ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science **2017** Vol. 27. No. 1. 15~22 DOI : https://doi.org/10.5352/JLS.2017.27.1.15

pH Stress Alters Cytoplasmic Membrane Fluidity and *atp*B Gene Expression in *Streptococcus mutans*

Chul Min Cho, Seung Il Jung, Myung Sup Kim, Sae A Lee and Jung Sook Kang*

Department of Oral Biochemistry and Molecular Biology, School of Dentistry, Pusan National University, Yangsan 50612, Korea

Received October 25, 2016 / Revised December 15, 2016 / Accepted December 22, 2016

Streptococcus mutans (*S. mutans*), which plays a major role in the etiology of human dental caries, is able to tolerate exposure to acid shock in addition to its acidogenicity. We investigated the effects of pH stress on membrane fluidity, activities and expression levels of F-ATPase, and proton permeability in *S. mutans*. Using 1,6-diphenyl-1,3,5-hexatriene, we observed membrane ordering at pH 4.8 and pH 8.8. The ordering effects were larger at pH 4.8 in cytoplasmic membranes isolated from *S. mutans* (CMSM). Increasing pH resulted in a decrease in the activities and expression levels of F-ATPase. The proton permeability was decreased at both acidic and alkaline pHs, and the lowest permeability was observed at pH 4.8. The lower permeability at pH 8.8 than pH 6.8 is likely to be caused by the decreased proton influx due to the decreased CMSM fluidity. In addition, it seems to be evident that extremely low permeability at pH 4.8 was caused by the decreased proton influx due to the decreased CMSM fluidity as well as the increased proton efflux due to the increased activity and expression level of F-ATPase. It is likely that CMSM fluidity and F-ATPase activity are two major key factors that determine proton permeability in *S. mutans*. We suggest that CMSM fluidity plays an important role in the determination of proton permeability, which sheds light on the possibility of using nonspecific membrane fluidizers, e.g., ethanol, for anti-caries purposes.

Key words : *atp*B gene expression, membrane fluidity, pH stress, proton permeability, *Streptococcus mutans*

Introduction

Today, dental caries remains one of the most common diseases afflicting humans throughout the world. Although 200-300 bacterial species have been found associated with dental biofilm, only the presence of *Streptococcus mutans* (*S. mutans*) has been consistently linked with the formation of human dental caries [17]. This gram-positive bacterium is able to demineralize dental enamel by generation of a localized acidic environment in plaque by rapid glycolysis of dietary sugars to lactic acid. In addition to its acidogenicity, *S. mutans* is able to tolerate exposure to continual and rapid cycles of acid shock [10]. It has been known that acid tolerance of *S. mutans* is primarily based on the presence of the membrane-bound, proton-pumping F-ATPase that can maintain the intracellular pH [5, 7, 11, 16, 25]. However, very few results have been reported for the expression of *S. mutans* F-ATPase in response to external pH.

The integrity of the bacterial cytoplasmic membrane is essential in maintaining the viability of cells and their metabolic functions particularly under stress. Bacteria actively adjust membrane fluidity through changes in lipid composition in response to alterations in temperature, pressure, ion concentrations, nutrient availability, xenobiotics and pH [19]. Unsaturated fatty acids (UFAs) have crucial roles in maintenance of membrane structure and function in many groups of aerobic bacteria. UFAs are key molecules in the regulation of cellular membrane fluidity. Quivey et al. [23] showed that *S. mutans* grown at pH 5.0 had increased levels of mono-unsaturated fatty acids and longer chain fatty acids than cells grown at pH 7.0. Fozo and Quivey [8] reported that as the growth pH is lowered there is an incremental change from short-chained, saturated fatty acids ($C_{14:0}$ and $C_{16:0}$) to long-chained, mono-unsaturated fatty acids ($C_{18:1}$ and $C_{20:1}$). However, no attempt has been made yet to determine membrane fluidity alterations in response to environmental acidification in *S. mutans*.

Proton permeability is an important factor permitting cells to maintain the proton-motive force (pmf) for vital energy

***Corresponding author**
Tel : +82-51-510-8226, Fax : +82-51-510-8228
E-mail : jsokang@pusan.ac.kr

transducing processes. pmf is essential for *S. mutans* activity. In addition to its high-affinity phosphotransferase sugar transport system, *S. mutans* has the second, low-affinity sugar uptake system, which is activated by pmf [12]. This is why *S. mutans* can survive under acidic conditions which are unfavorable to other bacteria. It has been shown that the proton permeability correlates with the physical state of the membrane; in particular, the permeability increases with increasing membrane fluidity [26].

