JOURNAL OF BACTERIOLOGY, Mar. 2003, p. 1525–1533 Vol. 185, No. 5
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.5.1525–1533.2003

Genetic and Biochemical Characterization of the F-ATPase Operon from *Streptococcus sanguis* 10904

Wendi L. Kuhnert[1]† and Robert G. Quivey, Jr.[1,2]*

Center for Oral Biology,[2] and Department of Microbiology and Immunology,[1] University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Received 19 August 2002/Accepted 4 December 2002

Oral streptococci utilize an F-ATPase to regulate cytoplasmic pH. Previous studies have shown that this enzyme is a principal determinant of aciduricity in the oral streptococcal species *Streptococcus sanguis* and *Streptococcus mutans*. Differences in the pH optima of the respective ATPases appears to be the main reason that *S. mutans* is more tolerant of low pH values than *S. sanguis* and hence pathogenic. We have recently reported the genetic arrangement for the *S. mutans* operon. For purposes of comparative structural biology we have also investigated the F-ATPase from *S. sanguis*. Here, we report the genetic characterization and expression in *Escherichia coli* of the *S. sanguis* ATPase operon. Sequence analysis showed a gene order of *atpEBFHAGDC* and that a large intergenic space existed upstream of the structural genes. Activity data demonstrate that ATPase activity is induced under acidic conditions in both *S. sanguis* and *S. mutans*; however, it is not induced to the same extent in the nonpathogenic *S. sanguis*. Expression studies with an *atpD* deletion strain of *E. coli* showed that *S. sanguis-E. coli* hybrid enzymes were able to degrade ATP but were not sufficiently functional to permit growth on succinate minimal media. Hybrid enzymes were found to be relatively insensitive to inhibition by dicyclohexylcarbodiimide, indicating loss of productive coupling between the membrane and catalytic subunits.

Streptococcus mutans and *Streptococcus sanguis* are oral microorganisms that are able to colonize the surfaces of teeth. Both bacteria survive by metabolizing dietary sugars such as sucrose into organic acids, particularly lactate. As salivary constituents, food particles, and bacteria accumulate on and around the tooth surface, the ecological niche known as dental plaque forms (45). Concomitant with the production of acid, localized plaque pH levels fall, resulting in the erosion of tooth enamel and the initiation of dental caries (40).

The relationship between *S. mutans* and *S. sanguis* in plaque appears to be an inverse one. Previous in vitro studies have shown that as mixed cultures of oral bacteria become acidified, *S. mutans* becomes a dominant member of the flora while the proportion of *S. sanguis* is greatly diminished (12, 13, 31). Supportive of the in vitro data, in vivo studies have repeatedly shown that *S. mutans* is a causative agent of dental disease (18). The ability of *S. mutans* to resist acidification appears to involve adaptive mechanisms which include a membrane-bound proton-translocating ATPase (F-ATPase, H^+ ATPase) (11) and, potentially, other proteins (49).

The function of the F-ATPase in streptococci is to regulate internal pH by pumping protons out of the cell (11, 17, 36, 37). This movement of protons results in an internal pH which is more basic than that of the plaque environment, thereby protecting relatively acid-sensitive glycolytic enzymes (15). Previous studies have shown that the pH optima of the *S. mutans* ATPase is approximately 6.0 while that of *S. sanguis* is approximately 7.0 (55). Thus, the *S. mutans* enzyme is well positioned to continue pumping at pH values below levels at which *S. sanguis* can survive. Further, *S. mutans* is also apparently able to up-regulate synthesis of its ATPase (8, 9, 31). The participation of the ATPase in the acid-adaptive response of *S. mutans* explains, in part, why the bacterium is a major contributor to dental caries and why *S. sanguis* cannot effectively compete at low pH values.

In order to better understand the molecular mechanisms by which *S. mutans* up-regulates its ATPase, Smith et al. had previously undertaken the characterization of the *S. mutans* ATPase operon, including its cloning and nucleotide sequence determination (53). The deduced amino acid sequences for the eight structural genes of the *S. mutans* ATPase operon showed that this enzyme is homologous to the well-characterized *Escherichia coli* ATPase as well as those of other bacteria (53).

The enzyme's mechanism has been the subject of intensive investigation, and recent structural studies have provided insights into the spatial relationship of the subunits and ATPase activity versus proton conduction through the membrane. Functionally, the enzyme is organized into two distinct, but physically linked, domains; one is cytoplasmic (F_1) and the other is membrane bound (F_0) (60). The cytoplasmic domain consists of three subunits, α, β, and γ, in a stoichiometry of 3:3:1. The function of the cytoplasmic domain is to catalyze synthesis of ATP when protons move from the outside of cells into the cytoplasm, through the membrane-bound domain, or to cleave ATP when protons are pumped out of the cell, as in the case of the oral streptococci. The F_1 subunit linking catalysis with proton conduction is the γ subunit, which functions as a rotor (6, 21, 46). The membrane-bound domain itself consists of five subunits, a, b, c, δ, and ε (1:2:9 to 10:1:1), and it

* Corresponding author. Mailing address: Center for Oral Biology, Box 611, 601 Elmwood Ave., Rochester, NY 14642. Phone: (585) 275-0382. Fax: (585) 506-0190. E-mail: Robert_Quivey@urmc.rochester.edu.

† Present address: Division of Viral Hepatitis, Centers for Disease Control, Atlanta, GA 30319-3103.

functions as the membrane-bound, proton-specific channel (24, 27, 43, 54, 64).

The genetic organization of bacterial ATPase operons is typically similar to that of *E. coli*, in which the membrane subunit genes appear first, followed by the genes encoding the catalytic subunits (60). However, notable differences were observed in the genetic organization of the *S. mutans* operon. For example, the gene order for the F_0 domain of the *E. coli* enzyme is *atpBEF* while that for *S. mutans* is *atpEBF*. Interestingly, the *S. mutans* operon did not contain an *atpI* gene equivalent upstream of the structural genes but rather contained an intergenic region of 239 bp. The functional significance of the DNA upstream of the structural genes remains to be elucidated; however, analysis of the region showed inverted repeats present in the DNA which are hypothesized to be involved in stem-loop structures (53).

Relatively little is known about the acid-adaptive abilities of *S. sanguis* or whether the regulatory mechanism for the ATPase operon is similar to that in *S. mutans*. Moreover, the availability of sequence data from the *S. sanguis* structural genes may be useful for comparison to the enzyme from the highly related *S. mutans*. As part of the effort towards understanding the role of the ATPase in the aciduricity of oral streptococci, we report here the cloning and expression in *E. coli* of the *S. sanguis* ATPase operon.

MATERIALS AND METHODS

Bacterial strains and media. *S. sanguis* ATCC 10904 was grown on Todd-Hewitt broth (30 g per liter) (Difco, Detroit, Mich.) for chromosomal DNA isolation and in a modified Todd-Hewitt broth (per liter: 37 g of brain heart infusion, 1 g of Bacto peptone, 2 g of NaCl, 0.4 g of disodium phosphate, 2.5 g of sodium carbonate, 0.5% glucose) when grown in continuous culture in the chemostat. On solid medium, *S. sanguis* was grown at 37°C in an atmosphere of 5% (vol/vol) CO_2. *E. coli* DH10B (Invitrogen, Carlsbad, Calif.) (30), GBE180 (48), or JP17 (38) was used for all cloning experiments, as indicated. *E. coli* strains were grown on Luria broth (10 g of tryptone [Difco], 5 g of yeast extract [Difco], 10 g of NaCl per liter) at 37°C (51). Selective antibiotics were added as follows: ampicillin, 100 μg/ml; chloramphenicol, 20 μg/ml. 5-Bromo-4-chloro-3-indolyl-β-D-galactosidase (X-Gal) was used at a concentration of 20 mg/ml in solid medium to facilitate screening of prospective clones.

Cloning of the F-ATPase operon. A 5- to 10-kbp subgenomic library of *S. sanguis* 10904 was prepared, from which plasmid pSSA9, containing the entire ATPase operon except the c subunit, was isolated. The library was created in the *Eco*RI site of vector pSU20 (7) and screened by colony hybridization with a PCR-amplified fragment of the *S. mutans* β subunit (*atpD*) as the probe. Probe labeling, colony hybridization, and autoradiography were performed as previously reported (50). A second subgenomic library was created by digestion of the *S. sanguis* 10904 chromosomal DNA with *Sau*3AI and *Sst*I. Fragments that were 1 to 4 kbp in size were cloned into pSU20. Southern hybridization of the clones to an *atpB* subunit probe from *S. mutans* indicated that plasmid pSSP19 contained the correct fragment. Sequencing confirmed that the insert carried the *atpE* and *atpB* subunits of the *S. sanguis* ATPase operon as well as the presumed promoter region.

