

Fluoride Inhibition of Proton-Translocating ATPases of Oral Bacteria

SCOTT V. W. SUTTON, GARY R. BENDER, AND ROBERT E. MARQUIS*

Departments of Microbiology and Immunology and of Dental Research, The University of Rochester, Rochester, New York 14642

Received 27 March 1987/Accepted 6 August 1987

The ATPases of isolated membranes of lactic acid bacteria were found to be inhibited by fluoride in a complex manner. Among the enzymes tested, that of *Streptococcus mutans* GS-5 was the most sensitive to fluoride, and the initial rate of hydrolysis of ATP was reduced 50% by approximately 3 mM fluoride. The enzyme of *Lactobacillus casei* ATCC 4646 was the most resistant, and about 25 mM fluoride was required for 50% inhibition. The response to fluoride appeared to involve reversible, noncompetitive inhibition during short exposure to low levels of fluoride and nonreversible inhibition at higher fluoride levels. In addition, kinetic studies of the effects of fluoride on the enzymes of membranes of *S. mutans* and *L. casei* indicated that reversible inhibition was at least partly overcome at high levels of either ATP or Mg. The effects of pH on fluoride inhibition of ATPases were markedly different from the effects of pH on inhibition of acid/base regulation of intact cells by fluoride. It appeared that formation of HF was not required for inhibition of the ATPases. F₁ ATPases isolated from the membranes by washing with buffers of low ionic strength proved to be less sensitive to fluoride than the membrane-associated F₁F₀ holoenzymes, and it was concluded that the F₀ or membrane sector of the holoenzyme is involved in fluoride inhibition.

Fluoride is considered to have played a major role in the reduction of dental caries in the past decades in the industrialized countries. It is added as an anticaries agent to a variety of vehicles, particularly drinking water and toothpastes. Current views of its anticaries action center on incorporation into the mineral phases of teeth with resultant reductions in acid solubilization and enhancement of remineralization. The early view of Bibby and van Kesteren (5) that the anticaries effect was related to antimicrobial actions was set aside for years because the low levels of fluoride found in saliva, ca. 5.5 μ M (21), were well below the levels needed for significant antimicrobial effects. However, Dawes et al. (7) found that dental plaque can concentrate fluoride to levels as high as about 2.5 μ mol/g (wet weight). Subsequent studies have confirmed these findings of fluoride concentration by plaque. Indeed, the level in plaque fluid after a rinse with 20 mM NaF solution has been found to rise to ca. 22 mM (21) and to remain at millimolar levels for long periods after exposure. In addition, fluoride in enamel of teeth can move into plaque during acid dissolution (15). The fluoride in plaque appears to be readily extracted with dilute acids (17). Overall, it seems that these high levels of fluoride in plaque cannot be ignored in relation to possible direct antimicrobial effects on plaque bacteria, although there is evidence that plaque bacteria may be able to adapt physiologically to function in the presence of initially inhibitory levels of fluoride (12).

The antimicrobial action of fluoride is itself complex. Fluoride affects many metabolic enzymes. Enolase is particularly sensitive, and inhibition of this enzyme can reduce the supply of phosphoenolpyruvate for use by the phosphotransferase system and so inhibit sugar uptake as well as glycolysis. Fluoride also inhibits processes such as glycogen synthesis (11) and peroxidase activity (22). Recently, the action of fluoride in dissipating Δ pH across the cell membrane has been demonstrated (8, 13), and fluoride has been

found to increase the proton conductivities of cell membranes (4), especially at the low pH values of carious dental plaque. The increases in transmembrane movements of protons caused by fluoride can be interpreted at least partly in terms of the weak-acid properties of fluoride, which has a pK_a of 3.15, and a carrier function for HF in bringing protons across the lipoidal barriers of the membrane from an acidified environment to a relatively alkaline cytoplasm. However, as described in this paper, in a previous short paper (16), and in a preliminary communication by Kashket (J. Dent. Res. 65:736, 1986), fluoride can also inhibit the proton-translocating ATPases of streptococcal cell membranes and may enhance net permeability of cells to protons by inhibiting proton transport out of the cell catalyzed by the enzymes.

