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Coenzyme F_{390} Synthetase from *Methanobacterium thermoautotrophicum* Marburg Belongs to the Superfamily of Adenylate-Forming Enzymes

PAUL VERMEIJ,[1]* RUUD J. T. VAN DER STEEN,[1] JAN T. KELTJENS,[1] GODFRIED D. VOGELS,[1] AND THOMAS LEISINGER[2]

Department of Microbiology, Faculty of Science, University of Nijmegen, NL-6525 ED Nijmegen, The Netherlands,[1] and Mikrobiologisches Institut, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland[2]

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Depending on the reduction-oxidation state of the cell, some methanogenic bacteria synthesize or hydrolyze 8-hydroxyadenylylated coenzyme F_{420} (coenzyme F_{390}). These two reactions are catalyzed by coenzyme F_{390} synthetase and hydrolase, respectively. To gain more insight into the mechanism of the former reaction, coenzyme F_{390} synthetase from *Methanobacterium thermoautotrophicum* Marburg was purified 89-fold from cell extract to a specific activity of 0.75 $\mu mol \cdot min^{-1} \cdot mg$ of protein^{-1}. The monomeric enzyme consisted of a polypeptide with an apparent molecular mass of 41 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *ftsA*, the gene encoding coenzyme F_{390} synthetase, was cloned and sequenced. It encoded a protein of 377 amino acids with a predicted M_r of 43,280. FtsA was found to be similar to domains found in the superfamily of peptide synthetases and adenylate-forming enzymes. FtsA was most similar to gramicidin S synthetase II (67% similarity in a 227-amino-acid region) and δ-(L-α-aminoadipyl)-L-cysteine-D-valine synthetase (57% similarity in a 193-amino-acid region). Coenzyme F_{390} synthetase, however, holds an exceptional position in the superfamily of adenylate-forming enzymes in that it does not activate a carboxyl group of an amino or hydroxy acid but an aromatic hydroxyl group of coenzyme F_{420}.

Methanogenesis, the coupling of methane formation to energy generation, represents a type of metabolism found only in the methanogenic archaea. Analysis of this central metabolic pathway has revealed that methanogens contain a set of unique cofactors for the reduction of various C_1 compounds to methane (5, 10, 36). One of these is the blue fluorescent 5-deazaflavin derivative, coenzyme F_{420} (7,8-didemethyl-8-hydroxy-5-deazaflavin-5′-phosphoryllactylglutamyl-glutamate) (Fig. 1) (6). It functions primarily as the central electron carrier both in methanogenesis and in a number of anabolic reactions (11, 30).

In a number of methanogens, coenzyme F_{420} was found to be converted into its 8-hydroxy-AMP and 8-hydroxy-GMP esters, coenzyme F_{390}-A and coenzyme F_{390}-G, upon exposure to oxygen (Fig. 1) (7, 32). After the reestablishment of anaerobic conditions, coenzyme F_{390} was reconverted into coenzyme F_{420} and AMP (13, 16). Studies with cell extracts from *Methanobacterium thermoautotrophicum* ΔH showed that the formation and degradation of coenzyme F_{390} were catalyzed by two separate enzymes, coenzyme F_{390} synthetase and coenzyme F_{390} hydrolase (12–14).

Recently, the enzymes catalyzing the formation and degradation of coenzyme F_{390} have been purified from *M. thermoautotrophicum* ΔH and characterized (33, 34). Coenzyme F_{390} synthetase was shown to be strongly inhibited by reduced coenzyme F_{420}. Thus, the activity of the enzyme is regulated by the ratio of reduced and oxidized coenzyme F_{420}. The activity of the coenzyme F_{390} hydrolase was found to be influenced by the redox potential of the environment. At low redox potentials, the enzyme showed optimal activity, while with an increasing redox potential, a lower activity was observed. From these data, it was concluded that the coenzyme F_{390} system is a sophisticated mechanism for methanogenic bacteria to sense the reduction and oxidation potential of the cell. Coenzyme F_{390} that is present after the redox potential of the cell increases may function as a signal compound, transferring information about the redox potential to a yet unknown site of action. We now have initiated studies on the genetics of coenzyme F_{390} metabolism by isolating the gene encoding coenzyme F_{390} synthetase from *M. thermoautotrophicum* Marburg and characterizing it.