To aid in sequencing the 6.2-kbp ATPase fragment, subclones were constructed by using *Eco*RI and *Hin*cII digestion products ligated into pGEM-5Zf(+) (Promega, Madison, Wis.). Sequence determinations were performed by using radioactive (Sequenase; USB Corp., Cleveland, Ohio) and fluorescence-based methods (LiCor, Lincoln, Nebr.). Alignment software packages (MacVector, AssemblyLign, and GCG) were utilized to analyze and assemble the contigs (Accelrys, Burlington, Mass.). Subunit homologies were estimated by using GCG software (Accelrys).

Primer extension analysis. Primer extension was performed on RNA isolated from *S. sanguis* 10904 cells grown at steady state in a chemostat (BioFlo 2000; New Brunswick Scientific, Edison, N.J.). Cells in continuous culture were harvested at pH 7.0 and 6.0 after a minimum of 10 generations at each pH value. Dilution rates for the cells in continuous culture were $D = 0.18\ h^{-1}$, which is equivalent to a 3.8-h generation time. Cells (60 ml), at an optical density at 600 nm of 1.4 to 1.5, were collected at each pH value. Harvested cells were collected by centrifugation and frozen at −80°C. RNA was isolated according to the procedure outlined by Gagnon et al. (28) with the following modifications. Cell pellets were resuspended in 1 ml of RNase-free Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.5]) and mixed with 1 ml of a 1:1 phenol–chloroform-isoamyl alcohol (24:1) solution. A 20% (wt/vol) solution of sodium dodecyl sulfate (62.5 μl) was added, and the cell solution was divided into aliquots such that 1 ml of cell solution was added to 1 g of chloroform-treated glass beads (150 to 212 μm in diameter; Biospec Products, Bartlesville, Okla.). The cells were lysed in a Mini-Bead Beater homogenizer (Biospec Products) for 1 min (beat for 30 s, ice for 30 s, beat for 30 s). After lysis, the beads were collected by centrifugation at 10,300 × *g* for 20 min. Supernatant solutions were removed, pooled, and extracted 5 times with a 1:1 solution of phenol–chloroform-isoamyl alcohol (24:1). Nucleic acid was precipitated with a 1/10-volume of 10 M LiCl and 2 volumes of ethanol. RNA precipitates were resuspended in 100 μl of DNase buffer (20 mM Tris [pH 8.0], 50 mM KCl, 2 mM $MgCl_2$) and treated with 30 to 50 U of RNase-free DNase (Invitrogen) for 15 min at room temperature. The digestion reaction was stopped by the addition of 2 μl of 0.5 M EDTA (pH 8.0) and extracted once with 1 volume of phenol and then once with 1 volume of chloroform-isoamyl alcohol (24:1). The Promega avian myeloblastosis virus reverse transcription primer extension kit (Madison, Wis.) was used to label oligonucleotide primers for use as probes. Primer (10 pmol) was end labeled with [γ-^{32}P]ATP (6,000 Ci/mmol) (Perkin Elmer, Boston, Mass.). In the primer extension reactions, 35 μg of RNA from each sample and 100 fmol of labeled primer were used. The primer sequence was 5′-CCTTCAGCAATTGATACGC C-3′, which was located between bp 39 to 58 of the *S. sanguis atpE* gene (c subunit). A second primer with a sequence of 5′-ATTTCTGGCTGACGAGAC GC-3′, located between bp 87 to 106 of the *atpE* gene, was used to confirm the start site. Extension and sequencing products were loaded and separated for 2.75 h at 73 W on 8% polyacrylamide gels.

Expression of enzymatic activity within ATPase-defective *E. coli* strains. We examined the ability of the cloned ATPase genes to complement defects in *E. coli* strain JP17, an *atpD* deletion mutant (38). Strain JP17 was transformed with selected plasmids containing streptococcal DNA (Table 1); the resulting strains were then assayed for their ability to release inorganic phosphate from ATP. Plasmid pDP31 contains *atpD* and *atpC* and was used as a positive control (47). Cells from each of the strains were permeabilized for ATPase assays by using a previously described procedure (8) with the following modifications. Fifty-milliliter batch cultures were grown overnight in Luria broth supplemented with 10 mM $MgSO_4$ and 0.1% glucose plus the appropriate antibiotic. Fifty milliliters of harvested cells was divided into 2 aliquots. Twenty-five milliliters was used for dry weight determinations, and 25 ml was permeabilized for assays. The cells to be assayed were washed once with membrane buffer (75 mM Tris [pH 7.0], 10 mM $MgSO_4$) and then resuspended in a volume of 1 ml. One hundred-microliter aliquots of the permeabilized cells were frozen at −80°C and used within 1 month. A modified Fiske-Subbarow procedure (10, 56) was used to assay thawed aliquots of permeabilized cells for their ability to release phosphate from ATP (0.5 M ATP at pH 6.0; final concentration, 5 μM) (Sigma Diagnostics, St. Louis, Mo.).

DCCD inhibition studies. ATPase assays were performed as described above. One molar stock solutions of the ATPase inhibitor dicyclohexylcarbodiimide (DCCD; Sigma Diagnostics) were made and diluted for use in the assays. Various concentrations of DCCD were tested, but maximal inhibition was achieved with a 1 mM concentration in all strains. The inhibitor was added to 3 ml of ATPase buffer (50 mM Tris-Maleate [pH 6.0], 10 mM $MgSO_4$) at the indicated concentrations prior to the addition of the permeabilized cells. The reactions were allowed to equilibrate at 37°C for 5 min and were initiated with the addition of 30 μl of 0.5 M ATP at pH 6.0. The amount of inorganic phosphate released was measured with a commercially available kit for the modified Fiske-Subbarow assay (Sigma Diagnostics). Assays were performed on the previously described strains JP17(pDP31) and JP17(pSSA9) (Table 1).

Nucleotide sequence accession number. The GenBank accession number for the F-ATPase from *S. sanguis* 10904 is AF001955.

RESULTS

Cloning the *S. sanguis* 10904 ATPase operon. A 930-bp *atpD* gene fragment from *S. mutans* was amplified by PCR and used as a probe to anneal to *S. sanguis* 10904 chromosomal DNA digested with various enzymes. The probe hybridized to an

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Phenotype or description	Reference or source
E. coli strains		
DH10B	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80Δ*lacZdM15* Δ*lacX74*, *deoR recA1 endA1 araD139* Δ(*ara leu*)*7697 galU galK* λ$^-$ *rpsL nupG*	Invitrogen
GBE180	F$^-$ *endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1* ϕ80Δ(*lacZ*)*M15 pcnB zad*::Tn*10*(Tet^r)λ$^-$	48
JP17	*atpD* (bp 60–998) *argH pyrE endA recA* (Tn*10* from MV1193) Tet^r	38
Plasmids		
pSU20	Cm^r; intermediate-copy-number plasmid derived from pACYC184, contains multiple cloning sites in *lacZ* for X-Gal screening of insertions	7
pGEM-5Zf(+)	Ap^r; high-copy-number plasmid with multiple cloning sites in *lacZ* for X-Gal screening	Promega
pSSA9	Cm^r; a 6.2-kbp *Eco*RI fragment from *S. sanguis* 10904 chromosome-cloned into the *Eco*RI site of pSU20; this insert contains 7 out of 8 ATPase structural genes (lacking a portion of the c subunit)	This study
pClone 1.1	Ap^r; pSSA9 subclone in pGEM-5 containing a 2.0-kbp *Eco*RI-*Hin*cII fragment containing the complete a, b, and δ subunits as well as 200 bp of the α subunit	This study
pClone 3.4	Ap^r; pSSA9 subclone in pGEM-5 containing a 1.8-kbp *Hin*cII fragment containing the 3′ 1,313 bp of the α subunit and the 5′ 540 bp of the γ subunit	This study
pClone 1.3	Ap^r; pSSA9 subclone in pGEM-5 containing a 568-bp *Hin*cII fragment containing the 3′ 341 bp of the γ subunit and the 5′ 187 bp of the β subunit	This study
pClone 1.8	Ap^r; pSSA9 subclone in pGEM-5 containing a 1.4-kbp *Hin*cII fragment containing the 3′ 1,219 bp of the β subunit and the 5′ 198 bp of the ε subunit	This study
pClone 1.10	Ap^r; pSSA9 subclone in pGEM-5 containing a 250-bp *Hin*cII fragment containing the rest (221 bp) of the ε subunit and 29 bp of downstream sequence	This study
pSSP19	Cm^r; a 2.1-kbp fragment of *S. sanguis* 10904 chromosome-cloned into pSU20 containing the carboxy terminus of the *pmi* gene, promoter sequences for the ATPase operon, the c and a subunits, and 458 bp of the b subunit	This study
pDP31	Ap^r; contains the β and ε subunits of the *E. coli* ATPase operon	47

