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A unique F-type H^+-ATPase from *Streptococcus mutans*: An active H^+ pump at acidic pH

Yuka Sasaki [a], Eri Nogami [a], Masatomo Maeda [b], Mayumi Nakanishi-Matsui [b], Atsuko Iwamoto-Kihara [a,*]

[a] Department of Bioscience, Nagahama Institute of Bioscience and Technology, 1266 Tamura, Nagahama, Shiga 526-0829, Japan
[b] Department of Molecular Biology, School of Pharmacy, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba, Iwate 028-3694, Japan

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ABSTRACT

We have shown previously that the *Streptococcus mutans* F-type H^+-ATPase (F_OF_1) *c* subunit gene could complement *Escherichia coli* defective in the corresponding gene, particularly at acidic pH (Araki et al., (2013) [14]). In this study, the entire *S. mutans* F_OF_1 was functionally assembled in the *E. coli* plasma membrane (SF_OF_1). Membrane SF_OF_1 ATPase showed optimum activity at pH 7, essentially the same as that of the *S. mutans*, although the activity of *E. coli* F_OF_1 (EF_OF_1) was optimum at pH ⩾ 9. The membranes showed detectable ATP-dependent H^+-translocation at pH 5.5–6.5, but not at neutral conditions (pH ⩾ 7), consistent with the role of *S. mutans* F_OF_1 to pump H^+ out of the acidic cytoplasm. A hybrid F_OF_1, consisting of membrane-integrated F_O and -peripheral F_1 sectors from *S. mutans* and *E. coli* (SF_OEF_1), respectively, essentially showed the same pH profile as that of EF_OF_1 ATPase. However, ATP-driven H^+-transport was similar to that by SF_OF_1, with activity at acidic pH. Replacement of the conserved *c* subunit Glu53 in SF_OF_1 abolished H^+-transport at pH 6 or 7, suggesting its role in H^+ transport. Mutations in the SF_OF_1 *c* subunit, Ser17Ala or Glu20Ile, changed the pH dependency of H^+-transport, and the F_O could transport H^+ at pH 7, as the membranes with EF_OF_1. Ser17, Glu20, and their vicinity were suggested to be involved in H^+-transport in *S. mutans* at acidic pH.

1. Introduction

The F-type H^+-ATPase (F_OF_1) family includes ATP synthases found in membranes of bacteria, mitochondria and chloroplasts [1–3]. An electrochemical proton gradient generated by a respiratory chain is converted to the chemical energy currency ATP through F_OF_1 [1–3]. The same enzyme can hydrolyze ATP to generate the electrochemical proton gradient, which is a physiological role of the enzyme in anaerobic bacteria [1].

Plasma membranes of various bacteria contain the simplest version of the enzyme that consists of membrane embedded F_O (ab_2c_{10-15}) and peripheral F_1 ($\alpha_3\beta_3\gamma\delta\varepsilon$) portions. F_1 has three catalytic sites formed from residues of α and β subunits, and F_O has a proton pathway at the interface of *a* and *c* subunits. The *c* subunit has a hairpin structure with two transmembrane helices 1 and 2 (TM1 and TM2), and 10–15 of them assemble to form a ring structure (*c*-ring) [1–3]. A conserved acidic Glu or Asp residue in TM2 is essential for the H^+-transport [3].

Intra-molecular rotation of F_OF_1 couples between H^+-transport and ATP synthesis. Direct observations of bacterial F_OF_1 has established ATP hydrolysis dependent rotation of the $\gamma\varepsilon c_{10-15}$ subunit assembly against the $\alpha_3\beta_3\delta ab_2$, functioning mechanically as a rotor and a stator, respectively [4,5]. Each catalytic site in the three β subunits changes its conformation accompanying ATP hydrolysis. The sequential ATP hydrolysis directs the γ subunit rotation in the central space of $\alpha_3\beta_3$ subunit hexamer. Simultaneously, *c*-ring in the $\gamma\varepsilon c_{10-15}$ complex rotates against the ab_2, leading to continuous H^+-transport through the interface of *a* and *c* subunits, in which the essential Glu or Asp residue in the *c* subunit carries H^+ between the cytoplasmic and periplasmic half channels [2].

Streptococcus mutans is implicated as the principal causative agent of human dental caries, which is one of the most common infectious diseases [6,7]. The bacterium metabolizes dietary sugars, producing lactic acids that are excreted by a lactate/proton co-transporter [8]. Acidification (pH < 5.5) causes demineralization of calcium phosphate from the enamel layer of teeth that initiates caries [6]. Thus, *S. mutans* is able to grow anaerobically and survive at an acidic pH environment. The acid tolerance system involves maintenance of macromolecules such as DNA and protein, regulation of phospholipid composition of the plasma membrane, and ion transport including H^+ efflux [7].