MATERIALS AND METHODS

Membrane isolation. The bacterial strains used for membrane isolation were *Bacillus megaterium* ATCC 13632, *Lactobacillus casei* ATCC 4646, *Streptococcus faecium* ATCC 9790, *Streptococcus lactis* ATCC 19435, *Streptococcus milleri* ATCC 9895, *Streptococcus mutans* GS-5, *Streptococcus salivarius* ATCC 13419, and *Streptococcus sanguis* NCTC 10904. In a typical procedure for isolation of membranes, *S. mutans* GS-5 was grown at 37°C statically in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 2% (wt/vol) glucose and 20 mM DL-threonine. Cells were harvested by centrifugation when the culture had reached the late exponential stage of growth and the medium pH was about 5.2. The cells were washed twice with cold water, and cells from 1 liter of culture were suspended in 40 ml of osmotic buffer containing 0.5 g lysozyme and 2,000 U of mutanolysin (Sigma Chemical Co., St. Louis, Mo.). The suspension was sonicated twice for 15 s each time. The suspension was then added to 160 ml of warm (37°C) osmotic buffer and incubated with gentle shaking at 37°C for 2 h. Osmotic buffer contained 0.4 M sucrose, 2 mM MgSO₄, and 74 mM Tris (pH 7.5).

* Corresponding author.

The resulting suspension of spheroplasts was centrifuged at $9,000 \times g$ for 20 min at 4°C . The pelleted cells were resuspended in 60 ml of Senior membrane buffer (19), and NaCl was added so that the final concentration in the suspension was 0.8 M. The suspension quickly became viscous. DNase and RNase were added from stock solutions stored at -20°C in membrane buffer to give a final concentration of 0.01 mg/ml for each. Senior's membrane buffer contained 40 mM ϵ -amino-*n*-caproic acid, 6 mM *p*-aminobenzamidine, 10 mM MgSO_4 , 10% (vol/vol) glycerol, and 50 mM Tris (pH 7.0). The suspension of lysing spheroplasts was incubated at room temperature for 30 to 45 min with occasional swirling. Membranes were pelleted by centrifugation at $31,000 \times g$ for 30 min at 4°C and were washed twice with 30-ml portions of Senior membrane buffer. Each wash was for 30 min at ice temperature. The final membrane pellet was frozen at -20°C and retained ATPase activity for several months.

Prior to use, each membrane pellet was thawed, resuspended in membrane buffer (20 ml/liter of original culture), and assayed for protein by the method of Lowry et al. (14), with bovine serum albumin as standard. This resuspended membrane retained activity for several weeks when stored at 4°C .

A number of modifications of this basic procedure were used for the other bacteria listed. The procedure of Abrams et al. (1) was used to obtain spheroplasts of *S. faecium* with use of only lysozyme and no mutanolysin. The spheroplasts were then induced to undergo lysis in Senior membrane buffer by use of 0.8 M NaCl. The final NaCl concentration for inducing lysis of spheroplasts of *S. sanguis* was 1.0 M. For all of the other lactic acid bacteria, the procedure used was exactly that used for *S. mutans*. Protoplasts of *B. megaterium* were prepared by the procedure described by Corner and Marquis (6).

ATPase assays. ATPase activities were assessed in terms of amounts of phosphate released from ATP. Materials for the Fiske-SubbaRow (9) assay of phosphate were obtained from the American Monitor Corp., Indianapolis, Ind. The reaction buffer for all assays contained 50 mM Tris maleate and 10 mM MgSO_4 . The reaction was generally started by addition of 5 mM Tris ATP to a suspension of membranes at 37°C . The unit of enzyme activity was defined in terms of micromoles of phosphate released per minute per milligram of membrane protein or total protein for the F_1 subcomponent.