approximately 6-kbp *Eco*RI fragment, apparently containing at least part of the ATPase operon. A second Southern hybridization was performed with an *atpA* gene probe from *S. mutans* to verify that the same *Eco*RI fragment contained a second gene in the operon. The hybridizing fragment was then cloned into an intermediate-copy-number vector, pSU20, and screened by colony hybridization with the *atpD* probe. A putative clone, designated pSSA9, was chosen for further characterization. Due to the empirical observation that the clone was unstable in commonly used *E. coli* hosts, *E. coli* strain GBE180 was used to maintain plasmid pSSA9 (48). *E. coli* GBE180 is strain DH5α (Invitrogen) but with the addition of the *pcnB* allele, which has been shown to reduce the plasmid copy number (41). Reduction of the plasmid copy number resulted in the stabilization of the 6.2-kbp clone.

Nucleotide sequence determination of the insert in pSSA9 demonstrated that the 5′ end of the clone was homologous to the *atpB* (a subunit) of *S. mutans* (53) and other previously sequenced ATPase *atpB* genes. The 3′ end of the fragment was also sequenced, but it did not show significant homology to any previously identified genes as indicated via BLAST searching of the available databases. The 6-kbp fragment was subsequently digested with *Hin*cII and subcloned into pGEM-5Zf(+) linearized with *Eco*RV. Putative clones, possessing fragments of various lengths, were manually sequenced by the method of Tabor and Richardson (58) or automatically with a LiCor sequencing apparatus (42). Assembly of the resulting contigs revealed the following gene order: *atpBFHAGDC*. The only gene missing in pSSA9 was the *atpE* (c subunit), which was known to be the first gene in the *S. mutans* ATPase operon (53).

In order to sequence the rest of the operon, chromosome walking was performed by digesting *S. sanguis* chromosomal DNA with a panel of enzymes and isolating a fragment which hybridized to a PCR fragment of the *S. sanguis atpB* (a subunit). An approximately 2.3-kbp *Sst*I/*Sau*3AI fragment was identified and cloned into pSU20. The resulting clone was named pSSP19. A BLAST search of the GenBank databases showed that the 5′ end of the insert in pSSP19 had significant homology to the mannose phosphate isomerase (*pmi*) gene previously identified in *S. mutans* (GenBank accession number D16594). Additional searches showed that the homology extended to other cloned *pmi* genes such as that from *Bacillus subtilis* (GenBank accession number Z99110). The 3′ end of the fragment aligned to the *atpF* gene in the *S. sanguis* ATPase operon as was expected from our restriction analysis. The gene order and size of the subunits of the operon is shown in Fig. 1. Complete sequencing of the contig revealed a 266-bp intergenic region between the *pmi* gene and the first structural gene of the ATPase operon (c subunit, *atpE*). The intergenic region is shown in more detail in Fig. 2.

Comparison of ATPases. Comparison of the *S. mutans* and *S. sanguis* ATPase operons showed various levels of homology within each of the subunits. The individual genes and their relative homologies to selected ATPases are shown in Table 2. In general, the subunits in the F_1 (cytoplasmic) domain showed a higher level of homology than the F_0 (membrane) domain. The data indicated that a high degree of homology existed at the amino acid level in the sequences of all of the ATPase genes and that, for the most part, the genetic organization of the operons has been maintained in the oral streptococci (Table 2).

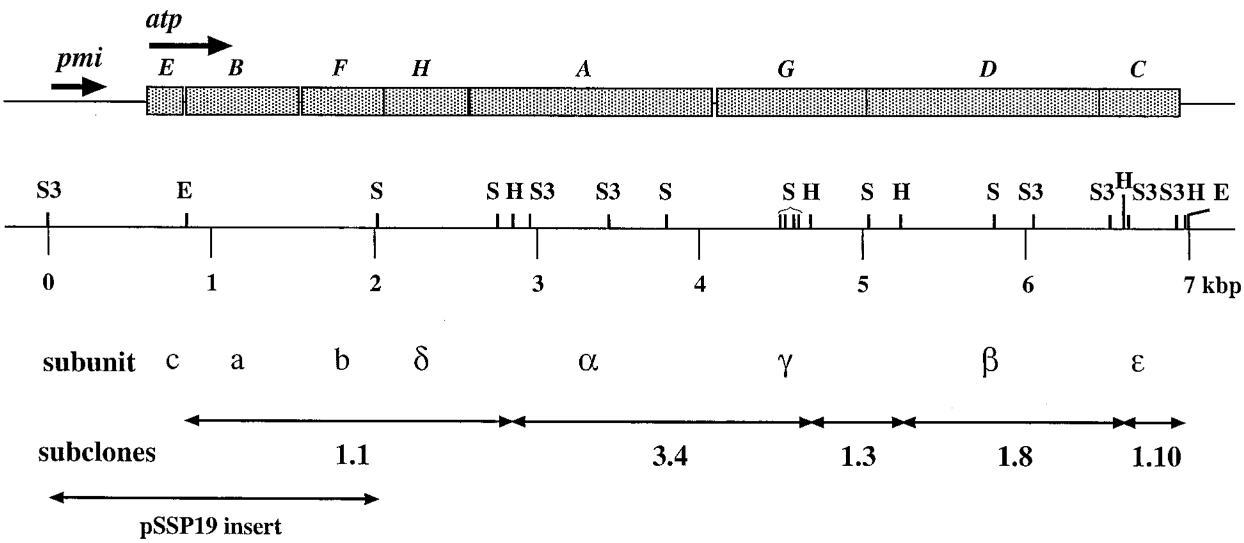

FIG. 1. Genetic organization of the *S. sanguis* ATPase operon. The map indicates the position and size of each of the genes with the *atp* designation in italics above and the subunit names below. The arrows indicate the direction of transcription for the operon as well as for the *pmi* gene. Restriction sites are shown on the kilobase pair scale with the following abbreviations: H, *Hin*cII; E, *Eco*RI; S, *Sst*I; S3, *Sau*3AI. The organization and relative size of each of the subclones is shown below. The GenBank accession number for the F-ATPase from *S. sanguis* 10904 is AF001955.

It has been speculated that the large intergenic regions seen upstream of the *S. mutans* (53) and *Enterococcus hirae* (52) ATPase operons may be involved in the regulation of the operon. Sequence analysis of the region has suggested the presence of potential stem-loops (52, 53). Interestingly, a similar analysis did not yield predicted stem-loop structures for the *S. sanguis* operon (data not shown). However, further examination of the upstream region revealed that all three streptococci possess unusually high adenosine-thymine content in this area. For example, the *S. sanguis* putative promoter DNA has 18 A or T bases in a 20-bp section located approximately 86 bp upstream of the *atpE* gene (Fig. 2).

