bacteria**—The ubiquity of the putative CoA-transferase gene(s) was monitored on chromosomal DNA by PCR amplification of the whole operon from nucleotides 73 to 1394 (Fig. 4A). All *H. pylori* strains tested (lanes 7–11) presented a strong positive signal. In contrast, no specific band was observed when using genomic DNA of *E. coli* JM 105, *C. jejuni*, or *H. felis*. To confirm the presence or the absence of similar sequences to that of *H. pylori* CoA-transferase in other bacteria, the bacterial DNAs were analyzed by slot blot and hybridized to the CoA-transferase AB probe using different stringency conditions (Fig. 4B). At the highest stringency conditions used (68 °C), only the CoA-transferase genes of *H. pylori* strains were detected; however, signals observed at 42 °C indicated that genes homologous to *H. pylori* CoA-transferases were present in *H. felis* and *E. coli* JM 105 genomes.

**Detection of the CoA-transferase Protein(s) in *H. pylori***—To confirm that the *H. pylori* enzyme was made of two subunits as suggested by the analysis of the nucleotide sequences, the *H. pylori* genes encoding the two ORFs were separately cloned into pQE11, overexpressed in *E. coli* M15 with six extra histidine residues at the amino end and purified by affinity chromatography using Ni$_2^+$-nitrilotriacetic acid beads. The purified recombinant proteins were injected into rabbits to yield specific antibodies against each ORF product. The anti-ORF1 and the anti-ORF2 antisera were then used to detect the endogenous *H. pylori* CoA-transferase. The antisera recognized two separate proteins of Mr; 26,000 (A subunit) and 24,000 (B subunit) (Fig. 5, lanes 1, A and B), respectively, confirming the dimeric structure of the putative CoA-transferase. The same results were

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**Fig. 2.** Nucleotide sequence and deduced amino acid residues of the putative *H. pylori* CoA-transferase A and B subunits. Potential ribosome binding sites are underlined. Putative transcriptional start sites and the first methionine of both subunits are in bold type. Stop codons are indicated with a bold asterisk. The sequences reported here have been deposited in the EMBL/GenBank™ data base under accession number AJ000086.
obtained when cellular lysates were prepared in the presence of phenylmethylsulfonyl fluoride (Fig. 5, lanes 2, A and B), thus ruling out a serine protease type digestion of a monomeric precursor as reported for the eukaryotic homologue (22).

Expression of H. pylori CoA-transferase Genes in E. coli—The whole DNA fragment containing the CoA-transferase operon of H. pylori was then inserted into pKK223-3 to yield a plasmid named pCoAT. Following isopropyl-β-D-thiogalactopyranoside induction of transformed E. coli cells, two distinct bands corresponding to proteins of approximately Mr 26,000 and 24,000 were detected by Western blotting with the anti-A and the anti-B subunit antibodies independent of the presence of phenylmethylsulfonyl fluoride (Fig. 5, lanes 3 and 4, A and B), confirming the fact that H. pylori putative CoA-transferase is

Fig. 3. Comparison of the amino acid sequences of the CoA-transferases. Alignment of the deduced amino acid sequences of the CoA-transferase subunits A and B of C. acetobutylicum (C. acet), A. calcoaceticus (A. calc), and P. putida (P. puti) with the two putative subunits of H. pylori (H. pyl) and the monomeric proteins from B. subtilis (B. subt) and pig and human heart mitochondria (Pig and Human). Identical residues among all aligned proteins are in bold type. The glycine cluster of each protein is underlined. The conserved active site glutamate is indicated with a bold asterix above the sequence. Numbers refer to the amino acid residues of the human heart SCOT.
Helicobacter pylori Succinyl CoA:Acetoacetate CoA-Transferase

CoA transferase (CoA T) activities were measured with the following substrates: succinyl CoA and \( \beta \)-keto dipate for the \( \beta \)-keto dipate CoA transferase, acet acetyl CoA and acetate for the acet acetyl CoA transferase, and succinyl CoA and acetate for the SCOT as CoA donors and acceptors, respectively. Specific activities were calculated as a function of the protein concentration of total bacterial lysates as described under “Experimental Procedures” and are expressed as the means \( \pm \) S.D. (\( n = 4 \)).