MATERIALS AND METHODS

Materials. *M. thermoautotrophicum* Marburg (DSM 2133) was grown at 65°C in a 12-1 fermentor containing 10-1 mineral medium (28) and H_2-CO_2 (80 and 20%, respectively, by volume). Before entering the stationary growth phase, cells were anaerobically harvested with a Sharpless continuous centrifuge and were stored at −70°C under nitrogen. Cell extract was prepared as documented before (12), with the exception that no dithiothreitol was added to the buffers. Coenzyme F_{420} was purified from *M. thermoautotrophicum* ΔH essentially as described before (6), except that a bonded-phase octadecyl column was used to obtain pure and salt-free preparations (29).

Procion Red HE-3B (ICI, Manchester, United Kingdom) column material was a gift of J. Visser, Department of Molecular Genetics, University of Wageningen (Wageningen, The Netherlands). The dye was immobilized (0.3 g of dye per 25 g of Sepharose CL-6B) as documented before (1). TSK-butyl 650(S) was purchased from Merck. The TSK-DEAE-5PW column was obtained from TosoHaas (Montgomeryville, Pa.). Centriprep YM-10 and Centricon 10 concentrators were from Amicon Division. ATP was purchased from Boehringer Mannheim. All other chemicals were of the highest grade available.

All DNA-modifying enzymes were purchased from Boehringer Mannheim, New England Biolabs, Pharmacia, Epicentre Technologies, and Stratagene. Radioactively labelled compounds were bought from Amersham. Synthetic oligonucleotides were from Microsynth AG (Windisch, Switzerland) and Eurogentec (Seraing, Belgium). Cloning vector pBluescript was from Stratagene. *Escherichia coli* Dh5α (GIBCO BRL) served as the host for the vectors.

Enzyme assays. F_{390} synthetase was routinely assayed in a 1-ml cuvette under air by simultaneously following the changes in A_{340} and A_{420} at 55°C (12). A typical assay mixture (750 μl) contained 100 mM potassium phosphate buffer

* Corresponding author. Mailing address: Department of Microbiology, Faculty of Science, University of Nijmegen, Toernooiveld 1, NL-6525 ED Nijmegen, The Netherlands. Phone: 31 24 3653437. Fax: 31 24 3553450. Electronic mail address: jankel@sci.kun.nl.

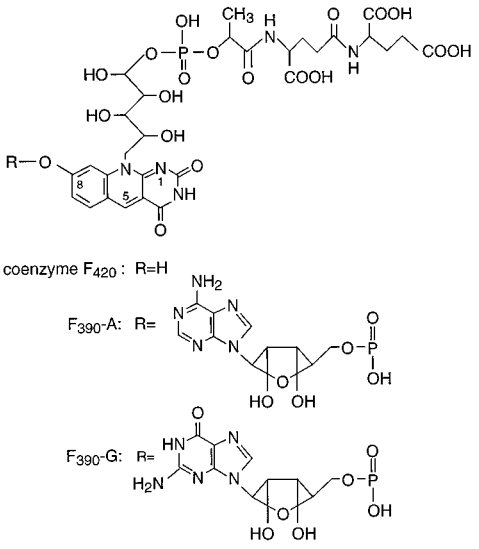

FIG. 1. Structures of coenzyme F_{420}, F_{390}-A, and F_{390}-G (6, 9).

(pH 6.4), 1.6 mM ATP, 16 mM $MgCl_2$, and an appropriate amount of enzyme fraction. The reaction was initiated by the addition of 40 μM F_{420}. Further additions or omissions were done as described in the text. The decrease of the A_{420} was used for the rate calculation, with the molar extinction coefficients for coenzyme F_{420} (ε_{420} = 21.8 $mM^{-1} \cdot cm^{-1}$) and coenzyme F_{390} (ε_{390} = 6.6 $mM^{-1} \cdot cm^{-1}$) being used (12, 23). The protein concentrations of the enzyme samples were determined with Bio-Rad protein reagent (Bio-Rad Laboratories, Richmond, Calif.), with bovine serum albumin being used as a standard.