Transcriptional start site of the ATPase operon. With the large intergenic region upstream of the operon, it was of interest to examine the transcriptional start site for the operon. The results of primer extension analysis identified a guanine at position −31 bp relative to the initial methionine for the *atpE* gene. We also evaluated whether the transcriptional start site

```
  1  TGGCAATCTTCGTGAGTTGCATTTGGAGCAGTCAATTGATGTTCTGACTATCGGTGAGCCTGCTAATAGCCATCCTGTTACCATTCAGGCAGATAGTTTG   100
      G  N  L  R  E  L  H  L  E  Q  S  I  D  V  L  T  I  G  E  P  A  N  S  H  P  V  T  I  Q  A  D  S  L

101  ACTTCTACTCTTTTGGTGGCCAATGACTTCTTTGCTGTTTACAAGTGGGATATTGCCGGTTCAGTTAATTTTAAAAAGACAGCGGACTATAGCTTGGTCA   200
     T  S  T  L  L  V  A  N  D  F  F  A  V  Y  K  W  D  I  A  G  S  V  N  F  K  K  T  A  D  Y  S  L  V  S

201  GTGTCTTGGAAGGTGTTGGTGAGCTAGAAGTTGATGGAGCTGTTTACCCGCTCGAAAAAGGGGACCATTTCATCCTGCCAAGCGACGTCACTGAATGGAC   300
      V  L  E  G  V  G  E  L  E  V  D  G  A  V  Y  P  L  E  K  G  D  H  F  I  L  P  S  D  V  T  E  W  T

301  CTTGTCTGGAGATTTGCAGCTGATTATCAGCCATCCATAATCATAGAAAAATCAAGAGCAAATAACCATTTTGCTCTTTTTTCTGTTAAAAATATAGGAA   400
      L  S  G  D  L  Q  L  I  I  S  H  P  *  ><—pmi

401  ATAAGTAAAATTCTAGACAGTTTCTTCTTATTTATATAGCAGAATAGTTTGTATGTTGATAATTATTTAATTGATTTTGGTAAAAAATTTTATATAACAA   500

501  AAGAAAAAAACAAAAAAACGCTTACAATTGTGTAAAAGATTGACTATTCGATATTTTAGGAGTAAACTAAGGAAGTAATTTAAAAAATATTAAGGAGAAG   600

601  TCATCATGAATTTAACATTTTTAGGACTTTGTTTTGCCTGCTTTGGCGTATCAATTGCTGAAGGTTTGATTATGAGTAATTTATTTAAGGCAGCGTCTCG   700
atpE —> M  N  L  T  F  L  G  L  C  F  A  C  F  G  V  S  I  A  E  G  L  I  M  S  N  L  F  K  A  A  S  R

701  TCAGCCAGAAATTATCGGTCAATTGAGAAGCCTCCTAATCTTGGGAATTGCCTTTGTCGAAGGTACTTTCTTTGTAACGCTGGCTATGGCTTTTGTTATC   800
      Q  P  E  I  I  G  Q  L  R  S  L  L  I  L  G  I  A  F  V  E  G  T  F  F  V  T  L  A  M  A  F  V  I

801  AAATAAGAAAGAATTCTCTTTAGAAAAAGGGGTGTCATCTGTTGGAAGAAAGTATCAATCCAACTATCAATGTCGGTCCTGTGACCTTTGATTTGACCCT   900
     K  *  >                        atpB —> L  E  E  S  I  N  P  T  I  N  V  G  P  V  T  F  D  L  T  L

901  GCTGGCCATGACCCTGCTGACGGTGCTTGTAACTTTTGGATTTGTTTATTGGGCAAGTCGAAAGATGACGATTAAGCCGTCACCAAAGCAGAATGCTCTG  1000
     L  A  M  T  L  L  T  V  L  V  T  F  G  F  V  Y  W  A  S  R  K  M  T  I  K  P  S  P  K  Q  N  A  L
```

FIG. 2. Partial nucleotide sequence of the *S. sanguis* ATPase operon including the 5′ upstream *pmi* gene as well as the intergenic region. The deduced amino acid sequence is shown in single-letter format, and the genes are indicated with bold italicized letters. An enlarged G with an arrow above it indicates the transcriptional start site at 575 bp. The single underline from bp 563 to 568 shows the presumptive −10 region, and the double underline from bp 540 to 545 indicates the presumptive −35 region. The dashed arrow above the sequence indicates the primer used in the primer extension reaction.

TABLE 2. Homology of the deduced *S. sanguis* F-ATPase subunit amino acid sequences compared to selected bacteria

Subunit[a]	*atp*[b]	% Identity (% similarity) to[c]:				
		S. mutans	*Streptococcus pyogenes*	*E. hirae*	*B. megaterium*	*E. coli*
a	E	51 (66)	ND	25 (37)	30 (42)	18 (33)
c	B	57 (69)	ND	44 (54)	33 (44)	26 (36)
b	F	49 (63)	50 (62)	41 (52)	38 (48)	25 (42)
δ	H	36 (48)	36 (49)	22 (40)	25 (36)	21 (32)
α	A	86 (90)	91 (94)	81 (87)	75 (80)	52 (63)
γ	G	68 (76)	70 (78)	67 (74)	52 (62)	35 (48)
β	D	92 (94)	93 (96)	88 (92)	78 (84)	66 (76)
ε	C	68 (81)	68 (80)	59 (73)	40 (59)	27 (42)

[a] Subunit designation of the genes of the ATPase operon.
[b] *atp* designation of the subunits of the ATPase operon.
[c] Sources of the deduced amino acid sequences are as follows: *S. mutans*, reference 53; *S. pyogenes*, reference 23; *E. hirae*, reference 52; *B. megaterium*, reference 14; *E. coli*, reference 60.

was affected by the growth pH or by growth at steady state. To do this, RNA was isolated from *S. sanguis* grown at steady state at pH 7.0 and 6.0 in a chemostat. The results showed that the start site was unaffected by the pH of the culture (Fig. 3). Once the start site was identified, sequence analysis of the region immediately upstream suggested a putative Pribnow box with a sequence of TAACT, which was similar to the *E. coli* consensus sequence of TATAAT. A potential −35 sequence was also tentatively identified that was nearly identical to the canonical *E. coli* −35 region of TTGACA (Fig. 3).

Induction of ATPase activity under acidic growth conditions. Previous data have shown that the *S. mutans* ATPase is up-regulated under acidic conditions (8). Maximal induction of activity was seen at pH 5, a level at which the nonpathogenic *S. sanguis* is not able to survive (8, 31). The ability of *S. mutans* to out-compete *S. sanguis* during growth in mixed cultures has raised questions about the acidurance of these organisms, that is, whether the up-regulation of F-ATPase contributes to the enhanced ability of *S. mutans* to survive at low pH values. To examine the issue more directly, *S. sanguis* was grown in a chemostat at steady state and the pH was dropped incrementally to 5.9; below this level the organism was unable to survive. ATPase levels from *S. sanguis* grown at pH 6.0 to 7.0 were compared to those of *S. mutans* grown at pH 5.0 to 7.0 (Fig. 4). The data show that ATPase activity was induced in both organisms; however, activity levels in *S. sanguis* did not reach the absolute levels seen in *S. mutans* at pH 5.0. Thus, it appeared that although both organisms possess acid-inducible enzymes, the *S. mutans* enzyme functions at a lower external pH and appears to have higher overall levels of activity, in agreement with related observations (55).

Expression of enzymatic activity in ATPase-defective *E. coli* strains. In order to determine whether streptococcal ATPase was actively expressed in *E. coli*, we transformed an ATPase-defective mutant, *E. coli* JP17, with the *S. sanguis* ATPase genes and measured the release of inorganic phosphate. The

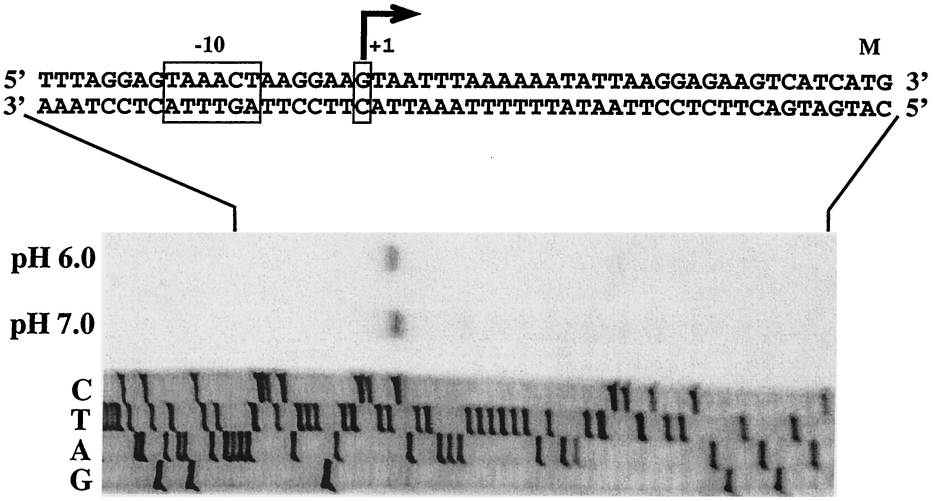

FIG. 3. Primer extension analysis of ATPase-specific RNA extracted from chemostat-grown *S. sanguis* 10904. The nucleotide ladder and extension products were generated with the same primer (see Materials and Methods). The lanes are marked for base content. Lanes with cDNA resulting from extension of the mRNAs are marked as pH 6.0 and 7.0, indicating the culture pH from which the RNA was prepared. The double-stranded nucleotide sequence from this region is shown above with an arrow indicating the site and direction of transcription initiation. Boxes are shown around the presumptive −10 region and the +1 nucleotide of the mRNA. An M above the ATG indicates the start codon for the c subunit (*atpE*).