| Plasmid          | Specific activity | CoA transferase activities reconstituted in E. coli JM 105 cells transfected with recombinant plasmids |
|------------------|-------------------|-----------------------------------------------------------------------------------------------------|
| pUreA            | 0.10 \( \pm \) 0.02 | 2.13 \( \pm \) 0.06 | 0.25 \( \pm \) 0.06 |
| pCoAT            | 1.0 \( \pm \) 0.1   | 44 \( \pm \) 4       | 2800 \( \pm \) 200   |
| Fold increase    | 10×                | 22×                   | 11000×                  |
| pUreA/CoAT       |                    |                       |                         |

Table II
The two \( H. \) pylori SCOT subunits are required for enzymatic activity

SCOT activity was measured by monitoring the increase in \( A_{250} \) corresponding to the formation of acet acetyl CoA in bacterial lysates expressing plasmids containing the A (pCoATA), the B (pCoATB), or both (pCoAT) CoA transferase subunits. Specific activities were reported to the protein concentration (mg) in bacterial lysates and expressed as the means \( \pm \) S.D. (\( n = 4 \)).

| E. coli strain expressing | Specific activity |
|---------------------------|-------------------|
| pCoAT         | 2800 \( \pm \) 200 |
| pCoATA        | 5 \( \pm \) 4      |
| pCoATB        | 9 \( \pm \) 6      |

**Notes:**
- The \( H. \) pylori lysates was the one leading to the formation of acet acetyl CoA from succinyl CoA and acetate in the presence of \( Mg^{2+} \) (Fig. 6).
- The rate of formation of acet acetyl CoA in \( H. \) pylori lysates was lower than the one measured in transformed \( E. \) coli and was not linear upon time (Fig. 6A), indicating that acet acetyl CoA was processed concomitantly. The addition of \( 2 \) mM iodoacetamide, an inhibitor of acet acetyl CoA thiolute (34) resulted in a 2.5-fold increase of CoA transferase specific activity (60 \( \pm \) 2 milliunits/mg versus 25 \( \pm \) 2 milliunits/mg with iodoacetamide) (Fig. 6B) and in a linear accumulation of acet acetyl CoA (Fig. 6A).
- Demonstration of the Ability of \( H. \) pylori to Generate Succinyl CoA—In most bacteria, succinyl CoA is generated through the oxidative or the reductive arm of the tricarboxylic acid cycle, by \( \alpha \)-ketoglutarate dehydrogenase and by a succinate synthetase, respectively. We therefore have determined whether the corresponding activities were present in \( H. \) pylori. Although both enzymatic activities could be measured, no CoA transferase activity could be detected (Table II). However, when equal amounts (measured as \( A_{250} \)) of A and B subunit expressing cells were mixed before preparing the lysates, part of the activity, corresponding to 1146 \( \pm \) 24 milliunits/mg, could be recovered.

**Notes:**
- The only CoA transferase activity detected in \( H. \) pylori lysates was the one leading to the formation of acet acetyl CoA from succinyl CoA and acetate in the presence of \( Mg^{2+} \) (Fig. 6).
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Helicobacter pylori Succinyl CoA:Acetoacetate CoA-Transferase

When the accumulation of succinyl CoA was measured in the presence of excess hydroxylamine by complexing the succino-hydroxamic acid formed to ferric salts, 8.2 μmol of succinyl CoA/mg of bacterial lysate was detected in H. pylori compared with 5.3 μmol/mg in E. coli after 30 min. In contrast to what was observed for E. coli, the generation of succinyl CoA in H. pylori was independent of the addition of succinate, CoA, or ATP, the substrates of succinyl CoA synthetase, thus demonstrating that in H. pylori, succinyl CoA is generated by other enzyme(s).

**DISCUSSION**

We describe here the gene cloning and biochemical characterization of a novel enzyme of H. pylori. On the basis of comparative analyses of the amino acid sequences of identified CoA-transferases and reconstitution of the activity in E. coli, the H. pylori enzyme is a succinyl CoA:Acetoacetate CoA-transferase. This study constitutes the first report of a bacterial SCOT.