Enzyme purification and amino acid sequence determination. F_{390} synthetase was purified under air at 25°C. All buffers were prepared in Milli-Q (Millipore). After the addition of 1 mM $MgCl_2$, 10 ml of cell extract was applied to a Procion Red HE-3B dye affinity chromatography column (1.0 by 6.0 cm; flow rate, 0.5 ml/min) equilibrated with 9 mM potassium phosphate buffer (pH 6.4) containing 1 mM $MgCl_2$ (buffer A). The column was stepwise developed with buffer A (50 ml), 250 mM KCl (30 ml), and 1 M KCl (30 ml), with the last two steps being with 10 mM potassium phosphate buffer (pH 6.4) (buffer B). Enzyme activity was recovered in the 250 mM KCl eluate. Active fractions from two separate Procion Red HE-3B columns were combined (18 ml), diluted (1:1 [vol/vol]) with 2 M KCl in buffer B, and loaded onto a TSK-butyl 650(S) column (1.0 by 6.0 cm; flow rate, 0.5 ml/min) equilibrated with buffer B containing 1 M KCl. The column was developed by washing it with equilibration buffer (30 ml) and then with a linear gradient of 1 to 0 M KCl in buffer B (200 ml). F_{390} synthetase activity was recovered in fractions containing 600 to 430 mM KCl. Active fractions were combined (45 ml), concentrated, and desalted with buffer B by ultrafiltration on an Amicon Centriprep YM-10 concentrator and loaded onto a TSK-DEAE-5PW column (0.75 by 7.5 cm) equilibrated with buffer B. The column was developed at 0.5 ml/min with 200 ml of a 0 to 500 mM KCl linear gradient in buffer B. F_{390} synthetase was eluted from the column at 360 mM KCl. F_{390} synthetase activity was concentrated by ultrafiltration to 1 ml with a Centriprep YM-10 concentrator. Half of the concentrated enzyme preparation was diluted (1:1 [vol/vol]) with native polyacrylamide gel electrophoresis (PAGE) sample buffer and loaded onto a PrepGel (preparative gel electrophoresis unit; Bio-Rad). The native gel (running gel, 30 ml of 10% polyacrylamide; stacking gel, 10 ml of 4% polyacrylamide) was run at constant current of 40 mA. Electrophoresis buffers were prepared as described by Laemmli (17). Further procedures were done according to the instructions of the manufacturer. Coenzyme F_{390} synthetase activity was recovered in fractions that eluted from the running gel after 200 to 210 min (R_f = 0.58). Active fractions were pooled, desalted, and concentrated by ultrafiltration on a Centriprep YM-10 and a Centricon 10 with buffer B until they reached a final volume of 100 μl, and then they were stored at −20°C until use. The second half of the concentrated TSK-DEAE pool was separated on a sodium dodecyl sulfate (SDS)–10% polyacrylamide gel, with the purified enzyme being used as a marker and then blotted onto a polyvinylidene difluoride membrane (Bio-Rad). The polyvinylidene difluoride blot was stained with Ponceau S. The area containing the 41-kDa coenzyme F_{390} synthetase was excised. Amino acid sequences were determined with a 470A protein-peptide sequencer from Applied Biosystems (Foster City, Calif.) according to the protocol given by the manufacturer.

Molecular biological methods. The sequences of the oligonucleotide probes fts1 [TCCATIGT(C/T)TCIAT(C/T)TC, corresponding to amino acids 8 through 13] and fts2 [TCIGG(G/A)TT(G/A)AA(G/A)TA(G/A)TT, corresponding to amino acids 3 through 8] were derived from the N-terminal amino acid sequence (see Fig. 4) of the coenzyme F_{390} synthetase. Genomic DNA from *M. thermoautotrophicum* was isolated as described previously (15). Labelling of oligonucleotides with ^{32}P, Southern hybridizations, and other techniques were done as described by Sambrook et al. (25) or as recommended by the manufacturer. Qiagen column-purified plasmid was used for sequencing. DNA fragments cloned into pBLUESCRIPT were sequenced by the dideoxynucleotide method (26), with the modified T7 DNA polymerase (Sequenase) or the T7 sequencing kit (Pharmacia) being used. Both strands of DNA were independently and completely sequenced. The nucleotide sequence was analyzed on the VAX with the University of Wisconsin Genetics Computer Group sequence analysis software package, version 8.0 (4).