The aims of this research are threefold: (1) to study the effects of pH stress on the fluidity of the cytoplasmic membranes isolated from *S. mutans* (CMSM); (2) to check the expression levels of *atp*B gene of *S. mutans* under pH stress; and (3) to examine the relationship between proton permeability, CMSM fluidity, and F-ATPase. We observed CMSM ordering at both acidic and alkaline pHs, however, the ordering effects were larger at acidic pH. Our results suggest that CMSM fluidity plays a pivotal role in the viability of *S. mutans* under pH stress.

Materials and Methods

Materials

S. mutans UA159 (#700610), a proven cariogenic dental pathogen [1], was purchased from ATCC (Manassas, VA, USA). Brain Heart Infusion (BHI) medium from Becton, Dickinson & Company (Franklin, NJ, USA); mutanolysin and phenylmethylsulfonyl fluoride (PMSF) from Sigma-Aldrich (St. Louis, MO, USA); DPH and Superscript™ First-Strand Synthesis System for RT-PCR from Life Technologies (NY, USA); and RNAProtect Bacteria Reagent and the RNeasy mini kit from Quiagen (Hilden, Germany).

Bacterial Culture

S. mutans was grown in 50 ml BHI medium for 16 hr at 37°C. The culture was used to inoculate 1 l flasks of prewarmed BHI medium and incubated at 37°C until an absorbance reading of 0.7 at 600 nm was reached. The cultivation was done at either pH 4.8, 6.8 or 8.8. During cultivation, the bacteria exhibited a tendency to acidify the cultivation medium. So, the pH value was continuously monitored and adjusted by a stepwise addition of 500 mM KOH, if necessary. The growth curves were measured every 15 minutes. The growth rates (c) were calculated as $c = \Delta \log_2(OD_{420})/\Delta t$ where OD_{420} is the optical density at 420 nm, and t is time.

Preparation of Bacterial Cytoplasmic Membranes (CMSM)

Unless otherwise specified all steps were carried out at 0-4°C. Cells were washed once in 0.1 M phosphate-buffered saline (PBS), twice in 5 mM ethylenediaminetetraacetic acid (EDTA), and once in distilled water. Cells were lysed by treatment with mutanolysin. The enzyme was added to a final concentration of 20 μg/ml and incubated for 1 hr at 37°C. And then the lysed cells were broken with glass beads. In all cases, 1 mM PMSF was added to inhibit the protease activity. The cell wall-membrane complexes were collected at 31,000× g for 1 hr after treatment of the lysates with ribonuclease and deoxyribonuclease (50 μg/ml each). They were washed extensively with 30 mM Tris-HCl containing 5 mM $MgCl_2$ and layered on a one-step gradient of 56% (w/v) sucrose-3 mM EDTA over a cushion of 70% (w/v) sucrose-3 mM EDTA. The gradient was centrifuged in a Beckman Optima™ LE-80K ultracentrifuge (Brea, CA, USA), using a SW28 rotor, at 40,000× g for 45 min. The material at the 56% (w/v) and 70% (w/v) sucrose/EDTA interface was collected with a Pasteur pipette, diluted with PBS, and pelleted by centrifugation at 31,000× g for 45 min. The pellet was washed extensively with PBS, divided into small aliquots, and stored at -70°C.

Cytoplasmic contamination was assessed by assaying the presence of glucokinase by the method of Porter and Chassy [22]. Total protein contents of membranes were determined by the method of Lowry et al. [18].

Fluorescence Measurements

Aliquots of CMSM were suspended in phosphate buffer (pH 4.8, 6.8 or 8.8) at a protein concentration of 50 μg/ml. Stock solution of DPH was made in dimethylformamide. The concentration of DPH was 1 μM and determined using molar extinction coefficients of 88,000 $M^{-1}cm^{-1}$ at 350 nm. For incorporation of DPH, aliquots of stock solutions were added to the membrane suspension, and the mixture was incubated at 37°C for 30 min in the dark. The excitation wavelength of DPH was 360 nm while emission was observed at 430 nm. Fluorescence measurements were carried out at 37°C using a Cary Eclipse fluorescence spectrophotometer (Varian Inc., Palo Alto, CA, USA).

The anisotropy of DPH is calculated by

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

where the intensity of the components of the fluorescence

that were parallel (I_{VV}) and perpendicular (I_{VH}) to the direction of the vertically polarized excitation light was determined by measuring the emitted light through polarizers oriented vertically and horizontally. The grating correction factor G is given by the ratio of the fluorescence intensities of the vertical (I_{HV}) to horizontal (I_{HH}) components when the exciting light is polarized in the horizontal direction.