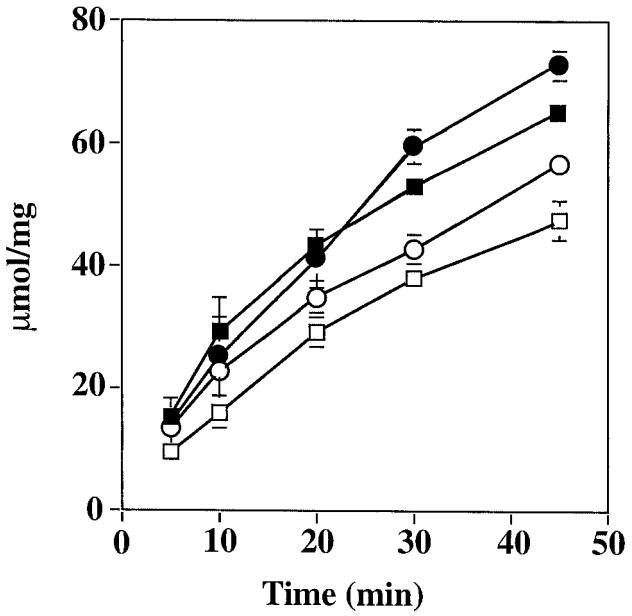

FIG. 4. ATPase activity in *S. sanguis* and *S. mutans* as a function of growth pH values. Extracts were prepared from steady-state cultures of *S. sanguis* 10904 and *S. mutans* UA159 grown at the indicated pH values. Error bars represent the ranges from six determinations. The circles represent the UA159 extracts while the squares represent the *S. sanguis* 10904 extracts. Open symbols represent growth at pH 7 while filled symbols represent growth at lower pH values (pH 6 for *S. sanguis* or pH 5 for *S. mutans*).

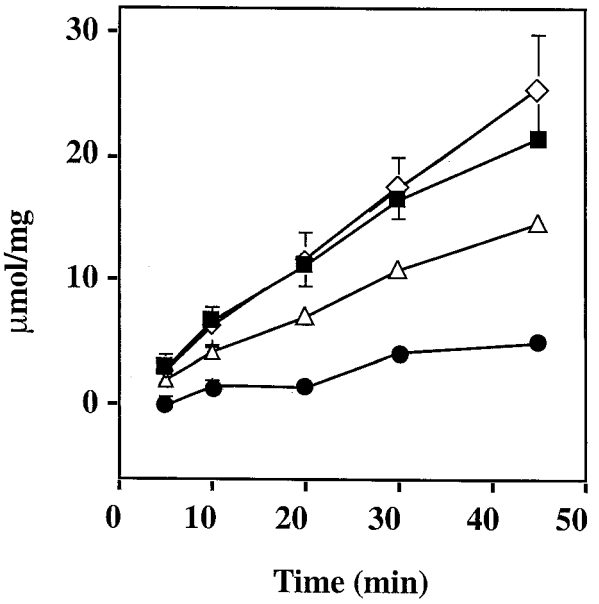

FIG. 5. Complementation of an *E. coli atpD* mutant strain with pDP31 (*E. coli*) and pSSA9 (*S. sanguis*) *atpD* genes expressed from plasmids. Plasmids and assay conditions are described in Materials and Methods. The extracts were prepared from overnight cultures of each strain. The error bars indicate six repeats with a minimum of two separate extract preparations. The filled squares represent the wild-type *E. coli* strain DH10B. The filled circles represent the *E. coli atpD* mutant JP17. The engineered strains are shown in by open diamonds for JP17(pSSA9) and open triangles for JP17(pDP31).

defective strain, JP17, had a deletion in the *atpD* gene (β subunit) of the operon and exhibited low levels of activity over time (Fig. 5). As a positive control, we transformed JP17 with a complementing *E. coli* plasmid, pDP31, which contains the complete *atpD* and *atpC* genes. The results show that the engineered strain can achieve approximately the same levels of activity seen in a wild-type *E. coli* strain, DH10B (Fig. 5). Strain JP17 containing plasmid pSSA9 showed ATPase activity at nearly wild-type levels. Interestingly, the ATPase activity seen when JP17 was complemented with the *S. sanguis* ATPase genes was typically higher than that observed in the strain containing pDP31. Since pSSA9 did not contain all of the genes, the extent of enzymatic activity could not have been due solely to the plasmid-borne subunits, suggesting the likelihood that hybrid enzymes had been formed.

DCCD partially inhibits *S. sanguis* ATPase expressed in *E. coli atp* mutants. DCCD is known to inhibit the action of proton-translocating ATPases via binding to an aspartate residue at position 61 (in *E. coli*) of the *atpE* gene (c subunit). Enzymatic activity data indicated the strong possibility that hybrid enzymes were functional in the JP17 background. However, the presence of the *S. sanguis* genes was insufficient to support growth of JP17 on minimal succinate-containing medium, indicating that the enzyme was at least partially uncoupled from oxidative phosphorylation (data not shown). It was of interest then to determine the DCCD susceptibility of the strains containing cloned material. Strain JP17 was not affected greatly by the addition of 1 mM DCCD due to the fact that this strain exhibited only background levels of activity in the assay (Fig. 6). When the defect in JP17 was repaired by the addition of a functional *E. coli atpD* gene (β subunit) carried on plasmid pDP31, activity was inhibited by the addition of 1 mM DCCD. However, the *S. sanguis*-derived activity from JP17(pSSA9) was not inhibited to the same extent as that seen with the *E. coli* enzyme. Control reactions with extracts prepared from wild-type *E. coli* (Fig. 6) and *S. sanguis* (data not shown) showed significant inhibition of ATPase by DCCD. Thus, it seems that the lack of inhibition seen in JP17(pSSA9) was probably not due to a difference in the products of the *S. sanguis* ATPase genes themselves. Rather, it appears that hybrid enzymes had formed that were not capable of coupled ATP cleavage and proton translocation, in agreement with the observations on minimal succinate medium.

DISCUSSION

Previously, Smith et al. reported the cloning and nucleotide sequence determination of the F-ATPase from *S. mutans* (53). The isolation and characterization of the homologous operon from *S. sanguis* reported here provides the necessary information for additional inquiry into the structural and functional differences of the F-ATPases from these organisms.

The genetic organization of the *S. sanguis* ATPase operon was identical to that seen previously in *S. mutans* (53), *E. hirae* (52), *Streptococcus oralis*, and *Streptococcus pneumoniae* (22). To date, all streptococci appear to possess an F_0 gene order consisting of *atpEBF* in comparison to that of *atpBEF*, which is seen in most other bacteria. The significance of the altered