Analytical PAGE. Denaturing SDS-PAGE, native PAGE, and Coomassie brilliant blue staining were performed with the PhastSystem (Pharmacia) or the Bio-Rad Protean II as described by the manufacturers. F_{390} synthetase activity staining was done by incubating a native PAGE gel in a reaction mixture and subsequently inspecting the gel under long-wave (366-nm) UV light as described before (33). For the determination of the subunit molecular mass of the denatured protein by SDS-PAGE, the following standards were used: rabbit muscle phosphorylase (97.4 kDa), bovine serum albumin (66.2 kDa), hen egg white albumin (45 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and hen egg white lysozyme (14.4 kDa). For native PAGE, urease (hexamer, 545 kDa; trimer, 277 kDa), bovine serum albumin (dimer, 132 kDa; monomer, 66 kDa), chicken egg albumin (45 kDa), and α-lactalbumin (14.2 kDa) served as molecular standards.

Nucleotide sequence accession number. The *ftsA* sequence (see Fig. 4) has been deposited in the EMBL data library under accession number X92347.

RESULTS

Purification of coenzyme F_{390} synthetase from *M. thermoautotrophicum* Marburg. The F_{390} synthetase was purified under air at 25°C by means of dye affinity chromatography on a Procion red HE-3B column, subsequent hydrophobic interaction chromatography on a TSK-butyl column followed by ion-exchange chromatography on a TSK-DEAE column, and finally by preparative gel electrophoresis on a PrepGel, as summarized in Table 1. The enzyme was purified 89-fold, with a final yield of 0.9%. Addition of 1 mM $MgCl_2$ to the cell extract and equilibration buffer proved to be essential for proper binding of the enzyme to the Procion Red HE-3B column. The enzyme could be eluted from the column with 250 mM KCl or 5 mM ATP. After TSK-DEAE column chromatography, an SDS-PAGE gel of the enzyme preparation showed the presence of three major bands with sizes of approximately 77, 41, and 33 kDa (Fig. 2A). To allocate one of these bands to the coenzyme F_{390} synthetase, half of the enzyme preparation was subjected to native PrepGel. SDS-PAGE of the concentrated PrepGel preparation showed a single band with an apparent molecular mass of 41 kDa (Fig. 2A). Activity staining of a native PAGE gel of the purified enzyme revealed an active band with a size of approximately 40 kDa. It appeared that the enzyme from *M. thermoautotrophicum* Marburg was about 10 kDa smaller than the same enzyme purified from strain ΔH (Fig. 2B). This experiment unequivocally proved that the coenzyme F_{390} synthetase from *M. ther-*

TABLE 1. Purification of F_{390} synthetase from *M. thermoautotrophicum* Marburg[a]

Step	Total protein (mg)	Total activity (U)[b]	Sp act ($U/10^3$ mg)[b]	Factor (fold)	Recovery (%)
Cell extract	810	6.80	8.4	1	100
Procion Red HE-3B	19	2.17	114	13	32
TSK-butyl 650(S)	11	1.77	161	19	26
TSK-DEAE	1.2	1.08	900	107	16
PrepGel	0.08	0.06	747	89	0.9

[a] Purification started with 20 ml of cell extract. Enzyme activity was determined as described in Materials and Methods.

[b] One unit (U) is the amount of enzyme catalyzing the conversion of 1 μmol of $F_{420} \cdot min^{-1}$.