F-ATPase assay

CMSM were added to Tris-maleate buffer (pH 7.0) with 10 mM $MgSO_4$, and the mixtures were warmed to 37°C. The ATPase reaction was initiated by addition of ATP (pH 7.0) and incubated at 37°C for 15 min. The reaction was stopped by adding 10% trichloroacetic acid. As the negative control, we did the same procedure, but added TCA at time zero in order to inactivate the enzyme first. The released phosphate was determined using the method of Bencini et al. [6].

RNA extraction and cDNA synthesis

Cells were harvested via centrifugation, resuspended and incubated in RNAProtect Bacteria Reagent at room temperature, and then centrifuged at 4,000× g for 10 min. The pellets were resuspended in 200 μl lysis buffer containing 20 mM Tris-HCl (pH 8.0), 20 mg/ml lysozyme, 1,000 U/ml mutanolysin, and 60 mAU/ml proteinase k. They were incubated at 37°C for 45 min, followed by two cycles of sonication for 15 sec twice.

RNA extractions were carried out with the RNeasy mini kit according to the manufacturer's instructions. The concentration of RNA was spectrophotometrically measured as A_{260}, with its purity confirmed by the value of A_{260}/A_{280}.

Total intact RNA obtained from *S. mutans* UA159 was reverse-transcribed into cDNA using a Superscript™ First-Strand Synthesis System for RT-PCR according to the manufacturer's instructions.

Real-time PCR

The resulting cDNA and negative controls were amplified by ABI PRISM 7700 real-time PCR system with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The primers were: 5′-AGGCCGACTTTACCACCTTT-3′ (forward) and 5′-TCCGGTTGGAAAAGAAACAC-3′ (reverse) for *atp*B; 5′-TTTGACGGTCTCCGATAACC-3′ (forward) and 5′-TGTTGATGGTCTGGGTGAAA-3′(reverse) for *atp*D; and 5′-AAGCAACGCGAAGAACCTTA-3′ (forward) and 5′-GTCTCGCTAGAGTGCCCAAC-3′(reverse) for 16S rRNA.

Each reaction tube contained 20 μl of reaction mixture, including 1X SYBR Green PCR Master Mix, cDNA, template, and 2 μl of 10 pM forward and reverse primers. The cycle profile was as follows: 1 cycle at 50°C for 5 min and initial denaturation at 95°C for 10 min, followed by 40 cycle amplification consisting of denaturation at 95°C for 15 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s.

The relative expression was calculated by normalizing the mRNA of the *atp*B and *atp*D genes with that of the 16S rRNA gene, which served as the reference gene.

Proton flux assay

The proton permeability was assessed using the procedures described previously [4, 21]. Cells were harvested by centrifugation and washed in 5 mM $MgCl_2$. They were suspended at a cell density of 5 mg/ml in 20 mM potassium phosphate buffer (either pH 4.8, 6.8, or 8.8) for 1 hr to deplete the endogenous catabolites. The cells were centrifuged again and suspended at a cell density of 25 mg/ml in 150 mM KCl + 1 mM $MgCl_2$. The suspensions were then titrated to pH 4.6 with 0.5 M HCl until the pH value stabilized, as indicated by no pH change for at least 2 min. Then 0.5 M HCl was added to decrease the pH values in approximately 0.4 units. The subsequent increase in pH associated with proton movements across the cell membrane into the cytoplasm was monitored using a glass electrode. 10% (v/v) butanol was added to the suspensions at 80 min to disrupt the cell membrane.

Statistical analysis

The data are presented as mean ± SEM. Student's *t*-test was performed to determine the significant difference. Values were considered statistically significant when P value was <0.05. The statistical analyses were performed using IBM SPSS Statistics 23 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Effects of pH stress on the fluidity of CMSM

First of all, we compared the growth rates of *S. mutans* cultured under pH stress. The measured growth rates, calculated as $c = \Delta\log_2(OD_{420} \times 1{,}000)/\Delta t$, are depicted in Fig. 1A. It is seen that pH stress did not allow a full recovery of