Sequence comparisons between the H. pylori CoA-transferase and other members of the family showed that both subunits of H. pylori CoA-transferase present a high degree of homology with the SCOT isolated from pig (22), human heart mitochondria (18), and an unclassified protein with no established function of B. subtilis (GenBank®). Furthermore, a significant degree of homology was detected with the other cloned members of the CoA-transferase family such as the butyrate-acetoacetate CoA-transferase (21) and the β-ketoadipate CoA-transferase (12), confirming the remarkable amino acid sequence conservation between CoA-transferases from very diverse organisms (12). If we exclude the Bacillus protein with no known function, all the bacterial CoA-transferases, including the H. pylori one, are made of two subunits, whereas the eukaryotic CoA-transferases are monomeric enzymes. Parales and Harwood (12) already noted that the A subunits of the prokaryotic proteins align with the N-terminal half of the mammalian protein, whereas the B subunits align with the C-terminal portion and that strong sequence similarity is seen throughout the length of the alignment with very few gaps.

Interestingly, the pig heart enzyme is susceptible to two proteolytic events. One of them is produced in the hydrophilic region and generates N- and C-terminal fragments that retain full catalytic activity (22). This highly hydrophilic region falls between the alignments of H. pylori CoA-transferase A and B subunits (Fig. 3), as already observed (12). Our results directly support the hypothesis of Parales and Harwood, who suggested then that a gene fusion probably occurred at some time during evolution of CoA-transferases. The second cleaved form of the pig heart CoA-transferase involves an autolytic fragmentation at the active site thiol ester, glutamate 344 (35), known to be conserved in all sequenced CoA-transferases (Fig. 3, bold asterix). The other region with striking homology between the mammalian enzyme and the A subunits of other CoA-transferases is that of glycine clusters (Fig. 3, underlined), which may be implicated in CoA binding to the transferase (12).

Although CoA-transferases are very conserved proteins, mechanistically and functionally similar in catalyzing the reversible transfer of one CoA from one carboxylic acid to another, their substrate ranges and their role in metabolism appear to be very different. Although other CoA-transferases are present in E. coli (36), no succinyl CoA:Acetoacetate CoA-transferase activity was detected in these bacteria, allowing us to monitor the H. pylori enzyme activity in reconstitution experiments after transfection of the H. pylori genes. Our results show that in the presence of succinyl CoA, the H. pylori enzyme is able to very efficiently convert acetoacetate into acetoacetyl CoA. No enzymatic activity could be detected when the structural genes encoding for the A and B subunits were expressed separately in E. coli, demonstrating that the two subunits are required for the function of the enzyme. It is of interest to note that genetic linkage of the H. pylori SCOT subunits is not required for the effective association of the two subunits into a functional heterodimer because activity can be restored by mixing E. coli cells expressing each subunit separately.

The succinyl CoA:Acetoacetate CoA-transferase is made constitutively in H. pylori when the bacteria are grown in vitro, on plates or in liquid cultures. We still do not know, however, the importance of the H. pylori CoA-transferase for Helicobacter survival/pathogenicity in vivo.

**TABLE III**

Determination of α-ketoglutarate dehydrogenase and succinate synthetase activities

Activities were measured in E. coli K12 and H. pylori as described under "Experimental Procedures." Specific activities were calculated in function of the protein concentration of total bacterial lysates and are expressed as the means of two separate determinations ± the statistical range. BD, below detection.

| Specific activity | α-Ketoglutarate dehydrogenase (EC 1.2.4.2) | Succinate synthetase (EC 6.1.2.5) |
|------------------|------------------------------------------|---------------------------------|
| E. coli K12      | 13.5 ± 1.2                               | 214 ± 45                        |
| H. pylori        | BD                                        | BD                              |

Fig. 6. Succinyl CoA:Acetoacetate CoA-transferase activity assay in H. pylori. H. pylori lysates were prepared (see "Experimental Procedures") and succinyl CoA:Acetoacetate CoA-transferase activities measured by spectrophotometry as formation of acetoacetyl CoA at 310 nm in 50 μl of lysates for 4 min. The results are expressed as the means of two activity determinations. Error bars represent the range of variation. A, no CoA donor (closed squares); 300 μM succinyl CoA (open circles); 300 μM succinyl CoA and 2 mM iodoacetamide (closed circles). B, the A<sub>310</sub> increment of the first minute was used to calculate specific activities. Black bar, no CoA donor; white bar, 300 μM succinyl CoA; hatched bar, 300 μM succinyl CoA and 2 mM iodoacetamide.
help us to understand the role of \( H. \) \textit{pylori} CoA-transferase.