Abbreviations: SF_OF_1, *S. mutans* F_OF_1 expressed in *E. coli* membranes; EF_OF_1, *E. coli* F_OF_1 expressed in *E. coli* membranes; SF_OEF_1, hybrid F_OF_1 formed from *S. mutans* F_O and *E. coli* F_1.

* Corresponding author. Fax: +81 749 64 8140.
E-mail address: a_iwamoto@nagahama-i-bio.ac.jp (A. Iwamoto-Kihara).

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The F-type ATPase is important for maintaining the cytoplasmic pH by H^+ secretion because oral streptococci are able to grow at acidic pH corresponding to the pH optima of their F-type ATPase activities [7,9]. Furthermore, transcription and translation of F_OF_1 genes increased at acidic conditions [10,11]. However, the molecular basis of H^+-transport in the *S. mutans* F_OF_1 is mostly unknown, including the H^+ transporting *c* subunit. Although the Glu residue for H^+-transport is conserved, the surrounding residues are different particularly among bacteria [3]. Thus, the *c* subunits of pathogenic bacteria are the possible target of anti-bacterial agents. In this regard, the binding site for diarylquinoline TMC207, a very promising candidate drug for tuberculosis, is the *c* subunit of *Mycobacterium tuberculosis* [12,13].

We have constructed an *Escherichia coli* cell that carries a hybrid H^+-ATPase gene comprising the *S. mutans c* subunit and *E. coli* residual subunits [14]. The cells could grow by oxidative phosphorylation, indicating that the *c*-ring of *S. mutans* is functional for ATP synthesis in *E. coli* membranes. These cells showed better growth at pH 5.5 than at pH 7.5, possibly due to optimal H^+-transport for acidic environment. However, the prepared inverted membranes did not display ATP-driven H^+-translocation between pH 5.5 and 8.0, although the H^+ transporting residue conserved among different *c* subunits is also found in the bacterium (Glu53). However, the membranes became capable of H^+-transport when Glu20 in the *c* subunit was substituted with Gln. These interesting results suggested that *S. mutans* had a unique pH-dependent property of H^+-transport compared with other bacteria such as *E. coli.*

In order to investigate the H^+-transport in more detail, we expressed the entire *S. mutans* F_OF_1 in *E. coli* membranes. The membrane vesicles demonstrated ATP-driven H^+-transport at acidic but not at neutral pH, similar to those of *S. mutans*. Mutational studies suggested that the key region imparting the pH-dependency is in the first transmembrane helix (TM1) containing Glu20 residue.

2. Materials and methods

2.1. Plasmids carrying F_OF_1 genes of S. mutans and E. coli

The 6.3-kb DNA fragment for *S. mutans* H^+-ATPase operon was inserted downstream of the *trc* promoter (pRSM1; 10,695 bp) (Fig. 1, Fig. S1). The plasmid pBWU13.X was a derivative of pBWU13 that carries all the F_OF_1 genes for *E. coli* [15]. To construct a hybrid, genes for *S. mutans* F_O were ligated to those for *E. coli* F_1 (Fig. 1A and B). Because the carboxyl-terminal region of the *b* subunit (F_O) interacted with the δ subunit (F_1) [16], the segment for *S. mutans* Glu54–Ala165 (carboxyl-terminus) of the *b* subunit was substituted with the corresponding *E. coli* segment (Glu48–Leu156) (Table 1).

2.2. Bacterial culture and membrane preparation

The *S. mutans* strain GS-5 cells were cultured in heart infusion broth (pH 7.0) (Becton Dickinson, Co. Ltd.) containing 0.2% glucose at 37 °C until late exponential phase where the medium pH was decreased to approximately 5. The genes for *S. mutans* F_OF_1 and its hybrid with *E. coli* F_OF_1 were introduced into the *E. coli* strain DK8 lacking F_OF_1 genes [15]. Bacterial culture grown to early logarithmic phase was treated with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 5 h to induce expression of the F_OF_1 genes (Fig. S2). The strain DK8/pBWU13.X and KY7230 (wild-type *E. coli*) was cultured without IPTG.

Inverted membrane vesicles of *E. coli* were prepared as described elsewhere [15]. *S. mutans* membranes were prepared as previously described [17].

2.3. Western blot analysis of membrane proteins

Membrane proteins separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) were transferred to a PVDF membrane. Rabbit antisera raised against the amino- and the carboxyl-terminal regions of the *S. mutans* β (STGKIAQVVGPVVPV) and the *a* (TTYIGKKVNIDTKGN) subunits, respectively, were used. Anti-*E. coli* F_1 and anti-myc antisera (Bethyl Laboratories, Inc., TX) were also used.