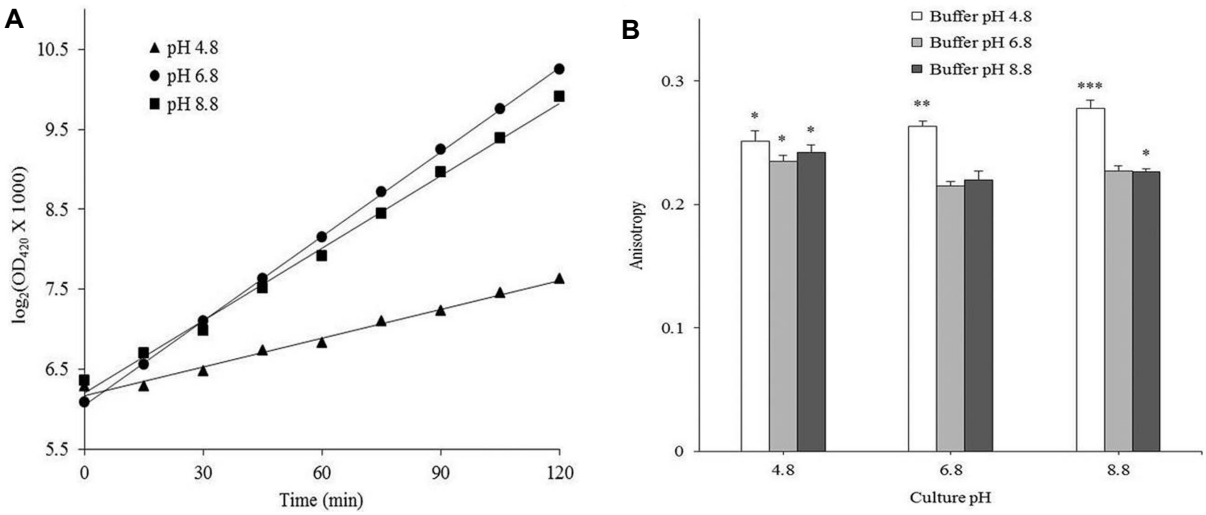

Fig. 1. Cell growth and cytoplasmic membrane fluidity. (A) Growth curves of *S. mutans* UA159 grown at pH 4.8 (filled triangle), pH 6.8 (filled circle) and pH 8.8 (filled square). The growth rates (c) were calculated as $c = \Delta\log_2(OD_{420})/\Delta t$ where OD_{420} is the optical density at 420 nm and t is time. Each point represents an average of three independent experiments. (B) Effects of pH stress on the anisotropy (*r*) of DPH in the cytoplasmic membranes isolated from *Streptococcus mutans* (CMSM). The anisotropy values were calculated using the equation in Materials and Methods. Each bar represents the mean ± SEM of 5 determinations. **p*<0.05, ***p*<0.01, ****p*<0.001 vs. control (culture pH 6.8 – buffer pH 6.8).

the growth rate measured at optimum pH of 6.8. Compared to pH 6.8 (c=0.0352 min^{-1}), the growth rates were reduced to 0.0119 and 0.0301 min^{-1} for *S. mutans* cultured at pH 4.8 and 8.8, respectively.

We measured CMSM fluidity using DPH anisotropy because DPH is the most widely used membrane probe which distributes evenly throughout the hydrophobic regions of membrane lipids. To evaluate the effect of pH, we manipulated the pH of both culture media and measuring phosphate buffer. In other words, CMSM was isolated from *S. mutans* grown in BHI media differing in pH (either 4.8, 6.8 or 8.8) and suspended in phosphate buffer (either pH 4.8, 6.8 or 8.8). Fig. 1B shows the effects of pH stress on the anisotropy values (*r*) of DPH in CMSM. At the same culture and buffer pHs, the order of anisotropy values was: pH 4.8 > pH 8.8 > pH 6.8. Again, when the CMSM differing in culture pHs were exposed to phosphate buffer of optimum pH 6.8, the order of anisotropy was: culture pH 4.8 > culture pH 8.8 > culture pH 6.8. In addition, at all culture pHs, the order of anisotropy values was: buffer pH 4.8 > buffer pH 8.8 ≥ buffer pH 6.8. Taken together, these results indicate CMSM rigidification by pH stress, especially acidic pH since a decrease of the anisotropy of DPH reflects an increase of the rotational mobility. This indicates CMSM rigidification by both acidic and alkaline pHs, which is opposite to the prediction of membrane fluidization by Petrackova et al. [20]. It was inferred mainly from the increase in the amount of monounsaturated fatty acids in *S. mutans*. The fact that there was concomitant increase in the amount of long chain fatty acids was overlooked. In addition, in the analysis of fatty acids, Quivey et al. [23] and Fozo and Quivey [8] used lipid extracts from whole bacteria, instead of CMSM. So, it is possible that the change in the lipid composition may not reflect exactly that of CMSM. The possibility of the presence of intracellular organelle lipids cannot be excluded. Membrane ordering at acidic pH is consistent with the results obtained previously using *Oenococcus oeni* [19] and *Bacillus subtilis* [20]. However, *Bacillus subtilis* showed membrane fluidization by alkaline pH [20], which does not coincide with our result. We assume that membrane effects of pH are different depending on the organisms.

Among the nine combinations of culture and buffer pHs, the CMSM with culture and buffer pH 6.8 were the most fluid. On the other hand, CMSM were the most ordered at culture pH 8.8 and buffer pH 4.8. When the CMSM of culture pH 4.8 were exposed to pH 8.8 buffer, the extent of CMSM ordering was much smaller. This may be due to the acid tolerance of *S. mutans*. Buffer pH resulted in a comparable fluidity change to culture pH. In view of the fact that CMSM are composed of not only lipids but also proteins, it is highly probable that the pH changes result in a significant change in protein conformations as well.