Isolation of F_1 ATPase. The F_1 enzyme was isolated from membranes by the method of Senior et al. (19), which involves washing the membranes with buffers of low ionic strength. The F_1 preparations were centrifuged at $100,000 \times g$ for 2 h prior to use. The F_1 enzyme was found to be insensitive to inhibition by dicyclohexylcarbodiimide.

RESULTS

Fluoride inhibition of membrane-associated ATPases. Figure 1 presents data on the inhibition by fluoride of ATP hydrolysis by isolated membranes of *S. faecium* ATCC 9790, *S. mutans* GS-5, *S. sanguis* NCTC 10904, and *L. casei* ATCC 4646. The general pattern of inhibition was similar for all of the organisms, although there were differences in the sensitivities of the various enzymes to fluoride. Similar data were obtained also for membranes isolated from cells of *S. milleri* ATCC 9895, *S. salivarius* ATCC 13419, *S. lactis* ATCC 19435, and *B. megaterium* ATCC 13632. Values for the approximate concentrations of fluoride required for 50% reduction in the initial rate of hydrolysis are presented in

Table 1. The range of sensitivities is from that of the enzyme of *S. mutans* GS-5, which was 50% inhibited by about 3 mM fluoride, to that of the enzyme from *L. casei* ATCC 4646, which was 50% inhibited by about 25 mM fluoride. The enzyme studied previously from *S. faecium* ATCC 9790 was less sensitive to fluoride than the enzymes from the other streptococci tested.

All of the curves in Fig. 1 show decreasing enzymatic activities with time, even though the initial ATP concentration was in excess at 5 mM. The basis for diminished activity did not appear to be enzyme inactivation in control suspensions but product inhibition by the ADP formed. The pattern of inhibition of ATP hydrolysis by ADP for membranes of *S. mutans*, *S. sanguis*, and *L. casei* is shown in Fig. 2.

The decrease in enzymatic activity with time was greatly increased by fluoride, and for *S. mutans*, 5, 10, or 20 mM fluoride acted to stop completely hydrolysis after about 10 min of incubation, well before ADP became inhibitory to any significant extent. The activity was not restored when the membranes were washed with fluoride-free buffer or were soaked in fluoride-free buffer overnight at 4°C , even though these procedures did not result in significant reduction in the activities of control enzymes. In essence, it seems that fluoride causes inhibition of the enzymes in two ways—a rapid, reversible manner, and a slower, not readily reversible manner. We refer to these two modes of inhibition as reversible and irreversible, even though the latter may possibly be reversed under some as yet undetermined set of conditions.

Dependence of inhibition on pH. The inhibition of membrane-associated ATPases by fluoride did not appear to have a strong dependence on pH in the range of values normally in dental plaque as shown by the data presented in Table 2. This relative insensitivity to pH changes contrasts with the responses of intact cells to fluoride (8).

Kinetics of short-term fluoride inhibition. Data are presented in Fig. 3 for assessment of kinetic parameters of the ATPases of isolated membranes from cells of *S. mutans* GS-5 and *L. casei* ATCC 4646. The points show initial rates of hydrolysis over ranges of concentrations of ATP and Mg; 1 mM fluoride was used to inhibit the enzyme of *S. mutans*, while 5 mM fluoride was used to inhibit the more resistant enzyme of *L. casei*. The Lineweaver-Burk plots show cooperative kinetics in relation both to ATP and Mg. Here the enzyme activities have been viewed in terms of two kinetic sites, one with higher affinities for the substrates and one with lower affinities.