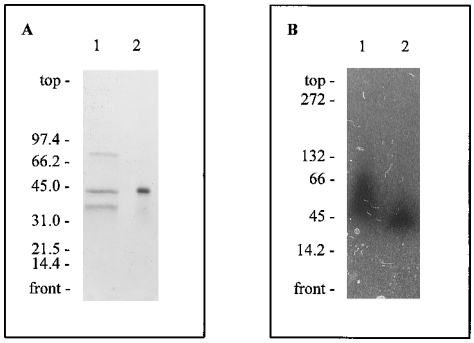

FIG. 2. Analysis of the purified F_{390} synthetase by PAGE. (A) SDS–12.5% PAGE and Coomassie brilliant blue staining. Lane 1, 1.9 μg of protein of the fraction which eluted from the TSK-DEAE column with 0.36 M KCl; lane 2, 1.6 μg of the purified F_{390} synthetase from *M. thermoautotrophicum* Marburg. (B) Native 12.5% PAGE and activity staining. Lane 1, 30 μg of cell extract from *M. thermoautotrophicum* ΔH; lane 2, 1.6 μg of the purified F_{390} synthetase from *M. thermoautotrophicum* Marburg. The numbers at the left of each gel refer to the molecular masses (in kilodaltons) of the protein standards.

moautotrophicum Marburg was composed of a single subunit with a size of 41 kDa.

Although PrepGel electrophoresis yielded homogeneous active enzyme, the purification procedure recovered amounts of coenzyme F_{390} synthetase that were too low to allow a determination of the N-terminal amino acid sequence. Subsequently, the synthetase was brought to homogeneity by SDS-PAGE of the TSK-DEAE fraction, and then the 41-kDa band was transferred onto a polyvinylidene difluoride membrane.

Cloning of the gene (*ftsA*) encoding the coenzyme F_{390} synthetase. DNA of *M. thermoautotrophicum* was double digested with the restriction endonucleases *Eco*RI and *Pst*I. The digestion products were separated on an agarose gel, after which they were transferred to a Hybond-N membrane (Amersham). Under the hybridization conditions employed (32.5°C, 6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.2% SDS), both synthetic oligonucleotides gave single, identical hybridization signals. The probes hybridized to a 323-bp fragment, which was ligated into the *Eco*RI and *Pst*I cleavage sites of vector pBluescript SK, resulting in plasmid pPV320.

Plasmid pPV320 was transferred into *E. coli* Dh5α. After confirmation that a part of the deduced amino acid sequence of the cloned fragment and the N-terminal sequence of the coenzyme F_{390} synthetase were identical, the 323-bp insert was used as probe to screen a pVK100 library of *M. thermoautotrophicum* DNA (10 to 20 kbp of DNA fragments) (18). For subcloning, one of the positive cosmids was isolated. Subsequently, a 1.65-kbp *Bam*HI fragment containing the whole gene and an overlapping 1.2-kbp *Hin*dIII fragment were cloned into pBluescript SK vector and sequenced. The restriction map of the sequenced DNA region is given in Fig. 3.

Nucleotide sequence of the *ftsA* gene and derived amino acid sequence. In the DNA region analyzed to obtain the *ftsA* gene sequence, an open reading frame of 377 amino acids encoding a 43.28-kDa protein was detected (Fig. 3 and 4). The open reading frame was identified as the *ftsA* gene by comparing its deduced amino acid sequence with the N-terminal amino acid sequence of the 41-kDa purified enzyme (Fig. 4). The start codon was preceded 8 bp upstream by sequence TGGAGG TG, representing the putative ribosome binding site (3, 24). Sequence TTTATCCA, found 36 bp upstream of the translational start, conforms to the common methanogen promoter box A element (4, 24). In vivo transcription experiments with *Haloferax volcanii* wild-type $tRNA^{Lys}$ promoter and mutant $tRNA^{Lys}$ promoters showed that the two cytosines at positions 523 and 524 (Fig. 4) do not influence transcription efficiency (22) and proved to be 100% functional compared with the archaebacterial consensus box A sequence (TTTAT ATA). Tetranucleotide GTGC is located 27 bp downstream of the putative box A, and it contains three of four bases of the archaebacterial box B consensus (4, 24). Immediately downstream of the stop codon, sequence AGGGTGTCACTGGAG TGTGAGGACCCT may form a stem-loop structure to function as a transcription terminator (20, 35). No transcription terminator motif that conforms to the tandemly arranged oligo(dT) sequences interspersed with C residues was found (4, 24). Approximately 300 bp upstream of the *ftsA* gene, a second open reading frame was detected (Fig. 4). Comparison of the derived amino acid sequence with sequences of known proteins in the database gave no indication of its nature.