2.4. Other procedures

H^+-transport was monitored by fluorescence quenching of 9-amino-6-chloro-2-methoxyacridine (ACMA) [18,19]. Tertiary structure of the *S. mutans c* subunit was obtained by homology modeling using MOE software system (Ryoka Systems Inc., Tokyo, Japan) based on the subunit structures of *Spirulina platensis* (PDB: 2WIE) [20], *Spinacia oleracea* (PDB: 2W5J) [21], *Bacillus pseudofirmus* (PDB: 2X2V) [22], and *Saccharomyces cerevisiae* (PDB: 3U2F, 3U32) [23].

3. Results and discussion

3.1. Assembly of the S. mutans F_OF_1 subunits in E. coli plasma membranes

An entire operon for *S. mutans* F_OF_1 with an intrinsic promoter was cloned and introduced into the *E. coli* strain DK8 with deleted endogenous F_OF_1 genes [15]. However, assembled F_OF_1 was hardly detectable by ATPase assay and western blotting of membranes, even though the *E. coli* promoter for F_OF_1 (P_3, a weak one) was inserted upstream of the operon (data not shown).

In this study, we introduced the inducible *trc* promoter upstream of the genes (Fig. 1A, Fig. S1). After induction with IPTG, presence of F_OF_1 derived from *S. mutans* genes (SF_OF_1) was estimated using antibodies against *a* and β subunits: membranes applied to the gel electrophoresis were 30 and 2 μg protein, respectively for *S. mutans* and *E. coli* with SF_OF_1. Since signals of the immunoblot were similar, SF_OF_1 expressed in *E. coli* membranes was 10-fold more than of those in *S. mutans* membranes (Fig. 1C, lanes 1 and 2). ATPase activity of the membranes with SF_OF_1 was 4.5 μmol/mg·min at pH 7 (Table 1), whereas original *S. mutans* membranes showed activity of 0.31 μmol/mg·min, consistent with the increased amounts of subunits.

We also expressed the hybrid enzyme SF_OEF_1 in *E. coli* membranes carrying the F_O and F_1 from *S. mutans* and *E. coli*, respectively (Fig. 1A and B). The *a* subunit of *S. mutans* was detected with the *E. coli* α and β subunits in the membranes (Fig. 1C, lane 3), indicating that SF_O and EF_1 were properly assembled. Membrane ATPase activity of the cells expressing *E. coli* F_OF_1 (EF_OF_1) with the intrinsic P_3 promoter was similar to cells containing SF_OF_1 (3.3 μmol/mg·min) at pH 7 (Fig. 1, Table 1).

3.2. Comparison of membrane ATPase activities among SF_OF_1, EF_OF_1, and hybrid SF_OEF_1

The pH profile of ATPase activities from *S. mutans* plasma membranes (Fig. 2A, note the vertical axis) was compared with that of *E. coli* membranes expressing various F_OF_1 (Fig. 2B). The membranes with SF_OF_1 showed a maximal activity at pH 7 similar to the *S. mutans* membranes, confirming the optimal pH of purified *S. mutans* F_1-ATPase [9].

Membranes expressing EF_OF_1 were more active in alkaline pH similar to those from the wild-type *E. coli* (Fig. 2). The hybrid SF_OEF_1 showed similar pH dependency with that of the EF_OF_1,