Effects of pH stress on the F-ATPase activities and the expression levels of the *atp*B gene in *S. mutans*

Although recent studies have shown that a large proportion of the *S. mutans* genome is required for acid tolerance [2, 3, 24], the F-ATPase is still considered as one of the key components of the acid tolerance response. The F-ATPase activity is critical for establishing and maintaining pmf across CMSM. The F-ATPase activity might be influenced by CMSM ordering under pH stress. The order of F-ATPase activity was: pH 4.8 > pH 6.8 > pH 8.8 (Fig. 2A). Our results agree with those of others who showed increased levels of F-ATPase activity in acidic environment [5, 7, 11, 16, 25]. The lowest F-ATPase activity was obtained with CMSM from *S. mutans* cultured at pH 8.8.

The F-ATPase operon promoter [13, 15] and *atp* genes [2, 9] of *S. mutans* were transcriptionally upregulated at acidic pH. We checked the relative quantities of mRNA expression of the *atp*B gene which encodes the catalytic β-subunit of F-ATPase (Fig. 2B). The mRNA expression levels were: pH 4.8 > pH 6.8 > pH 8.8. The *S. mutans* F-ATPase was transcriptionally upregulated at pH 4.8 and downregulated at pH 8.8. Similar results were obtained for the expression of *atp*D (regulatory α-subunit gene). Increasing pH resulted in a decrease in the activities and the expression levels of F-ATPase.

Relationship between fluidity, proton permeability and F-ATPase activity

Petrackova et al. [20] reported decreased proton permeability at both acidic and alkaline pH in *Bacillus subtilis*. Hence, it is highly probable that the above rigidification is related to CMSM's permeability to protons and/or other ions. We performed an acidic pH-jump assay in intact cells adapted to pH 4.8, 6.8 and 8.8. Fig. 3A shows the results of the proton flux assay. It can be seen that, following the initial pH pulse, the pH of the cell suspension starts to gradually increase as a consequence of cellular proton uptake. The rate of the pH increase was found to be the highest for *S. mutans* cultivated at pH 6.8 and the lowest at pH 4.8.

We examined the relationship between cytoplasmic membrane fluidity, F-ATPase activity and proton permeability in *S. mutans*. The upper model of Fig. 3B shows the proton flux in *S. mutans* when we consider only F-ATPase activity. Without considering CMSM fluidity, equal amounts of protons will pass through CMSM at all three pHs. Under these circumstances, the order of proton permeability will be: pH 8.8 > pH 6.8 > pH 4.8. Of course, this was not the case. Under our experimental conditions, *S. mutans* grown at pH 6.8 showed the highest proton permeability (Fig. 3A). The lower permeability at pH 8.8 than pH 6.8 is likely to be caused by decreased proton influx due to decreased CMSM fluidity. The decreased proton influx seems to offset the effect of the lowest proton efflux. In addition, very low permeability of *S. mutans* grown at pH 4.8 can be better explained when considering CMSM fluidity together (lower model in Fig. 3B). It seems to be evident that extremely low permeability at pH 4.8 was caused by decreased proton influx due to decreased CMSM fluidity as well as increased proton efflux due to the increased activity and expression level of F-ATPase. Thus, it is likely that CMSM fluidity and F-ATPase activity are two major key factors that determine