DISCUSSION

Coenzyme F_{390} synthetase was purified from *M. thermoautotrophicum* Marburg by the four-step procedure shown in Table 1. The purified enzyme was composed of a single 41-kDa polypeptide, which is 10 kDa smaller than the anologous enzyme from the ΔH strain. A comparison of the procedures used for the purification of the two enzymes suggests that the strain ΔH synthetase has a much more hydrophobic nature than that of the Marburg strain (reference 33 and this paper). It is expected that the additional 10-kDa stretch will contain a substantial number of hydrophobic amino acids. The differences with respect to coenzyme F_{390} synthetase provide further evidence that *M. thermoautotrophicum* Marburg and strain ΔH are only distantly related (2) and have to be considered representatives of different species of the genus *Methanobacterium* (21).

Following enzyme purification, the *ftsA* gene was cloned and sequenced. Repeated expression experiments in *E. coli* in which the *ftsA* gene was under the control of both the *lacI* promoter and the *E. coli* ribosome binding site were unsuccessful. Upon induction, all growth ceased, indicating that FtsA may be toxic for *E. coli* (data not shown). Homology searches with the amino acid sequence derived from the *ftsA* gene sequence showed a high degree of similarity to domains found in enzymes belonging to the superfamily of peptide synthetases and adenylate-forming enzymes. All adenylate-forming enzymes contain one to four of these domains, which all show a high degree of similarity. The FtsA sequence was found to be most similar to that of the first of the four conserved and repeated domains, each with a size of about 600 amino acids, found within gramicidin S synthetase II (GrsB) from *Bacillus brevis* (31) (Fig. 5) and that of the second of three conserved and repeated domains found within δ-(L-α-aminoadipyl)-L-cysteine-D-valine synthetase (PcbA) from *Acremonium chrysogenum* (8) (Fig. 5). Though similarity holds for the complete FtsA sequence, it was especially high within a

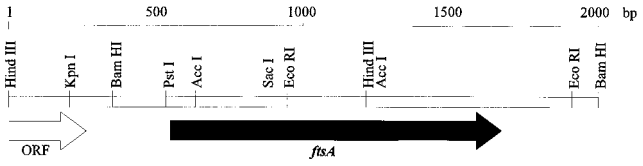

FIG. 3. Restriction map of the sequenced genome region containing the *ftsA* gene. ORF, open reading frame.