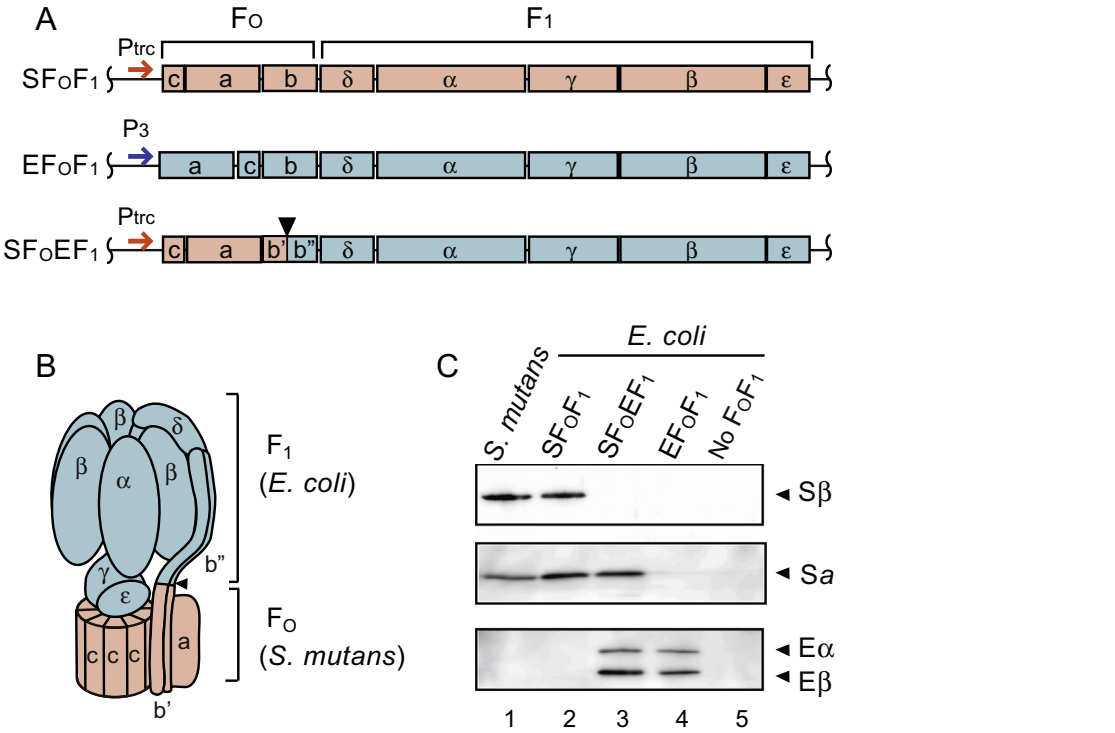

Fig. 1. Expression of the F_OF_1 genes of *S. mutans*, *E. coli*, and their hybrid in *E. coli* plasma membranes. (A) Gene clusters (operon) for F_OF_1 of *S. mutans* (SF_OF_1), *E. coli* (EF_OF_1), and their hybrid (SF_OEF_1). IPTG inducible promoter *trc* (P_{trc}) was introduced upstream of the operon for SF_OF_1 and SF_OEF_1. Genes for EF_OF_1were expressed from an intrinsic weak promoter (P_3). To construct the hybrid gene cluster, the segment for the amino-terminal part of the *S. mutans b* subunit gene (*b*'; Met1–Ala53) was ligated to that of the carboxyl-terminal part of *E. coli* gene (*b*"; Glu48–Leu156) in the tether region of the subunit (arrow head) [16]. All genes were introduced into *E. coli* DK8 strain lacking all F_OF_1 genes. (B) The schematic model of SF_OEF_1. (C) Membrane fractions from *S. mutans* GS-5 (lane 1) and *E. coli* DK8 harboring genes for SF_OF_1 (lane 2), SF_OEF_1 (lane 3), and EF_OF_1 (lane 4) were prepared. Membranes of *E. coli* without F_OF_1 genes also prepared as a control (lane 5). Membrane proteins (30 and 2 μg for lanes 1 and 2–5, respectively) were subjected to SDS–PAGE and the *S. mutans* β (Sβ), the *S. mutans a* (*Sa*), and the *E. coli* α and β subunits (Eα and Eβ) were detected using corresponding antibodies.

Table 1
Properties of the *E. coli* strains containing various F_OF_1 comprising *S. mutans* and *E. coli* subunits. The *E. coli* H^+-ATPase (F_OF_1) deletion strain (DK8) harboring recombinant plasmids carrying the gene cluster for F_OF_1 of *S. mutans* and *E. coli* and their hybrid. The *b* subunit of SF_OEF_1 was a fusion of *S. mutans* (*b*′) and *E. coli* (*b*″) (Section 2, and Fig. 1). Membrane ATPase activities were assayed at various pHs. Growth of each strain was tested (72 h) on minimal medium containing 0.2% glucose or 0.4% succinate as the sole carbon source, in the presence of IPTG.

F_OF_1	Subunits		ATPase (μmol/mg·min)		Growth	
	S. mutans	*E. coli*	pH 6	pH 7	Glc	Suc
SF_OF_1	*cab*δαγβε	–	3.8	4.5	+	–
SF_OEF_1	*cab*′	*b*″δαγβε	0.8	1.9	+	–
EF_OF_1	–	*acb*δαγβε	1.3	3.3	+	+
None	–	–	0.02	0.02	+	–

indicating that F_1 sector is mainly responsible for pH dependency of ATP hydrolysis. The specific activities of SF_OF_1 in the *E. coli* membranes were higher than that of EF_OF_1 below pH 7 (Fig. 2B). This result was not due to the difference in enzyme stabilities between SF_OF_1 and EF_OF_1 in acidic conditions (Fig. S3).

3.3. ATP-driven H^+-transport in membranes with *S. mutans* F_OF_1

Although H^+ secretion is important for *S. mutans* in acidic environments, ATP-driven H^+-transport of this bacterium was not studied previously in cells or membrane vesicles.