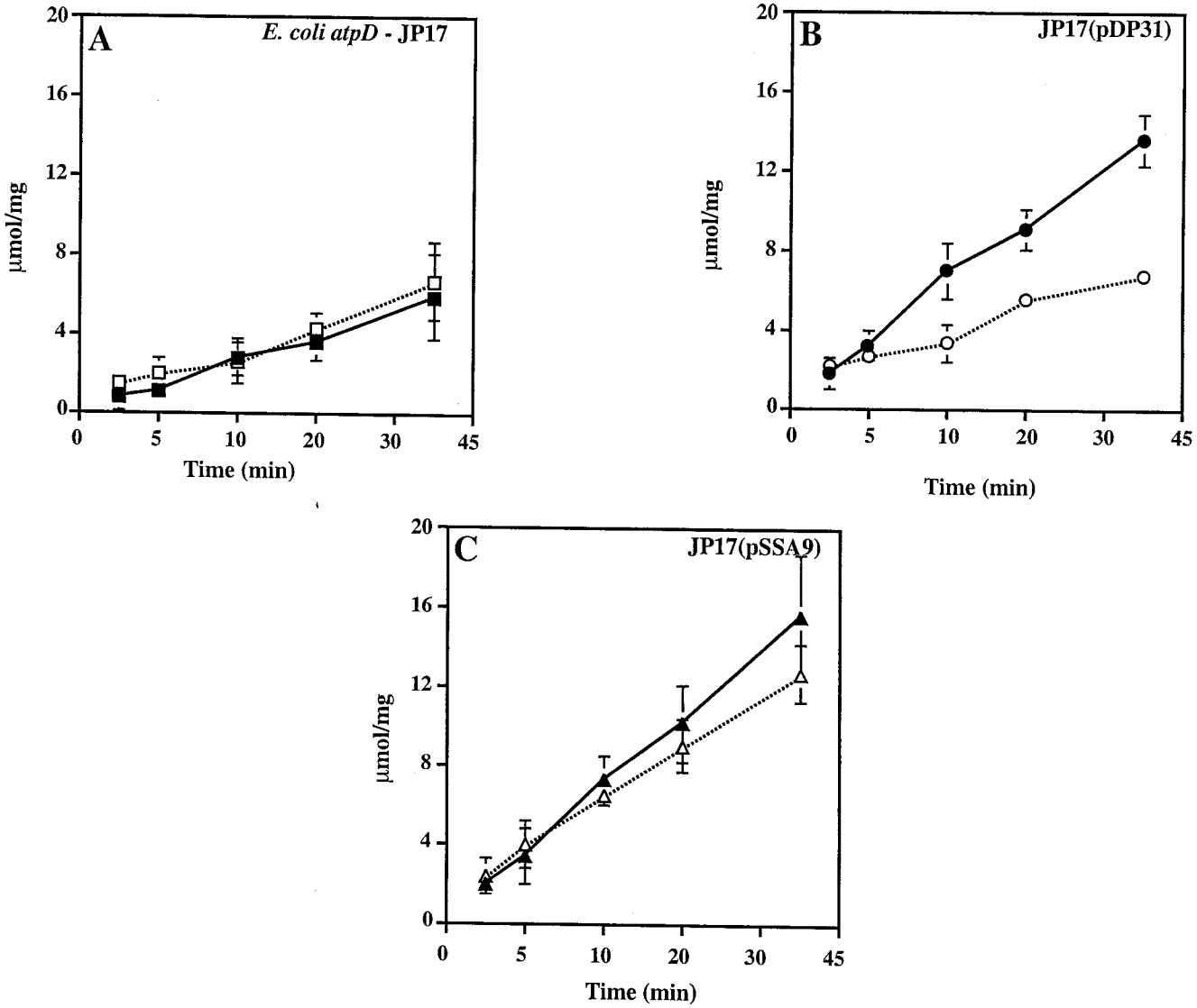

FIG. 6. DCCD does not inhibit *S. sanguis-E. coli* ATPase hybrids in *E. coli*. Extracts were incubated with 1 mM DCCD for 5 min prior to the addition of ATP. Assay time points were as shown. (A) *E. coli atpD* strain JP17 with (■) and without (□) 1 mM DCCD. (B) Strain JP17(pDP31) with (●) and without (○) 1 mM DCCD. (C) Strain JP17(pSSA9) with (▲) and without (△) 1 mM DCCD.

gene order for the membrane-bound subunits is currently unclear.

The wealth of information regarding the structure of F-ATPases and the functional role of specific residues provide a basis for evaluating differences between the two oral streptococcal forms of the enzyme. The Walker-A sequence (61) found in *E. coli* is similar to domains found in other nucleotide binding proteins such as *ras* p21, adenylate cyclase (19, 20, 26, 29), and *ercA* (5). In *E. coli*, the domain is located at positions 149 to 156 of the *atpD* gene. The Gly-Gly-Ala-Gly-Val-Gly-Lys-Thr sequence is completely conserved in *S. sanguis* and *S. mutans*, although the position is modestly shifted to residues 155 to 162. Similarly, the nucleotide binding region sequence, Gly-Asp-Arg-Gln-Thr-Gly-Lys-Thr, established for the α subunit (*atpA*), is conserved in both position and sequence at residues 169 to 176 in both streptococci. The conservation of sequence in the catalytic domains extended well beyond the information in the nucleotide binding clefts, thereby providing additional evidence of the strong pressure in maintaining the ATPase structure in the streptococci. For example, coupling of proton conduction with ATP synthesis has been shown to involve, at least, the interaction of the β380DELSEEDβ386 sequence with the γM23 residue in the *E. coli* F-ATPase (*E. coli* F_1F_0) enzyme (1, 4, 35). The γM23 residue was conserved in both of the oral streptococci. The β subunit sequence, though somewhat rearranged as DELSDDE in *S. sanguis* and DELSDEE in *S. mutans* (53), still contains information appropriate for interaction with the γ subunit. Residues involved with the rotation of γ-ε have been identified via cysteine cross-linking mutagenesis (1, 3), two of which, αS411 and βE381, are conserved in the *S. sanguis* and *S. mutans* homologues. Two additional residues implicated in the rotational mechanism,

γC87 and εS108, were not conserved in the oral streptococcal sequences (1–3). Glutamate residues were deduced at position 108 of the ε sequence for *S. mutans* and *S. sanguis*, such that it seems possible that the carboxyl OH might play a role similar to that of the serine residue in *E. coli* F_1F_0. It is difficult to reconcile the absence of cysteine in the γ subunit of both streptococcal forms. However, in both enzymes, position 87 is occupied by serine residues, suggesting the possibility of hydrogen bonding via the hydroxyl group.

Conservation of functionally important residues extends into the membrane-bound residues as well. Nuclear magnetic resonance data have shown that the c subunit (*atpE*) contains two helical regions connected by a polar loop of three amino acids: Arg-41, Gln-42, and Pro-43 (25). All three of the loop amino acids are conserved in the streptococcal enzymes. Proton-binding known to occur at site D61 in *E. coli* is also likely conserved in the c subunits of the streptococcal enzymes. The R210 residue in helix 4 of the a subunit is known to be physically close to cD61 in *E. coli* F_1F_0 (33, 59). *S. sanguis* and *S. mutans* enzyme forms have also retained the residue. Likewise, the highly conserved residue, βR36, which is necessary for the retention of coupling (16), has been similarly conserved in the streptococcal forms of the subunit. In the *E. coli* F_1F_0 enzyme, cQ42 is shielded from *N*-ethylmaleimide labeling by the presence of the γ and ε subunits (62), apparently by association with γY205 (63). The cQ42 residue has been conserved in *S. sanguis*, and a tyrosine residue at position 207 of the γ subunit was deduced from the nucleotide sequence. The corresponding position in *S. mutans* is an asparagine (53), and its ability to participate in bonding with cQ42 is an open question for the present.

It is clear from the strong conservation of relevant amino acids found in the oral streptococci that the mechanisms of function are likely to be identical to those described thus far for the *E. coli* F_1F_0 enzyme. What remains to be determined is the basis for the difference in pH optima for the two enzymes. Previous data have indicated that the kinetic parameters for the *E. coli* F_1 enzyme are unaffected by the presence or absence of F_0 (39). Results from studies with F_1 and F_1F_0 preparations from *S. sanguis* and *S. mutans* indicated that pH profiles for F_1 enzyme preparations were more alike than those using membrane-bound or membrane-free preparation of F_1F_0 (55). The conclusion derived from the experiments with the streptococcal forms was that the association of F_1 with F_0 led to enhanced acid tolerance of enzymatic activity. Mixing experiments have not yet been reported, so it is not yet possible to say that either the F_1 or F_0 domain of the *S. mutans* enzyme confers the pH advantage over the *S. sanguis* enzyme and which residues contribute to the difference.

Conservation of deduced amino acid sequences suggested the probability that the *S. sanguis* ATPase could be biochemically active in an *E. coli* strain lacking some or all of the ATPase subunits. Subsequent experiments with a strain defective in the *atpD* gene showed that cloned material from *S. sanguis* did confer on the mutant the ability to hydrolyze ATP. The constructs used in our experiments did not include the entire operon from *S. sanguis*, indicating the ability of the participating subunits to productively interact with those produced by *E. coli* as far as ATP cleavage was concerned. Our results were similar to those shown previously from experiments with clones from *E. hirae* (57) and *Bacillus megaterium* (32). DCCD sensitivity of the wild-type *S. sanguis* ATPase indicated a normally functioning enzyme, with ATP cleavage coupled to proton export such that in operon-lacking strains of *E. coli*, it might be possible to obtain completely functioning F_1-F_0 enzymes from oral streptococci. Importantly, it suggests the possibility that in future experiments, hybrid ATPase operons of *S. mutans* and *S. sanguis* genes might be constructed in a strain lacking ATPase subunits in *E. coli*, thereby permitting analyses of pH optima in a well-defined genetic background. Stable hybrids might then set the stage for evaluating the enzymes in streptococcal backgrounds for their effects on acid resistance and pathogenesis in the rodent model.