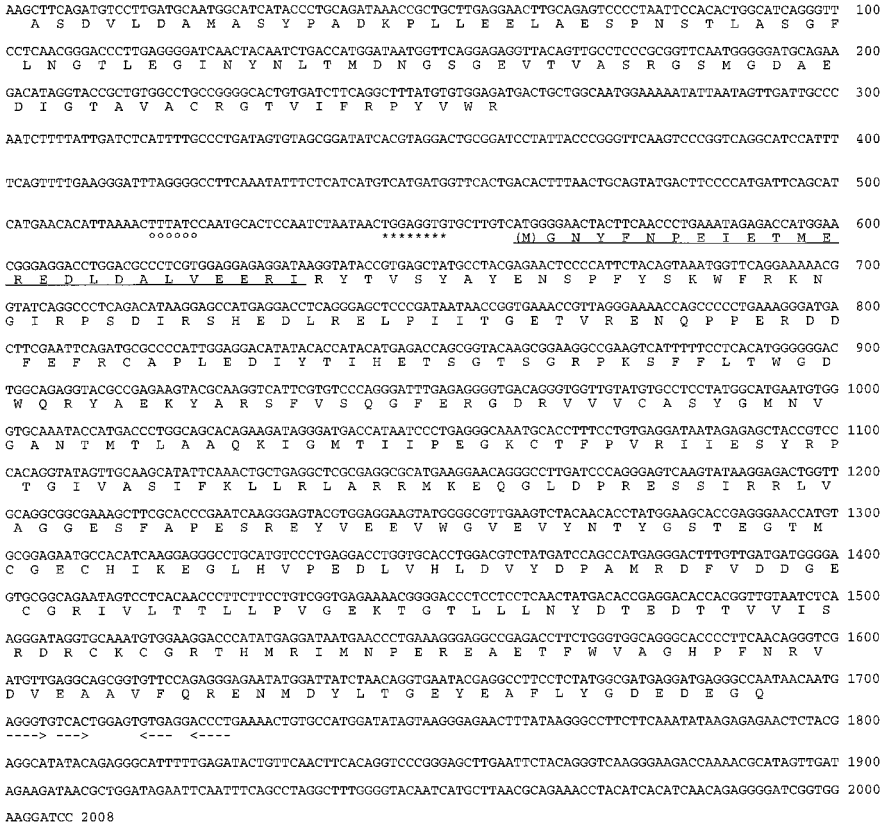

FIG. 4. Nucleotide sequence and derived amino acid sequence of the genome region containing the *ftsA* gene. The protein sequence determined by Edman degradation is emphasized by underlining. The potential ribosome binding site is labelled with asterisks. The nucleotides that conform to consensus archaeal promoter element box A are labelled with small circles. The arrows indicate a stem-loop structure with a putative transcription terminator function.

stretch of approximately 230 amino acids (residues 53 to 280). Here, FtsA was 18.9% identical and 66.7% homologous to GrsB. In this region (residues 53 to 246), FtsA showed 19.6% identity and 57.2% homology to PcbA. As a comparison, the first domain of the GrsB sequence and the second domain of the PcbA sequence were 35.0% identical (31). All domains found within the adenylate-forming enzymes contain four particularly conserved regions which are located in a defined order. These consensus sequences, TGTTG, YGD, TGD, and NGK, were suggested to represent nucleotide binding folds of either an ATP or an acyladenylate-binding site (31). FtsA contained only three of these highly conserved regions (Fig. 5). Despite the overall homology, FtsA contained at least four regions (residues 31 to 45, 279 to 289, 311 to 321, and 339 to 351) which are not found in the domain of GrsB and PcbA and which could be a part of the coenzyme F_{420}-binding site. However, comparison of these particular regions as well as of the whole FtsA sequence with other coenzyme F_{420}-dependent enzymes showed no apparent homology. This could be due to two reasons: first, a difference in the type of reactions at the coenzyme F_{420} chromophore, and second, a difference in substrate-binding properties. In all coenzyme F_{420}-dependent enzymes sequenced thus far (19), the chromophore is involved in a redox reaction (hydride transfer). In the coenzyme F_{390} synthetase reaction, the chromophore is the place where an AMP (or GMP) residue is bound. Coenzyme F_{390} synthetase is highly selective for its coenzyme F_{420} substrate; only coenzyme F_{420} derivatives which contain at least two glutamyl moieties are active in the reaction (12). The other coenzyme F_{420}-utilizing enzymes are less specific in this respect.

$$\mathrm{R\text{-}C(=O)\text{-}O^- + ATP \rightleftharpoons R\text{-}C(=O)\text{-}O\text{-}AMP + PP_i} \quad (1)$$

$$\mathrm{R\text{-}C(=O)\text{-}O\text{-}AMP + E\text{-}SH \rightleftharpoons E\text{-}S \sim C(=O)\text{-}R + AMP} \quad (2)$$

(R-COO-, substrate amino or hydroxy acid; E-SH, catalytic thiol group of the adenylate-forming enzyme.)