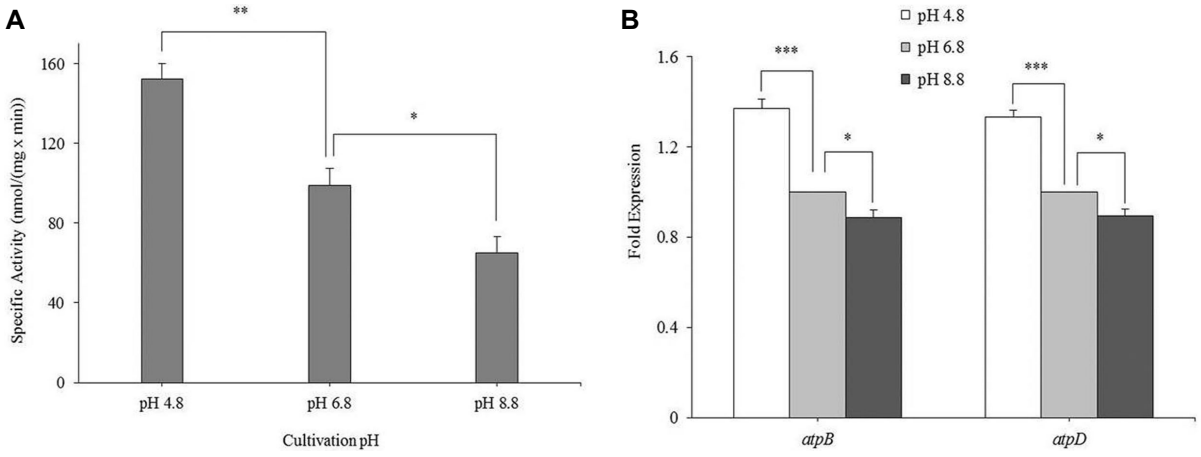

Fig. 2. Activities and expression levels of F-ATPase. (A) Specific activities of F-ATPase in CMSM. The ATPase reaction was initiated by addition of ATP. (B) Relative mRNA levels for the *atp*B and *atp*D genes of *S. mutans* UA159. The relative values were obtained by normalizing the *atp*B and *atp*D mRNA with expression of 16S rRNA gene. The Data are reported as mean ± SEM of triplicate analysis of three preparations. *p<0.05, **p<0.01, ***p<0.001.

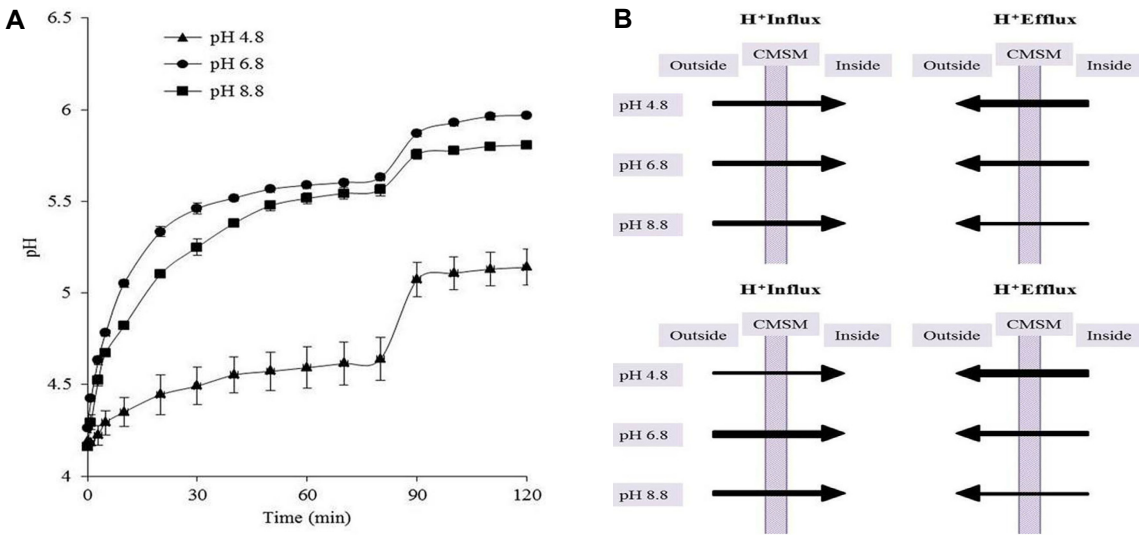

Fig. 3. Proton permeability and two hypothetical models. (A) Proton flux assay of intact *S. mutans* UA159 grown at pH 4.8 (filled triangle), pH 6.8 (filled circle) and pH 8.8 (filled square). 10% (v/v) butanol was added at 80 min to disrupt the cell membranes. Data are reported as mean ± SEM of triplicate analysis of three preparations. (B) Two hypothetical models of proton flux in *S. mutans* UA159. Proton efflux (right panels) depends on F-ATPase activity. The CMSM isolated from *S. mutans* adapted to pH 4.8 removes the largest number of protons out of the cells because of the highest activity. For the CMSM from cells grown at pH 8.8, the smallest number of protons is extruded. Suppose that proton influx (left panels) may or may not be dependent on membrane fluidity. We assume that protons enter the CMSM in uncharged, acid form. In the upper model, CMSM fluidity was not considered. At all pHs, equal amount of protons pass through CMSM. The lower model shows the proton flux through CMSM when we consider both membrane fluidity and F-ATPase activity. Depending on the fluidity, different amounts of protons pass through CMSM. For convenience, three different widths of the lines of arrows were arbitrarily chosen. They do not show the exact difference among them.

proton permeability in *S. mutans*.