With the enzyme from *S. mutans*, fluoride had more dramatic effects when ATP was the limiting substrate with excess Mg than when Mg was the limiting substrate with excess ATP. At low concentrations, less than about 1 mM, fluoride acted to decrease the K_m , but only from about 0.38 to 0.30 mM, and to decrease V_{\max} , from about 0.33 to 0.21 μmol of phosphate per min per mg of membrane protein. At higher substrate concentrations also, fluoride acted to decrease the K_m somewhat, and although the intercept with the ordinate axis of the figure shows a reduced V_{\max} for the enzyme exposed to 1 mM fluoride, still the action of fluoride resulted in an increase in the slope of the line relating $1/\text{velocity}$ to $1/\text{ATP concentration}$. Thus, it seems that much of the inhibition caused by fluoride at low substrate concentrations is reversed at high substrate concentrations. Similar effects of fluoride are apparent in the data for hydrolysis with Mg as the limiting substrate in the presence of excess ATP, but the effects are not as marked as those with ATP as limiting substrate.

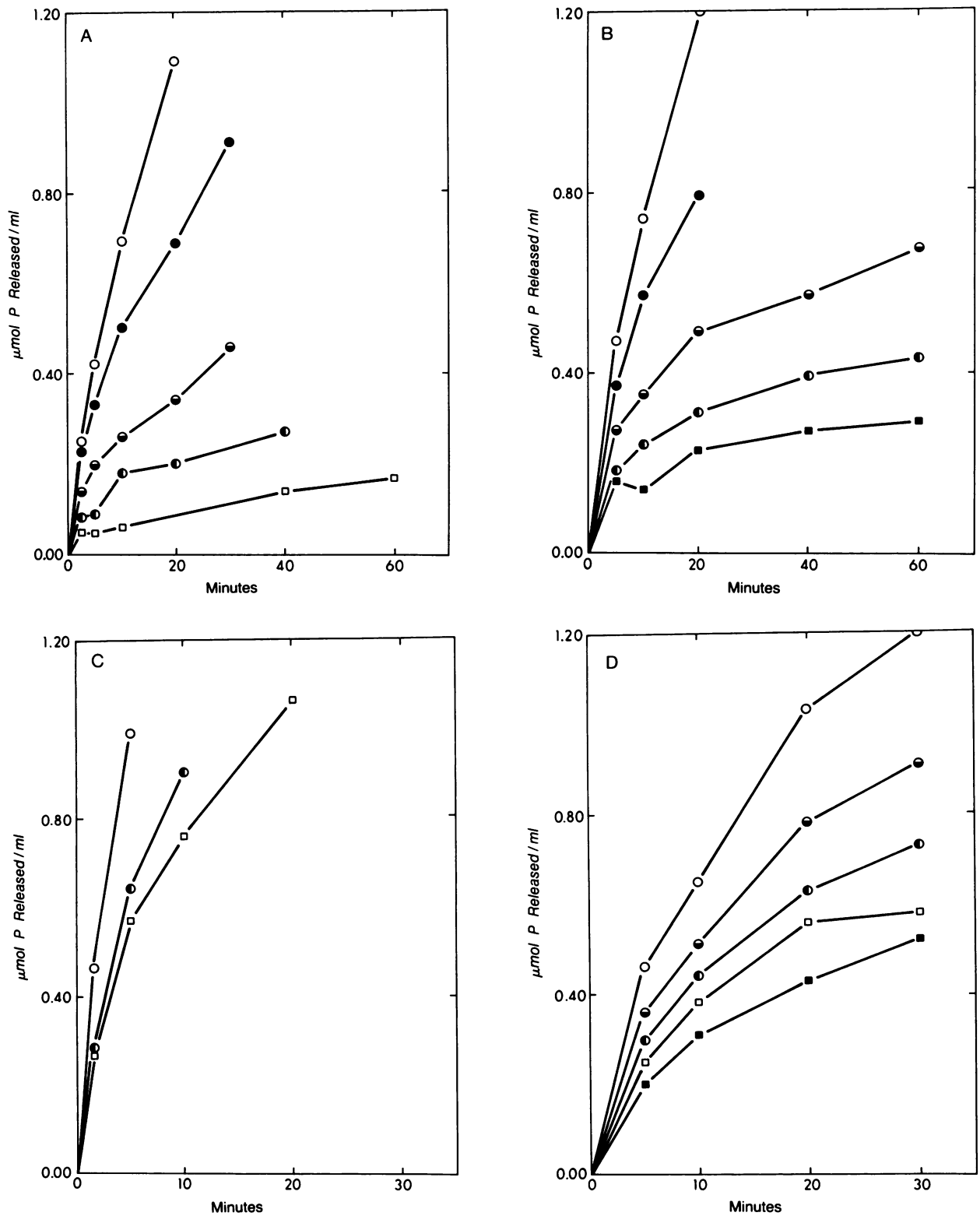


FIG. 1. Inhibition of membrane ATPases by fluoride. Reaction mixtures included isolated membranes, 10 mM Mg^{2+} , fluoride at the indicated concentrations, and 50 mM Tris maleate at pH 6.0 for *S. mutans* (A), pH 7.5 for *S. sanguis* (B), pH 6.5 for *L. casei* (C), and pH 7.0 for *S. faecium* (D). The mixture was incubated for 10 min at 37°C, and the reaction was started by addition of Tris ATP to yield a final concentration of 5 mM. Fluoride concentrations (millimolar) were 0 (○), 1 (●), 2.5 (○), 5 (●), 10 (□), and 20 (■).

TABLE 1. Fluoride inhibition of membrane-associated ATPases

Organism	Fluoride concn (mM) for 50% reduction in initial rate of hydrolysis of ATP ^a