We tested ATP-dependent H^+-transport in *S. mutans* membrane vesicles using ACMA fluorescence quenching at different pH 5–7.5 (Fig. 3A). The membranes showed significant ATP-driven quenching in the range of pH 5.5–6.5, but no quenching at pH 7.0 or 7.5. The *E. coli* membrane carrying SF_OF_1 showed essentially the same

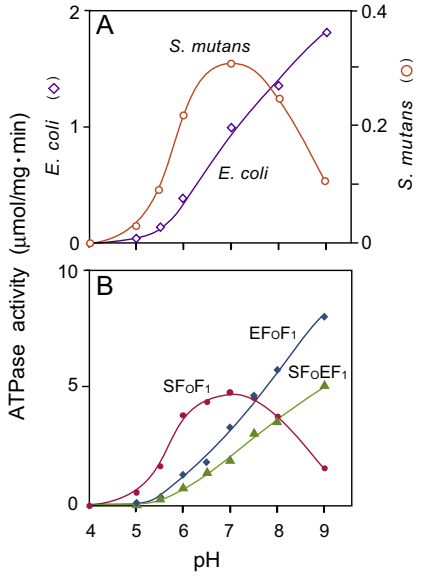

Fig. 2. pH-dependency of the membrane ATPase activities. (A) ATPase activities of the *S. mutans* GS-5 (orange circle) and *E. coli* KY7230 (purple diamond) membranes were assayed at different pHs (note the difference of vertical axis for the two bacteria). (B) ATPase activities of the *E. coli* DK8 membranes expressing SF_OF_1 (red circle), EF_OF_1 (blue diamond), and SF_OEF_1 (green triangle) were assayed at 37 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

results (Fig. 3B), in which the quenching extent was larger than *S. mutans* membranes corresponding to an increase in the ATPase activity (Fig. 2A and B).

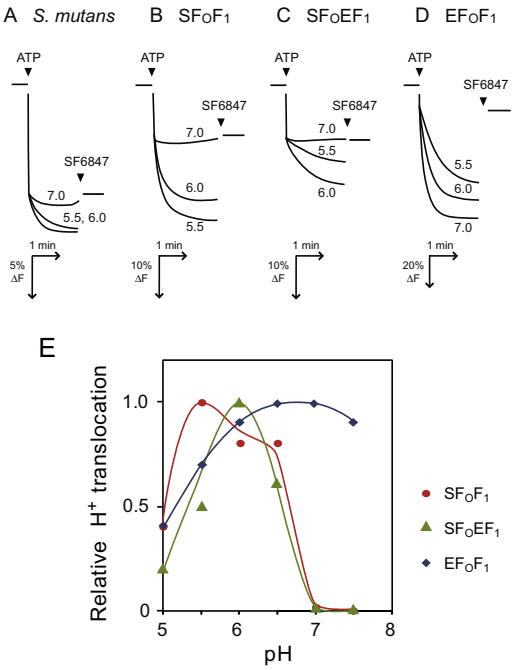

Fig. 3. pH dependent ATP-driven H^+-transports of the membranes. (A–D) Membrane H^+-transport monitored by ACMA fluorescence quenching. Inverted membranes (300 μg protein) from *S. mutans* (A) and *E. coli* expressing SF_OF_1 (B), SF_OEF_1 (C), and EF_OF_1 (D) were suspended in 2 ml of buffer at various pH (pH 5.0–7.5) containing 1 μM ACMA, 10 mM $MgCl_2$, and 140 mM KCl at 25 °C. Additions of ATP (1 mM) and a H^+ conductor SF6847 (12.5 μM) [18,19] are indicated with arrowheads, respectively. Results at pH 5.5, 6.0, and 7.0 are shown. E. The pH-dependency of the H^+-transports through *E. coli* membrane vesicles containing SF_OF_1 (red circle), SF_OEF_1 (green triangle) and EF_OF_1 (blue diamond). Relative H^+-translocations are indicated as the ratio to the maximal quenching of SF_OF_1, SF_OEF_1, and EF_OF_1 at pH 5.5, 6.0, and 6.5, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Since the membrane vesicles tested were inverted (inside-out), pH of the buffer corresponds to that of the cytoplasm. As described above, membrane vesicles with SF_OF_1 transported H^+ inside the vesicles efficiently at acidic pH. Furthermore, the quenching extents were not affected by the pH inside the vesicles, corresponding to that of the medium of *S. mutans* cells (data not shown). These results suggested that enzymes in *S. mutans* plasma membranes transport H^+ outside the cell when the cytoplasm becomes acidic at pH 6.5 or even lower.

Membranes with SF_OF_1 showed very low fluorescence quenching at pH 7.0 compared with those of EF_OF_1 (Fig. 3B, D and E), although they have similar ATPase activity (Fig. 2B). Western blotting confirmed that the SF_1 portion was not released from the membranes even after incubation at pH 7 (data not shown). Thus, ATP hydrolysis in SF_1 did not couple efficiently to H^+-translocation through F_O at neutral pH. These results suggest that the proton secretion of *S. mutans* is physiologically low when its cytoplasmic pH is neutral. This result may be consistent with little expression of the F_OF_1 genes in neutral conditions, as suggested previously [10,11].