However, because we have found that the genes encoding the CoA-transferase are preceded by a gene showing high homology to a thiolase (data not shown), we postulate that the bacteria convert the substrate to acetoacetyl CoA, which is then cleaved to two acetyl CoA (Fig. 7). These two molecules of acetyl CoA may then provide energy by a metabolic cycle in which one of them regenerates succinyl CoA from succinate and the other provides the anhydride energy required for ATP synthesis. Gene disruption experiments should allow us to establish whether a mutant without these genes is able to survive/grow and infect hosts.

As already mentioned, succinyl CoA:3-oxoacid CoA-transfases have been identified in mammalian tissues such as the brain and the heart where ketone bodies such as acetoacetate and \( \beta \)-hydroxybutyrate constitute a major metabolic fuel. In rats, it was found that in the gastric glandular mucosa, the enzyme activity in mitochondria was as high as that in heart and kidney, and two to four times greater than in other regions of the gastrointestinal tract. It was suggested that acetoacetate metabolism might support acid secretion on refeeding after a period without food (41, 42). Acetoacetate is definitively a substrate that is available in the gastric mucosa; however, our results do not allow us yet to assign a precise role to the succinyl CoA:acetoacetate CoA-transferase in \( H. \) \textit{pylori} metabolism. A great deal of information about the kinetic of the mammalian enzyme and about the action of metabolic inhibitors is available (17, 43) that should allow us to better understand the bacterial counterpart and use it as a potential target for new therapies against \textit{Helicobacter} infection.

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\textbf{Note Added in Proof}—The SCOT A subunit DNA sequence has been corrected (5 nucleotides). The updated version is available under the same accession number (AJ000086).