<i>S. mutans</i> GS-5	3
<i>S. milleri</i> ATCC 9895	4
<i>S. sanguis</i> NCTC 10904	5
<i>S. lactis</i> ATCC 19435	5
<i>S. salivarius</i> ATCC 13419	10
<i>S. faecium</i> ATCC 9790	12
<i>B. megaterium</i> ATCC 13632	20
<i>L. casei</i> ATCC 4646	25

^a All assays were carried out with membranes in suspension with 5 mM ATP, 10 mM Mg²⁺, and 50 mM Tris maleate buffer at the optimal pH for each enzyme.

The enzyme of *L. casei* was more fluoride resistant than that of *S. mutans* but did show cooperation and different effects of fluoride at low compared with high substrate levels. However, for the *L. casei* enzyme, the cooperative effects were more dramatic when Mg was the limiting substrate than when ATP was limiting (Fig. 3). At a low Mg concentration, fluoride at a level of 5 mM acted to decrease the K_m from about 0.76 to 0.39 mM and to decrease the V_{max} from about 1.00 to 0.24 μmol of phosphorus released per min per mg of membrane protein. At high substrate concentrations, fluoride acted to increase the K_m , and again, the enhancement of cooperation resulted in a V_{max} for the fluoride-treated membranes that was about the same as that for the control membranes. The effects of fluoride when ATP was limiting were less dramatic.

The V_{max} values for the *L. casei* membranes were about 3.1 μmol of phosphate per min per mg of membrane protein when the ATP level was varied and about 4.0 when the Mg level was varied. These values were higher than the values of about 1.0 for the membranes of *S. mutans* obtained when the level of either of the substrates was varied. In essence, the membranes of *L. casei* appeared to have greater ATPase capacities (per milligram of membrane protein) than did the membranes of *S. mutans*.