H^+-translocation of the membranes having hybrid SF_OEF_1 was observed at pH 5.5–6.5 but not at $\geqslant$7, similar to those of SF_OF_1; although the extent of H^+-transport at pH 5.5 was reduced (Fig. 3C and E), possibly due to the low ATPase activity of SF_OEF_1 at acidic pH (Fig. 2B). A similar amount of EF_1 was found in membranes treated at pH 6 or 7. These results suggest that pH dependencies of ATPase and H^+-transport were due to F_1 and F_O portions, respectively. These findings prompted us to study the domains of F_O responsible for the pH dependence of ATP-driven H^+-transport.

3.4. Mutational analysis of the c subunit of SF_OF_1

Since the *c* subunit was forming a proton channel with subunit *a*, we focused on the residue responsible for H^+-transport in this subunit. The amino acid sequence of the *S. mutans c* subunit was aligned with those of various bacteria and revealed that *S. mutans* Glu53 located in TM2 corresponded to *E. coli* Asp61 forming H^+-pathway [3,14] (Fig. S4). Thus, we replaced each residue between Val49 and Val58 with the corresponding *E. coli* residue (Fig. 4). We also substituted Ala17, Glu20, and a five-amino acid segment (Val16–Glu20) in TM1 by the *E. coli* residues or segment, which is adjacent to Glu53 in the predicted hairpin structure (Fig. 4A).

The amounts of the β and *a* subunits (data not shown) and ATPase activities (Table S1) of the membranes were not significantly changed by those amino acid substitutions. Replacements of Glu53 and four other residues (Ala50, Ile52, Thr55, and Phe56) in TM2 abolished H^+-transport at pH 6 (Fig. 4B). These results

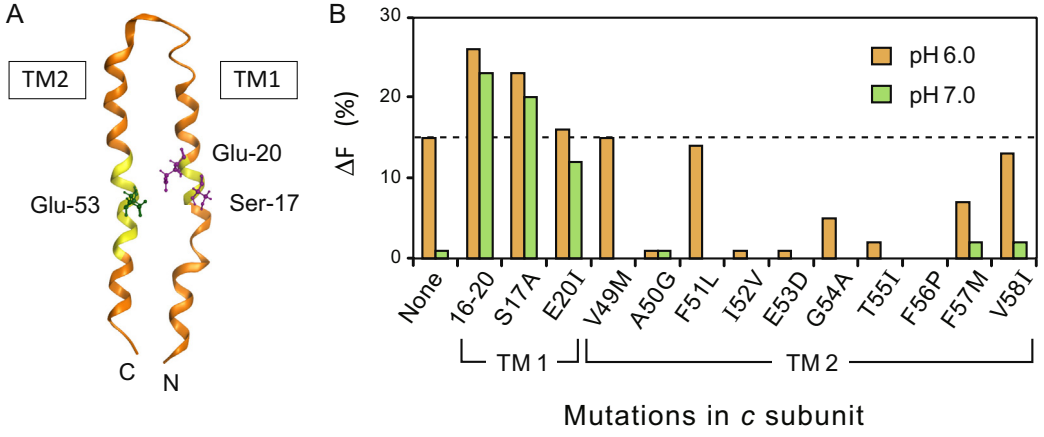

Fig. 4. Effects of mutations in the SF_OF_1 *c* subunit on ATP-driven H^+-transport through membrane. (A) Homology modeling of the *S. mutans c* subunit tertiary structure. The ribbon model of *S. mutans* subunit was predicted based on the crystal structure of *S. platensis c*-oligomer (PDB: 2WIE) [20]. The residues of Ser17, Glu20, and Glu53 were shown by stick-and-ball models. The regions introduced mutations are shown in yellow. (B) Effects of *c* subunit mutations in SF_OF_1 on ACMA fluorescence quenching at pH 6 and 7. The mutation sites in TM1 and TM2 are indicated. Replacement of the 5-residue segment between Val16 and Glu20 in TM1 (*S. mutans* VSLGE → E. *coli*, AAIGI) is indicated as 16–20. Quenching extents by ATP-driven H^+-transport at pH 6.0 and 7.0 are shown with orange and green bars, respectively. A dashed-line indicates the quenching extent with SF_OF_1 at pH 6.0. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

suggested the importance of Glu53 and the adjacent region to form an H^+-pathway, consistent with our previous results showing that the hybrid F_OF_1 (*c* subunit, *S. mutans*; other subunits, *E. coli*) could not grow by oxidative phosphorylation when a Glu53Gln substitution was introduced [14]. The conservation of Glu/Asp at this position suggests a role of Glu53 carboxyl group as part of the H^+-pathway [3]. Thus, according to the proposed H^+-transporting mechanism [2], the H^+ provided by the cytoplasmic half channel bound to the carboxyl group of Glu53, which should be released at the periplasmic half channel after a H^+-carrying *c*-ring rotation, results in H^+ efflux. Although the pK_a value for the homologous Asp61 in *E. coli* F_OF_1 was estimated at approximately 7 [24], the value for *S. mutans* Glu53 should be lower in order to release H^+ into acidic environment.