The suggestion that CMSM fluidity plays an important role in determination of proton permeability sheds light on the possibility of the use of nonspecific membrane fluidizers, e.g., ethanol, for anti-caries purposes. Ethanol might influence *S. mutans* viability, or, at least, be used as an adjunct to fluoride dentifrices and anti-plaque mouthrinses. However, it should be pointed out that we used *S. mutans* in suspensions. More convincing results can be obtained using *S. mutans* in biofilms which contain the extracellular polysaccharide matrix [14]. Additionally, only steady-state fluorescence measurements were performed. Time-resolved anisotropy decay will give us information about the rate and range of rotational motions of DPH within CMSM.

To our knowledge, this report is the first to examine CMSM fluidity and to elucidate the relationship between membrane fluidity, the activities and the expression levels of F-ATPase, and proton permeability in *S. mutans* under pH stress.

Acknowledgement

This work was supported by a 2-Years Research Grant of Pusan National University.

References

1. Ajdić, D., McShan, W. M., McLaughlin, R. E., Savić, G., Chang, J., Carson, M. B., Primeaux, C., Tian, R., Kenton, S., Jia, H., Lin, S., Qian, Y., Li, S., Zhu, H., Najar, F., Lai, H., White, J., Roe, B. A. and Ferretti, J. J. 2002. Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. *Proc. Natl. Acad. Sci. USA* **99,** 14434-14439.
2. Baker, J. L., Abranches, J., Faustoferri, R. C., Hubbard, C. J., Lemos, J. A., Courtney, M. A. and Quivey, R. G. 2015. Transcriptional profile of glucose-shocked and acid-adapted strains of *Streptococcus mutans*. *Mol. Oral Microbiol.* **30**, 496-517.
3. Baker, J. L., Faustoferri, R. C. and Quivey, R. G. 2016. Acid-adaptive mechanisms of *Streptococcus mutans*-the more we know, the more we don't. *Mol. Oral Microbiol.* doi: 10.1111/omi.12162.
4. Ban, S. H., Kim, J. E., Pandit, S. and Jeon, J. G. 2012. Influences of Dryopteris crassirhizoma extract on the viability, growth and virulence properties of *Streptococcus mutans*.

Molecules **17,** 9231-9244.
5. Belli, W. A. and Marquis, R. E. 1991. Adaptation of *Streptococcus mutans* and *Enterococcus hirae* to acid stress in continuous culture. *Appl. Environ. Microbiol.* **57,** 1134-1138.
6. Bencini, D. A., Shanley, M. S., Wild, J. R. and O'Donovan, G. A. 1983. New assay for enzymatic phosphate release: application to aspartate transcarbamylase and other enzymes. *Anal. Biochem.* **132,** 259-264.
7. Bender, G. R., Sutton, S. V. and Marquis, R. E. 1986. Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. *Infect. Immun.* **53,** 331-338.
8. Fozo, E. M. and Quivey, R. G. 2004. Shifts in the membrane fatty acid profile of *Streptococcus mutans* enhance survival in acidic environments. *Appl. Environ. Microbiol.* **70,** 929-936.
9. Gong, Y., Tian, X. L., Sutherland, T., Sisson, G., Mai, J., Ling, J. and Li, Y. H. 2009. Global transcriptional analysis of acid-inducible genes in *Streptococcus mutans*: multiple two-component systems involved in acid adaptation. *Microbiology* **155,** 3322-3332.
10. Gutierrez, J. A., Crowley, P. J., Cvitkovitch, D. G., Brady, L. J., Hamilton, I. R., Hillman, J. D. and Bleiweis, A. S. 1999. *Streptococcus mutans* ffh, a gene encoding a homologue of the 54kDa subunit of the signal recognition particle, is involved in resistance to acid stress. *Microbiology* **145,** 357-366.
11. Hamilton, I. R. and Buckley, N. D. 1991 Adaptation by *Streptococcus mutans* to acid tolerance. *Oral Microbiol. Immun.* **6,** 65-71.
12. Hamilton, I. R. and St, Martin, E. J. 1982. Evidence for the involvement of proton motive force in the transport of glucose by a mutant of *Streptococcus mutans* strain DR0001 defective in glucose-phosphoenopyruvate phosphotransferase activity. *Infect. Immun.* **36,** 567-575.
13. Hwang, G., Liu, Y., Kim, D., Sun, V., Aviles-Reyes, A., Kajfasz, J. K., Lemos, J. A. and Koo, H. 2016. Simultaneous spatiotemporal mapping of in situ pH and bacterial activity within an intact 3D microcolony structure. *Scientific Reports.* doi:10.1038/srep32841.
14. Koo, H., Falsetta, M. L. and Klein, M. I. 2013. The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. *J. Dent. Res.* **92,** 1065-1073.
15. Kuhnert, W. L., Zheng, G., Faustoferri, R. C. and Quivey, R. G. 2004. The F-ATPase operon promoter of *Streptococcus mutans* is transcriptionally regulated in response to external pH. *J. Bacteriol.* **186,** 8524-8528.
16. Lemos, J. A., Abranches, J. and Burne, R. A. 2005. Responses of cariogenic streptococci to environmental stresses. *Curr. Issues Mol. Biol.* **7,** 95-108.
17. Loesche, W. J. 1986. Role of *Streptococcus mutans* in human dental decay. *Microbiol. Rev.* **50,** 353-380.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265-275.
19. Mykytczuk, N. C., Trevors, J. T., Leduc, L. G. and Ferroni, G. D. 2007. Fluorescence polarization in studies of bacterial cytoplasmic membrane fluidity under environmental stress. *Prog. Biophys. Mol. Biol.* **95,** 60-82.
20. Petrackova, D., Becer, J., Svobodova, J. and Herman, P. 2010. Long-term adaptation of *Bacillus subtilis* 168 to extreme pH affects chemical and physical properties of the cellular membrane. *J. Membr. Biol.* **233,** 73-83.
21. Phan, T. N., Buckner, T., Sheng, J., Baldeck, J. D. and Marquis, R. E. 2004. Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. *Oral Microbiol. Immunol.* **19,** 31-38.
22. Porter, E. V. and Chassy, B. M. 1982. Glucokinase from *Streptococcus mutans*. *Methods Enzymol.* **90,** 25-30.
23. Quivey, R. G., Faustoferri, R., Monahan, K. and Marquis, R. 2000. Shifts in membrane fatty acid profiles associated with acid adapation of *Streptococcus mutans*. *FEMS Microbiol. Lett.* **189,** 89-92.
24. Quivey, R. G., Grayhack, E. J., Faustoferri, R. C., Hubbard, C. J., Baldeck, J. D., Wolf, A. S., MacGilvray, M. E., Rosalen, P. L., Scott-Anne, K., Santiago, B., Gopal, S., Payne, J. and Marquis, R. E. 2015. Functional profiling in *Streptococcus mutans*: construction and examination of a genomic collection of gene deletion mutants. *Mol. Oral Microbiol.* **30,** 474-495.
25. Quivey, R. G., Kuhnert, W. L. and Hahn, K. 2000. Adaptation of oral streptococci to low pH. *Adv. Microb. Physiol.* **42,** 239-274.
26. Zhang, G. J., Liu, H. W., Yang, L., Zhong, Y. G. and Zheng, Y. Z. 2000. Influence of membrane physical state on the lysosomal proton permeability. *J. Membr. Biol.* **175,** 53-62.