Fluoride inhibition of isolated F₁ ATPases. F₁ ATPases were isolated from cell membranes by means of washing with buffer of low ionic strength (20). The isolated F₁ enzymes were fully capable of hydrolyzing ATP but were resistant to the inhibitory action of dicyclohexylcarbodiimide. Unexpectedly, the F₁ enzymes were less sensitive to fluoride than were the membrane-associated F₁F₀ holoenzymes. Typical data for the F₁ enzyme from *S. mutans* are shown in Fig. 4. The pattern of inhibition differs from that

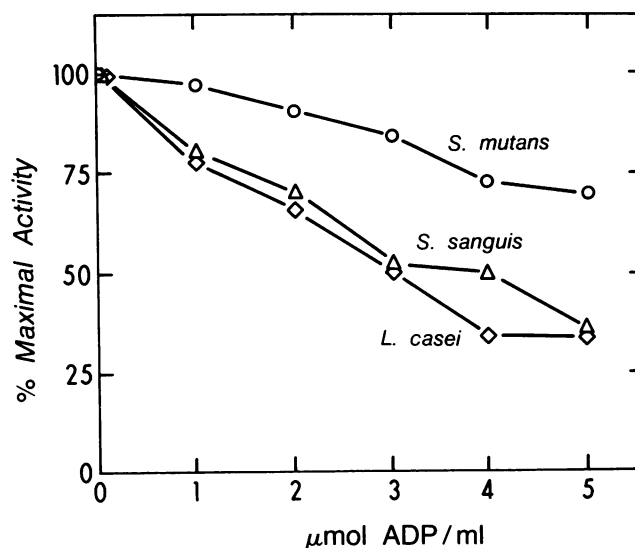


FIG. 2. Inhibition of membrane ATPases by ADP. The reaction mixtures were the same as those described in the legend to Fig. 1 except that ADP at the concentrations indicated was added before addition of ATP; 100% activities in enzyme units were 0.43 for *S. sanguis*, 0.82 for *S. mutans*, and 3.44 for *L. casei*.

shown in Fig. 1. With the F₁ enzyme, fluoride at a concentration of 5 mM had little effect on initial rate of hydrolysis (only 4% inhibition at 1 min), and even after 15 min, the inhibition was only about 16%. Moreover, fluoride at higher levels, including 50 mM, which completely inhibited the holoenzyme attached to the membrane could actually be stimulatory for the F₁ enzyme, mainly in terms of initial rates of reaction.

As indicated by the data presented in Table 3, a similar pattern of responses was found for F₁ enzymes from *S. sanguis* and *L. casei*. Fluoride at a concentration of 5 mM had little effect on initial rates of hydrolysis. Fluoride at higher levels of 10 or 20 mM was inhibitory; at a level of 50 mM, fluoride was actually stimulatory. Fluoride did cause inhibition over longer times, but there was less final inhibition by fluoride at a concentration of 50 mM than at 10 or 20 mM.

DISCUSSION

The development of techniques for isolation of membranes from a variety of oral bacteria (4, 18) has opened the way for comparative studies of the proton-translocating ATPases of the bacteria, which appear to play major roles in

TABLE 2. pH dependence of fluoride inhibition of membrane-associated ATPases

pH	% Inhibition at fluoride level (mM) ^a :											
	<i>S. mutans</i>				<i>S. sanguis</i>				<i>L. casei</i>			
	1.0	2.5	5.0	10.0	1.0	2.5	5.0	10.0	1.0	2.5	5.0	10.0
7	11	15	13	43	14	67	56	66	-3	29	18	43
6	24	39	56	64	2	7	65	78	-11	38	58	69
5	11	45	62	81	32	61	77	91	9	58	77	87
4	15	49	71	74					34	67	82	86

^a All assays were carried out with the standard assay mixture to which KF solutions were added. Phosphate analyses were carried out after 30 min of incubation. For *S. mutans* membranes, activity was maximal at pH 6.0; the activity was reduced 37% at pH 7.0, 24% at pH 5.0, and 41% at pH 4.0. For *S. sanguis* membranes, activity was maximal at pH 7.0; the activity was reduced 6% at pH 6.0 and 5.0, but 88% at pH 4.0. For *L. casei* membranes, activity was maximal at pH 6.0; the activity was reduced 3% at pH 7.0, 7% at pH 5.0, and 27% at pH 4.0. The percent inhibition in each case was calculated as the percent of the zero fluoride value at the specific pH.