\textbf{REFERENCES}

1. Marshall, B. J. (1995) \textit{J. Am. Med. Assoc.} 274, 1064–1066
2. De Koster, E., Buset, M., Fernandes, E., and Deltenre, M. (1994) \textit{Eur. J. Cancer Prev.} 3, 247–257
3. Logan, R. P. H. (1994) \textit{Lancet} 344, 1078–1079
4. Forman, D., Webb, P., and Parsonnet, J. (1994) \textit{Lancet} 343, 2160–2161
5. Barker, H. A., Jeng, J.-M., Neff, N., Robertson, J. M., Tan, F. K., and Hossaka, S. (1978) \textit{J. Bacteriol.} 134, 1219–1225
6. Wiesenborn, D. P., Rudolph, F. B., and Papoutsakis, E. T. (1989) \textit{Appl. Environ. Microbiol.} 55, 323–329
7. Buckel, W., Dorn, U., and Semmler, R. (1981) \textit{Eur. J. Biochem.} 118, 315–321
8. Sramek, S. J., and Freerman, F. E. (1975) \textit{Arch. Biochem. Biophys.} 171, 14–26
9. Yeh, W.-K. and Ornston, L. N. (1981) \textit{J. Biol. Chem.} 256, 1565–1569
10. Tung, K. K., and Wood, W. A. (1970) \textit{J. Bacteriol.} 124, 1462–1474
11. Schifer, C., and Buckel, W. (1991) \textit{Appl. Environ. Microbiol.} 57, 2699–2702
12. Parales, R. E., and Harwood, C. S. (1995) \textit{J. Bacteriol.} 174, 4675–4676
13. Blair, J. B. (1969) \textit{J. Biol. Chem.} 244, 851–854
14. Russell, J. J., and Patel, M. S. (1982) \textit{J. Neurochem.} 38, 1446–1452
15. Hersh, L. B., and Jencks, W. P. (1967) \textit{J. Biol. Chem.} 242, 339–340
16. Sharp, J. A., and Edwards, M. R. (1978) \textit{Biochem. J.} 173, 759–765
17. White, H., and Jencks, W. P. (1976) \textit{J. Biol. Chem.} 251, 1688–1699
18. Kassovska-Bratinova, S., Fukao, T., Song, X.-Q., Duncan, A. M. V., Chen, S., Kondo, N., and Mitchell, G. A. (1996) \textit{Am. J. Hum. Genet.} 55, 951–954
19. Hrubec, E. J., Shapiro, M. K., Haughton, J. E., and Ornston, L. N. (1988) \textit{Am. J. Med. Genet.} 316, 58–62
20. Doten, R. C., Ngi, K. L., Mitchell, D. J., and Ornston, L. N. (1987) \textit{J. Bacteriol.} 169, 3168–3174
21. Cary, J. G., Petersen, D. J., Papoutsakis, E. T., and Bennett, G. N. (1990) \textit{Appl. Environ. Microbiol.} 56, 1576–1583
22. Lin, T. W., and Bridge, W. A. (1992) \textit{J. Biol. Chem.} 267, 975–978
23. White, H., and Jencks, W. P. (1976) \textit{J. Biol. Chem.} 251, 1708–1711
24. Fenselau, A., and Wallis, K. (1974) \textit{Science} 182, 323–329
25. Saudubray, J. M., Specola, N., Middleton, B., Lombes, A., Bonnefont, J. P., Jakobs, C., Vassault, A., Charpentier, C., and Day, R. (1987) \textit{Enzyme} 32, 80–90
26. Hua, J., Birac, C., and Mégraud, F. (1996) in Helicobacter pylori: Techniques for Clinical Diagnosis & Basic Research (Lee, A., and Mégraud, F., eds) pp.
27. Zhu, K. Y., and Clark, J. M. (1995) Biotechniques 18, 222–224
28. Labigne, A., Cussac, V., and Courreux, P. (1991) J. Bacteriol. 173, 1920–1931
29. Howard, J. B., Zieske, L., Clarkson, J., and Rathe, L. (1986) J. Biol. Chem. 261, 60–65
30. Gibson, G. E., Sheu, K. F., Blass, J. P., Baker, A., Carlson, K. C., Harding, B., and Perrino, P. (1988) Arch. Neurol. 45, 836–840
31. Reeves, H. C., Rabin, R., Wegener, W. S., and Ajl, S. J. (1971) in Methods in Microbiology (Norris, J. R., and Ribbons, D. W., eds) Vol. 6A, pp. 446–448, Academic Press, London
32. Bridger, W. A., Ramaley, R. F., and Boyer, P. D. (1969) Methods Enzymol. 13, 70–71
33. Kowalchuk, G. A., Hartnett, G. B., Benson, A., Houghton, J. E., Ngai, K. L., and Ornston, L. N. (1994) Gene (amst.) 146, 23–30
34. Stewart, P. R., and Rudney, H. (1966) J. Biol. Chem. 241, 1222–1225
35. Rochet, J. C., and Bridger, W. A. (1994) Protein Sci. 3, 975–981
36. Jenkins, L. S., and Nunn, W. D. (1987) J. Bacteriol. 169, 42–52
37. Chalk, P. A., Roberts, A. D., and Blows, W. M. (1994) Microbiology 140, 2085–2092
38. Mendz, G. L., Burns, B. P., and Hazell, S. L. (1995) Biochim. Biophys. Acta 1244, 269–276
39. Clayton, C. L., Tay, A., O’Donnell, C., and Chalk, P. A. (1995) Gut 37, A67
40. Mendz, G. L., Hazell, S. L., and Srinivasan, S. (1995) Arch. Biochem. Biophys. 321, 153–159
41. Hanson, P. J., and Carrington, J. M. (1981) Biochem. J. 200, 349–355
42. Carrington, J. M., and Hanson, P. J. (1981) Biochem. Soc. Trans. 9, 55
43. Fenselau, A., and Wallis, K. (1974) Biochemistry 13, 3884–3888