초록 : pH stress가 *Streptococcus mutans*의 형질막 유동성 및 *atp*B 유전자 발현에 미치는 영향

조철민 · 정승일 · 김명섭 · 이새아 · 강정숙*
(부산대학교 치의학전문대학원 구강생화학교실)

치아우식의 주원인균인 *Streptococcus mutans* (*S. mutans*)는 산 생성 뿐 아니라 산에 대한 탁월한 저항성을 나타낸다. 본 연구에서는 *S. mutans*가 pH stress에 노출될 때 형질막 유동성, F-ATPase 활성과 발현 및 양성자 투과성 변화와 그 상관관계를 규명하였다. *S. mutans*로부터 형질막을 분리한 후 1,6-diphenyl-1,3,5-hexatriene을 사용하여 pH stress가 형질막 유동성 변화에 미치는 영향을 측정하였다. pH 4.8과 pH 8.8에서 배양한 *S. mutans*는 pH 6.8에서 배양한 *S. mutans*에 비하여 형질막 유동성이 감소되었다. F-ATPase 활성과 발현은 pH 4.8에서 가장 높았고, pH 8.8에서 가장 낮았다. 양성자 투과성은 pH 4.8과 pH 8.8에서 모두 감소되었으며, 특히 pH 4.8에서의 감소가 컸다. F-ATPase 활성만으로 양성자 투과성이 결정된다면 pH 8.8에서 가장 높아야 하나 pH 6.8보다 감소하는 것은 형질막 유동성 감소에 기인된 양성자 세포내 유입 감소와 관련된 것으로 추정한다. 또한 pH 4.8에서 양성자 투과성이 아주 낮은 것은 높은 F-ATPase 활성에 의한 양성자 세포외 유출 증가 뿐 아니라 형질막 유동성 감소에 의한 양성자 세포내 유입 감소에 기인된 것으로 추정한다. 따라서 pH stress에 의한 형질막 유동성 감소는 *S. mutans*가 세포내 pH 를 유지하는데 중요한 역할을 하는 것으로 생각되며 에탄올을 포함하여 비특이적으로 세포막 유동성을 증가시키는 약물들은 항우식제에 활용될 수 있을 것으로 추정한다.