Additional analysis of the *S. sanguis* F-ATPase operon showed that, like *S. mutans*, F-ATPase activity increased as the growth pH decreased from a value of 7 to 6. Since the increase in specific activity was not due to a change in growth rate, we conclude that the means by which the regulation of ATPase transcription is controlled in *S. sanguis* differs somewhat from that seen for the *E. coli* operon (34).

In conclusion, we have identified, cloned, and determined the nucleotide sequence for the F-ATPase from the oral streptococcus *S. sanguis*. The enzyme, while exhibiting sequence homology to other sequenced ATPases, does possess unique elements. The streptococci lack the *atpI* gene and have an altered F_0 gene order. Future experiments with more-defined contributions by streptococcal subunits will permit a clearer picture of whether catalytic and membrane subunits might productively interact with those from *E. coli*.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health/PHS (DE-01627). W.L.K. was supported by a predoctoral fellowship in the Rochester Cariology Training Program (NIH/PHS T32-DE07165).

We thank Roberta Faustoferri for assistance with the chemostat cultures and Robert E. Marquis, Alan Senior, and Joachim Weber for helpful discussions throughout.

REFERENCES

1. **Aggeler, R., and R. A. Capaldi.** 1996. Nucleotide-dependent movement of the epsilon subunit between alpha and beta subunits in the *Escherichia coli* F1F0-type ATPase. J. Biol. Chem. **271:**13888–13891.
2. **Aggeler, R., G. Gruber, and R. A. Capaldi.** 1998. Trapping of conformations of the *Escherichia coli* F1 ATPase by disulfide bond formation. A state of the enzyme with all three catalytic sites of equal and low affinity for nucleotides. FEBS Lett. **426:**37–40.
3. **Aggeler, R., I. Ogilvie, and R. A. Capaldi.** 1997. Rotation of a gamma-epsilon subunit domain in the *Escherichia coli* F1F0-ATP synthase complex. The gamma-epsilon subunits are essentially randomly distributed relative to the alpha3beta3delta domain in the intact complex. J. Biol. Chem. **272:**19621–19624.
4. **Al Shawi, M. K., and R. K. Nakamoto.** 1997. Mechanism of energy coupling in the FOF1-ATP synthase: the uncoupling mutation, gammaM23K, disrupts the use of binding energy to drive catalysis. Biochemistry **36:**12954–13960.
5. **Amano, T., M. Yoshida, Y. Matsuo, and K. Nishikawa.** 1994. Structural model of the ATP-binding domain of the F1-beta subunit based on analogy to the RecA protein. FEBS Lett. **351:**1–5.
6. **Arechaga, I., and P. C. Jones.** 2001. The rotor in the membrane of the ATP synthase and relatives. FEBS Lett. **494:**1–5.
7. **Bartolome, B., Y. Jubete, E. Martinez, and F. de la Cruz.** 1991. Construction and properties of a family of pACYC184-derived cloning vectors compatible with pBR322 and its derivatives. Gene **102:**75–78.
8. **Belli, W. A., and R. E. Marquis.** 1991. Adaptation of *Streptococcus mutans* and *Enterococcus hirae* to acid stress in continuous culture. Appl. Environ. Microbiol. **57:**1134–1138.
9. **Belli, W. A., and R. E. Marquis.** 1994. Catabolite modification of acid tolerance of *Streptococcus mutans* GS-5. Oral Microbiol. Immun. **9:**29–34.
10. **Bencini, D. A., M. S. Shanley, J. R. Wild, and G. A. O'Donovan.** 1983. New assay for enzymatic phosphate release: application to aspartate transcarbamylase and other enzymes. Anal. Biochem. **132:**259–264.