The membranes with SF_O transport H^+ at pH 6, but not at 7, as shown above (Fig. 3), consistent with the role of the enzyme in *S. mutans*. However, amino acid replacements in TM1 altered the pH dependency, and membranes became capable of H^+ transport at pH 7 (Fig. 4B). ATPase activities of the mutant membranes containing Ser17Ala, Glu20Ile, and the five residues replacement between Val16 and Glu20 at pH 7 were 94%, 88%, and 80% of the wild type, respectively (Table S1). Thus, ion transports in neutral pH were not caused by the stimulation of ATPase activities. In the *E. coli c* subunit, Ala24Asp or Ile28Glu mutation in TM1 inhibited active and passive H^+-transports at alkaline pH [18,19]. The negatively charged carboxyl group close to the essential Asp61 was considered to affect the pH-dependency [18]. It was also suggested that the carboxyl group of *S. mutans* Glu20, corresponding to position Ile28 of *E. coli*, possibly affected the H^+-transport in response to pH [19].

Therefore, our results suggested that at least Ser17 and Glu20 residues in the *S. mutans* were probably involved in protonation/deprotonation of the Glu53 at acidic pH.

3.5. Roles of the residues in TM1 for H^+-transport at acidic pH

The tertiary structures of *c*-rings from various H^+-transporting F_OF_1 have been reported [20–23]. Thus, we carried out homology modeling of the *S. mutans c* subunit based on the *S. platensis* subunit. Residues of the *S. platensis c* subunit were substituted by those of the *S. mutans* subunit and followed by energy minimization. The obtained structure predicted that the Glu20 side-chain faces that of the Glu53 (Fig. 4A), and the distance between two carboxyl carbons of the side-chains is approximately 5.4–5.7 Å. It is consistent that the pK_a value of the Glu53 was reduced by the negative charges located nearby [14,19], and thus the *S. mutans* F_OF_1 could transport H^+ at acidic pH in the cytoplasm (Fig. 3). The effect of the Ser17Ala mutation is possibly due to the indirect conformational change of Glu20: the hydroxyl group of Ser17 formed a hydrogen bond with the main-chain of Val13, as predicted by the model (Fig. 4A). Thus, Ser17Ala replacement could alter a local conformation around Glu20, moving its side-chain away from Glu53.

The F_OF_1 of *S. mutans* should function physiologically as a H^+-pump, but not as an ATP synthase because this anaerobic bacterium has no respiratory chain or other apparent proton pumps to drive ATP synthesis with F_OF_1 [25]. Because the ATPase activity and ATP-driven H^+-transport were observed in membranes expressing SF_OF_1, we tested their ATP synthesis. However, *E. coli* cells with assembled SF_OF_1 could not grow by oxidative phosphorylation (Table 1), although the *S. mutans c* subunits complemented *E. coli* lacking the corresponding gene [14]. On the other hand, *E. coli* H^+-ATPase can synthesize and hydrolyze ATP coupling with the electrochemical H^+ gradient.

These results may suggest that the *S. mutans* H^+-ATPase could not synthesize ATP possibly due to the intrinsic lack of ATP synthase activity. Further studies are being planned to understand this result. It will be of interest to study the roles of H^+-ATPase in other anaerobic bacteria, which function in establishing the electrochemical H^+ gradient.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.025.

References

[1] M. Futai, G.H. Sun-Wada, Y. Wada, Proton translocating ATPase: including unique enzymes coupling catalysis and proton translocation through mechanical rotation, in: M. Futai, Y. Wada, J. Kaplan (Eds.), Handbook of ATPases: Biochemistry, Cell Biology, Pathology, Wiley-VCH Verlag, Weinheim Germany, 2004, pp. 237–260.

[2] R.H. Fillingame, C.M. Angevine, O.Y. Dmitriev, Mechanics of coupling proton movements to *c*-ring rotation in ATP synthase, FEBS Lett. 555 (2003) 29–34.

[3] M. Maeda, ATP synthases: bioinformaticts based insights into how their electrochemically driven motor comprised of subunits *a* and *c* might serve as a drug target, J. Bioenerg. Biomembr. 40 (2008) 117–121.

[4] M. Tanabe, K. Nishio, Y. Iko, et al., Rotation of a complex of the γ subunit and *c* ring of *Escherichia coli* ATP synthase. The rotor and stator are interchangeable, J. Biol. Chem. 276 (2001) 15269–15274.

[5] K. Nishio, A. Iwamoto-Kihara, A. Yamamoto, et al., Subunit rotation of ATP synthase embedded in membranes: *a* or β subunit rotation relative to the *c* subunit ring, Proc. Natl. Acad. Sci. USA 99 (2002) 13448–13452.