The superfamily of peptide synthetases and adenylate-forming enzymes can be divided into two families (31). In the first family, which includes, among others, gramicidin S synthetase II and δ-(L-α-aminoadipyl)-L-cysteine-D-valine synthetase, the constituent substrates, amino acids, or hydroxy acids are activated as acyladenylates according to a multienzyme thiotemplate mechanism (reaction 1). Next, the activated substrates are bound via carboxyl thioester linkages to the corresponding peptide-synthesizing active sites (reaction 2). Subsequent peptide bond formation and transpeptidation require the aid of 4′-phosphopantetheine. The second family includes those enzymes that activate their substrates as acyladenylates but that do not perform thioester formation. Members of this family lack a typical LGGXS sequence, which in the first family is thought to be the site active in the covalent binding of thioester-activated substrates (27). Since coenzyme F_{390} synthetase

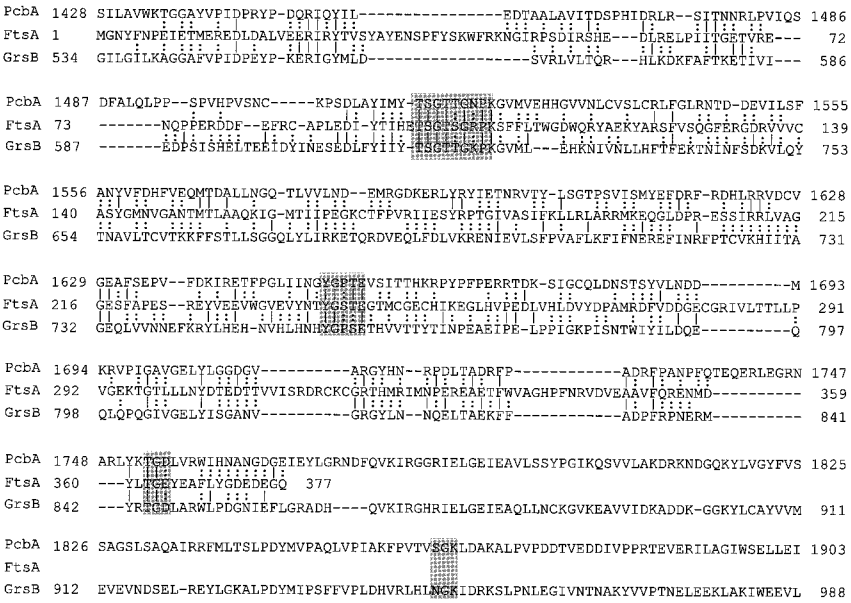

FIG. 5. Alignment of the amino acid sequences of the first of four functional domains within gramicidin S synthetase II (GrsB) from *B. brevis* (30), the second of three functional domains within α-aminoadipyl-cysteinyl-valine synthetase (PcbA) from *Cephalosporium acremonium* (8), and FtsA from *M. thermoautotrophicum* Marburg. Alignment of the sequences of the domains of GrsB and PcbA which exhibited the highest similarity with the amino acid sequence of FtsA is shown. Identical amino acids are connected by vertical lines, and conservative replacements are indicated with colons. Insertions made during alignment optimization are indicated with dashes. The four consensus sequences are shaded.

only catalyzes the formation of adenylylated coenzyme F_{420} and since the LGGXS consensus sequence is absent, one would expect coenzyme F_{390} synthetase to be placed in the second subgroup. Yet, it is most similar to members of the first family. It may be worthwhile to mention that *M. thermoautotrophicum* contains a second enzyme in coenzyme F_{390} metabolism which hydrolyzes the AMP-coenzyme F_{420} bond (13). In this enzyme, coenzyme F_{390} hydrolase, a redox-sensitive SH group is involved in catalysis (13, 34). It is tempting to speculate that the hydrolase harbors the LGGXS consensus sequence and that coenzyme F_{390} synthetase and hydrolase evolved from a single enzyme that catalyzed both reactions.

In summary, sequence analysis provided compelling evidence that coenzyme F_{390} synthetase is a member of the superfamily of adenylate-forming enzymes. The synthetase holds an exceptional position in that superfamily, since it is structurally more related to the first subgroup but is functionally homologous to the second subgroup. In addition, coenzyme F_{390} is the first representative of the superfamily that activates an aromatic hydroxy group rather than an amino acid or hydroxy acid.

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