11. **Bender, G. R., S. V. Sutton, and R. E. Marquis.** 1986. Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. Infect. Immun. **53:**331–338.
12. **Bowden, G. H., and I. R. Hamilton.** 1987. Environmental pH as a factor in the competition between strains of the oral streptococci *Streptococcus mutans*, *S. sanguis*, and "*S. mitior*" growing in continuous culture. Can. J. Microbiol. **33:**824–827.
13. **Bradshaw, D. J., P. D. Marsh, C. Allison, and K. M. Schilling.** 1996. Effect of oxygen, inoculum composition and flow rate on development of mixed-culture oral biofilms. Microbiology **142:**623–629.
14. **Brusilow, W. S., M. A. Scarpetta, C. A. Hawthorne, and W. P. Clark.** 1989. Organization and sequence of the genes coding for the proton-translocating ATPase of *Bacillus megaterium*. J. Biol. Chem. **264:**1528–1533.
15. **Casiano-Colon, A., and R. E. Marquis.** 1988. Role of the arginine deiminase system in protecting oral bacteria and an enzymatic basis for acid tolerance. Appl. Environ. Microbiol. **54:**1318–1324.
16. **Caviston, T. L., C. J. Ketchum, P. L. Sorgen, R. K. Nakamoto, and B. D. Cain.** 1998. Identification of an uncoupling mutation affecting the b subunit of F1F0 ATP synthase in *Escherichia coli*. FEBS Lett. **429:**201–206.
17. **Dashper, S. G., and E. C. Reynolds.** 1992. pH regulation by *Streptococcus mutans*. J. Dent. Res. **71:**1159–1165.
18. **De Stoppelaar, J., J. Van Houte, and D. O. Backer.** 1969. The relationship between extracellular polysaccharide-producing streptococci and smooth surface caries in 13-year-old children. Caries Res. **3:**190–199.
19. **Duncan, T. M., and R. L. Cross.** 1992. A model for the catalytic site of F1-ATPase based on analogies to nucleotide-binding domains of known structure. J. Bioenerg. Biomembr. **24:**453–461.
20. **Duncan, T. M., and A. E. Senior.** 1985. The defective proton-ATPase of uncD mutants of *Escherichia coli*. Two mutations which affect the catalytic mechanism. J. Biol. Chem. **260:**4901–4907.
21. **Elston, T., H. Wang, and G. Oster.** 1998. Energy transduction in ATP synthase. Nature **391:**510–513.
22. **Fenoll, A., R. Munoz, E. Garcia, and A. G. de la Campa.** 1994. Molecular basis of the optochin-sensitive phenotype of pneumococcus: characterization of the genes encoding the F0 complex of the *Streptococcus pneumoniae* and *Streptococcus oralis* H(+)-ATPases. Mol. Microbiol. **12:**587–598.
23. **Ferretti, J. J., W. M. McShan, D. Ajdic, D. J. Savic, G. Savic, K. Lyon, C. Primeaux, S. Sezate, A. N. Suvorov, S. Kenton, H. S. Lai, S. P. Lin, Y. Qian, H. G. Jia, F. Z. Najar, Q. Ren, H. Zhu, L. Song, J. White, X. Yuan, S. W. Clifton, B. A. Roe, and R. M. McLaughlin.** 2001. Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. USA **98:** 4658–4663.
24. **Fillingame, R. H., M. E. Girvin, W. Jiang, F. Valiyaveetil, and J. Hermolin.** 1998. Subunit interactions coupling H+ transport and ATP synthesis in F1F0 ATP synthase. Acta Physiol. Scand. Suppl. **643:**163–168.
25. **Fillingame, R. H., M. E. Girvin, and Y. Zhang.** 1995. Correlations of structure and function in subunit c of *Escherichia coli* F0F1 ATP synthase. Biochem. Soc. Trans. **23:**760–766.
26. **Fry, D. C., S. A. Kuby, and A. S. Mildvan.** 1986. ATP-binding site of adenylate kinase: mechanistic implications of its homology with *ras*-encoded p21, F1-ATPase, and other nucleotide-binding proteins. Proc. Natl. Acad. Sci. USA **83:**907–911.
27. **Futai, M., H. Omote, Y. Sambongi, and Y. Wada.** 2000. Synthase (H(+) ATPase): coupling between catalysis, mechanical work, and proton translocation. Biochim. Biophys. Acta **1458:**276–288.
28. **Gagnon, G., C. Vadeboncoeur, L. Gauthier, and M. Frenette.** 1995. Regulation of *pts*H and *pts*I gene expression in *Streptococcus salivarius* ATCC 25975. Mol. Microbiol. **16:**1111–1121.
29. **Garboczi, D. N., A. H. Fox, S. L. Gerring, and P. L. Pedersen.** 1988. Beta subunit of rat liver mitochondrial ATP synthase: cDNA cloning, amino acid sequence, expression in *Escherichia coli*, and structural relationship to adenylate kinase. Biochemistry **27:**553–560.
30. **Grant, S. G., J. Jessee, F. R. Bloom, and D. Hanahan.** 1990. Differential plasmid rescue from transgenic mouse DNAs into *Escherichia coli* methylation-restriction mutants. Proc. Natl. Acad. Sci. USA **87:**4645–4649.
31. **Hamilton, I. R., and N. D. Buckley.** 1991. Adaptation by Streptococcus mutans to acid tolerance. Oral Microbiol. Immunol. **6:**65–71.
32. **Hawthorne, C. A., and W. S. Brusilow.** 1986. Complementation of mutants in the *Escherichia coli* proton-translocating ATPase by cloned DNA from *Bacillus megaterium*. J. Biol. Chem. **261:**5245–5248.
33. **Jiang, W., and R. H. Fillingame.** 1998. Interacting helical faces of subunits a and c in the F1F0 ATP synthase of *Escherichia coli* defined by disulfide cross-linking. Proc. Natl. Acad. Sci. USA **95:**6607–6612.
34. **Kasimoglu, E., S. J. Park, J. Malek, C. P. Tseng, and R. P. Gunsalus.** 1996. Transcriptional regulation of the proton-translocating ATPase (*atp*IBEFHAGDC) operon of *Escherichia coli*: control by cell growth rate. J. Bacteriol. **178:**5563–5567.
35. **Ketchum, C. J., M. K. Al Shawi, and R. K. Nakamoto.** 1998. Intergenic suppression of the gammaM23K uncoupling mutation in F0F1 ATP synthase by betaGlu-381 substitutions: the role of the beta380DELSEED386 segment in energy coupling. Biochem. J. **330:**707–712.
36. **Kobayashi, H.** 1985. A proton-translocating ATPase regulates pH of the bacterial cytoplasm. J. Biol. Chem. **260:**72–76.
37. **Kobayashi, H., T. Suzuki, and T. Unemoto.** 1986. Streptococcal cytoplasmic pH is regulated by changes in amount and activity of a proton-translocating ATPase. J. Biol. Chem. **261:**627–630.
38. **Lee, R. S., J. Pagan, S. Wilke-Mounts, and A. E. Senior.** 1991. Characterization of *Escherichia coli* ATP synthase beta-subunit mutations using a chromosomal deletion strain. Biochemistry **30:**6842–6847.
39. **Lobau, S., J. Weber, and A. E. Senior.** 1998. Catalytic site nucleotide binding and hydrolysis in F1F0-ATP synthase. Biochemistry **37:**10846–10853.
40. **Loesche, W. J.** 1986. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev. **50:**353–380.
41. **Lopilato, J., S. Bortner, and J. Beckwith.** 1986. Mutations in a new chromosomal gene of *Escherichia coli* K-12, pcnB, reduce plasmid copy number of pBR322 and its derivatives. Mol. Gen. Genet. **205:**285–290.
42. **Middendorf, L. R., J. C. Bruce, R. C. Bruce, R. D. Eckles, D. L. Grone, S. C. Roemer, G. D. Sloniker, D. L. Steffens, S. L. Sutter, J. A. Brumbaugh, et al.** 1992. Continuous, on-line DNA sequencing using a versatile infrared laser scanner/electrophoresis apparatus. Electrophoresis **13:**487–494.
43. **Nakamoto, R. K.** 1996. Mechanisms of active transport in the FOF1 ATP synthase. J. Membr. Biol. **151:**101–111.
44. **Needleman, S. B., and C. D. Wunsch.** 1970. A general method applicable to the search for similarities in the amino acid sequence of two proteins. J. Mol. Biol. **48:**443–453.
45. **Nikiforuk, G.** 1985. Understanding dental caries, vol. 1. Etiology and mechanisms. Karger, Basel, Switzerland.
46. **Noji, H., R. Yasuda, M. Yoshida, and K. J. Kinosita.** 1997. Direct observation of the rotation of F1-ATPase. Nature **386:**299–302.
47. **Parsonage, D., M. S. Wilke, and A. E. Senior.** 1987. Directed mutagenesis of the beta-subunit of F1-ATPase from *Escherichia coli*. J. Biol. Chem. **262:** 8022–8026.
48. **Pierais, V. L., and G. J. Barcak.** 1999. Development of *E. coli* host strains tolerating unstable DNA sequences on ColE1 vectors. Focus **21:**18–19.
49. **Quivey, R. G., Jr., W. L. Kuhnert, and K. Hahn.** 2000. Adaptation of oral streptococci to low pH. Adv. Microbial Physiol. **42:**239–274.
50. **Quivey, R. G., Jr., R. C. Faustoferri, W. A. Belli, and J. S. Flores.** 1991. Polymerase chain reaction amplification, cloning, sequence determination and homologies of streptococcal ATPase-encoding DNAs. Gene **97:**63–68.
51. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
52. **Shibata, C., T. Ehara, K. Tomura, K. Igarashi, and H. Kobayashi.** 1992. Gene structure of *Enterococcus hirae* (*Streptococcus faecalis*) F1F0-ATPase, which functions as a regulator of cytoplasmic pH. J. Bacteriol. **174:**6117–6124.
53. **Smith, A. J., R. J. Quivey, and R. C. Faustoferri.** 1996. Cloning and nucleotide sequence analysis of the *Streptococcus mutans* membrane-bound, proton-translocating ATPase operon. Gene **183:**87–96.
54. **Stock, D., C. Gibbons, I. Arechaga, A. G. Leslie, and J. E. Walker.** 2000. The rotary mechanism of ATP synthase. Curr. Opin. Struct. Biol. **10:**672–679.
55. **Sturr, M. G., and R. E. Marquis.** 1992. Comparative acid tolerances and inhibitor sensitivities of isolated F-ATPases of oral lactic acid bacteria. Appl. Environ. Microbiol. **58:**2287–2291.
56. **Sutton, S. V., and R. E. Marquis.** 1987. Membrane-associated and solubilized ATPases of *Streptococcus mutans* and *Streptococcus sanguis*. J. Dent. Res. **66:**1095–1098.
57. **Suzuki, T., C. Shibata, A. Yamaguchi, K. Igarashi, and H. Kobayashi.** 1993. Complementation of an *Enterococcus hirae* (*Streptococcus faecalis*) mutant in the alpha subunit of the H(+)-ATPase by cloned genes from the same and different species. Mol. Microbiol. **9:**111–118.
58. **Tabor, S., and C. C. Richardson.** 1987. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. Proc. Natl. Acad. Sci. USA **84:**4767–4771.
59. **Valiyaveetil, F. I., and R. H. Fillingame.** 1997. On the role of Arg-210 and Glu-219 of subunit a in proton translocation by the *Escherichia coli* F0F1-ATP synthase. J. Biol. Chem. **272:**32635–32641.
60. **Walker, J. E., M. Saraste, and N. J. Gay.** 1984. The *unc* operon. Nucleotide sequence, regulation and structure of ATP-synthase. Biochim. Biophys. Acta **768:**164–200.
61. **Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. **1:**945–951.
62. **Watts, S. D., and R. A. Capaldi.** 1997. Interactions between the F1 and F0 parts in the *Escherichia coli* ATP synthase. Associations involving the loop region of C subunits. J. Biol. Chem. **272:**15065–15068.
63. **Watts, S. D., C. Tang, and R. A. Capaldi.** 1996. The stalk region of the *Escherichia coli* ATP synthase. Tyrosine 205 of the gamma subunit is in the interface between the F1 and F0 parts and can interact with both the epsilon and c oligomer. J. Biol. Chem. **271:**28341–28347.
64. **Weber, J., and A. E. Senior.** 1997. Catalytic mechanism of F1-ATPase. Biochim. Biophys. Acta **1319:**19–58.