[6] W.J. Loesche, Role of *Streptococcus mutans* human dental decay, Microbiol. Rev. 50 (1986) 353–380.

[7] R.G. Quivey Jr., W.L. Kuhnert, K. Hahn, Genetics of acid adaptation in oral streptococci, Crit. Rev. Oral Biol. Med. 12 (2001) 301–314.

[8] S.G. Dashper, E.C. Reynolds, Lactic acid excretion by *Streptococcus mutans*, Microbiology 142 (1996) 33–39.

[9] S.V.W. Sutton, R.E. Marquis, Membrane-associated and solubilized ATPases of *Streptococcus mutans* and *Streptococcus sanguis*, J. Dent. Res. 66 (1987) 1095–1098.

[10] W.L. Kuhnert, G. Zheng, R.C. Faustoferri, et al., The F-ATPase operon promoter of *Streptococcus mutans* is transcriptionally regulated in response to external pH, J. Bacteriol. 186 (2004) 8524–8528.

[11] A.C. Len, D.W.S. Harty, N.A. Jacques, Stress-responsive proteins are upregulated in *Streptococcus mutans* during acid tolerance, Microbiology 150 (2004) 1339–1351.

[12] K. Andries, P. Verhasselt, J. Guillemont, et al., A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*, Science 307 (2005) 223–227.

[13] E. Segala, W. Sougakoff, A. Nevejans-Chauffour, et al., New mutations in the mycobacterial ATP synthase: new insights into the binding of the diarylquinoline TMC207 to the ATP synthase *c*-ring structure, Antimicrob. Agents Chemother. 56 (2012) 2326–2334.

[14] M. Araki, K. Hoshi, M. Fujiwara, et al., Complementation of the F_O *c* subunit of *Escherichia coli* with that of *Streptococcus mutans* and properties of the hybrid F_OF_1 ATP synthase, J. Bacteriol. 195 (2013) 4873–4878.

[15] A. Iwamoto, H. Omote, H. Hanada, et al., Mutations in Ser174 and the glycine-rich sequence (Gly149, Gly150, and Thr156) in the β subunit of *Escherichia coli* H^+-ATPase, J. Biol. Chem. 266 (1991) 16350–16355.

[16] S.D. Dunn, D.J. Chipriano, P.A. Del Rizzo, ATP synthase stalk subunits *b*, δ and ε: structures and functions in energy coupling, in: M. Futai, Y. Wada, J. Kaplan (Eds.), Handbook of ATPases: Biochemistry, Cell Biology, Pathology, Wiley-VCH Verlag, Weinheim Germany, 2004, pp. 311–338.

[17] A. Hasona, K. Zuobi-Hasona, P.J. Crowley, et al., Membrane composition changes and physiological adaptation by *Streptococcus mutans* signal recognition particle pathway mutants, J. Bacteriol. 189 (2007) 1219–1230.

[18] Y. Zhang, R.H. Fillingame, Essential aspartate in subunit *c* of F_1F_0 ATP synthase. Effect of position 61 substitutions in helix-2 on function of Asp24 in helix-1, J. Biol. Chem. 269 (1994) 5473–5479.

[19] P.C. Jones, Introduction of a carboxyl group in the first transmembrane helix of *Escherichia coli* F_1F_O ATPase subunit *c* and cytoplasmic pH regulation, J. Bacteriol. 183 (2001) 1524–1530.
[20] D. Pogoryelov, O. Yildiz, J.D. Faraldo-Gómez, et al., High-resolution structure of the rotor ring of a proton-dependent ATP synthase, Nat. Struct. Mol. Biol. 16 (2009) 1068–1073.
[21] M. Vollmar, D. Schlieper, M. Winn, et al., Structure of the c_{14} rotor ring of the proton translocating chloroplast ATP synthase, J. Biol. Chem. 284 (2009) 18228–18235.
[22] L. Preiss, O. Yildiz, D.B. Hicks, et al., A new type of proton coordination in an F_1F_O-ATP synthase rotor ring, PLoS Biol. 8 (2010) e1000443.
[23] J. Symersky, V. Pagadala, D. Osowski, et al., Structure of the c_{10} ring of the yeast mitochondrial ATP synthase in the open conformation, Nat. Struct. Mol. Biol. 19 (2012) 485–491.
[24] F.M. Assadi-Porter, R.H. Fillingame, Proton-translocating carboxyl of subunit *c* of F_1F_O H^+-ATP synthase: the unique environment suggested by the pKa determined by ^1H NMR, Biochemistry 34 (1995) 16186–16193.
[25] D. Ajdić, W.M. McShan, R.E. McLaughlin, et al., Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen, Proc. Natl. Acad. Sci. USA 99 (2002) 14434–14439.