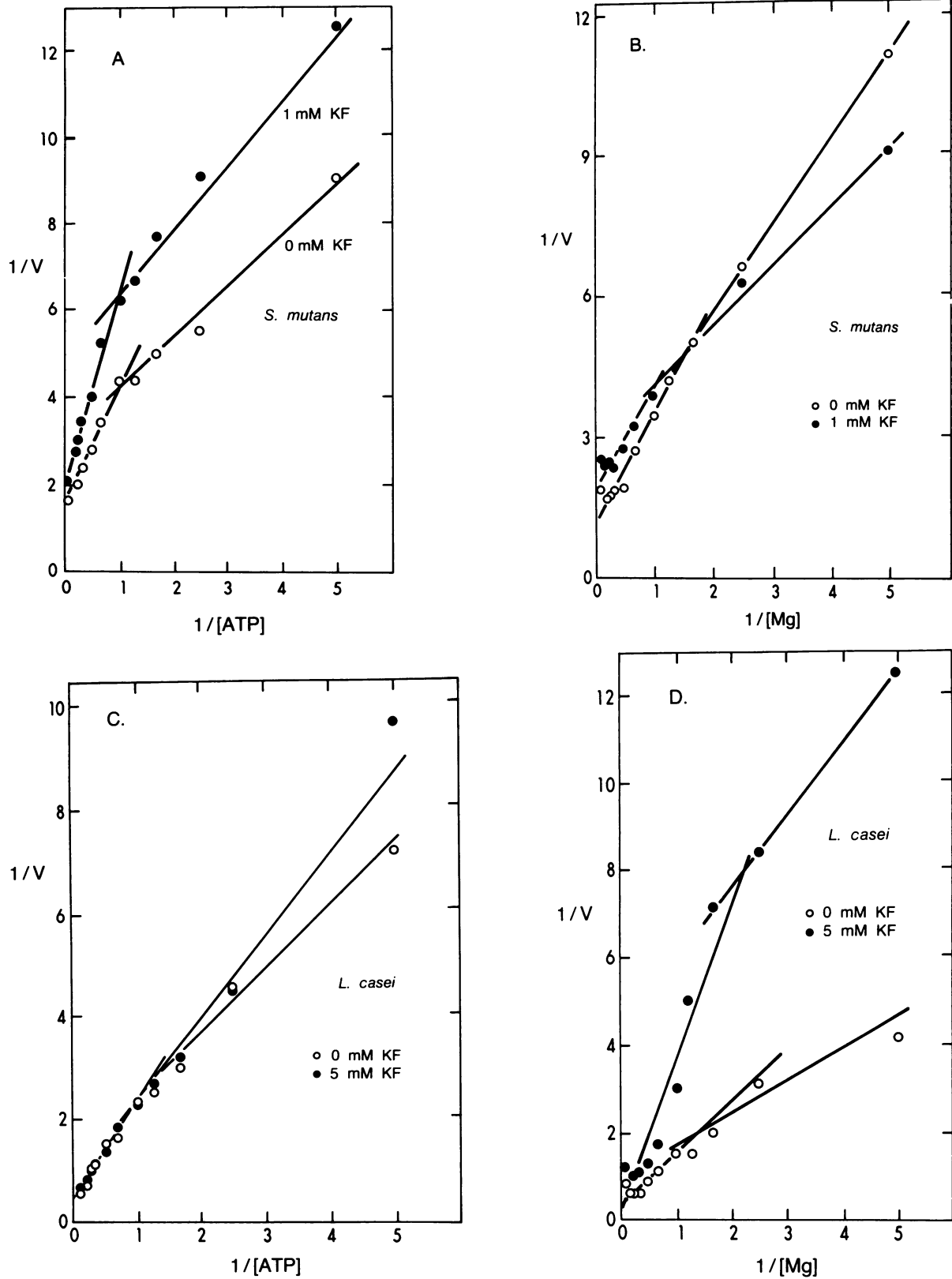


FIG. 3. Lineweaver-Burk plots for ATP hydrolysis by isolated membranes of *S. mutans* and *L. casei*. The units for Mg and ATP concentrations were millimolar, and velocities of reaction (V) were expressed in terms of micromoles of phosphate released per minute per milligram of membrane protein.

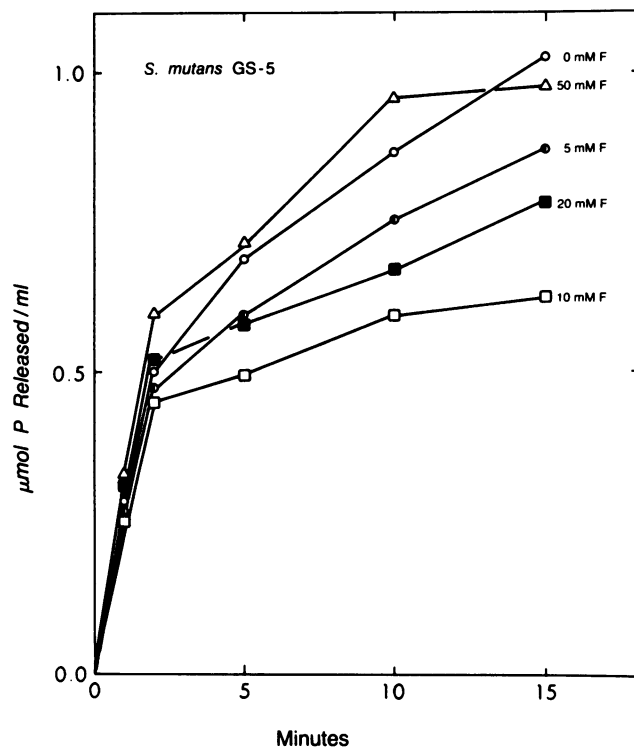


FIG. 4. Fluoride inhibition of the F_1 ATPase of *S. mutans*.

their acid/base physiology. In this paper, we compared sensitivities to fluoride and have also considered nonoral bacteria, particularly *S. faecium* ATCC 9790 (16). The ATPases of *S. mutans*, *S. milleri*, *S. sanguis*, and *S. lactis* appeared to be more sensitive to fluoride than the enzyme of *S. faecium*, while the enzyme of *S. salivarius* had similar sensitivity, and that of *L. casei* was less sensitive. Recently, Kashket (J. Dent. Res. 65:736, 1986) has reported that the membrane ATPase of *S. rattus* BHT was inhibited 50% by about 2.6 mM fluoride and so appears to have approximately the same sensitivity as the enzyme of *S. mutans* GS-5.

The greater sensitivity of the enzyme of *S. mutans* suggests that direct inhibition of proton translocation by fluoride could play a significant role in the antimicrobial action of fluoride against this organism, especially the reduction in acid tolerance. This reduction has been interpreted primarily in terms of the weak-acid properties of fluoride. The pK_a of fluoride is 3.15, and in acidified cultures of *S. mutans*, part of the fluoride would be in the HF or protonated form. Gutknecht and Walter (10) found that the permeability coefficient of synthetic membranes for HF was some 10^7 times that for F^- . Therefore, even at pH values well above

the pK_a of fluoride, the predominant movement of fluoride into the cell would be as HF. Once inside the cell, in the relatively alkaline cytoplasm, the HF would dissociate to yield the enzyme inhibitor F^- , but also H^+ , which would act to acidify the cytoplasm and reduce ΔpH across the cell membrane. It seems that this movement of HF into the cell in response to ΔpH across the cell membrane is the basis for fluoride concentration by cells and for the subsequent release of fluoride when ΔpH has been dissipated (23). In fact, the dissipation of ΔpH by fluoride has been assessed quantitatively (8, 14). However, it now appears that fluoride may dissipate ΔpH not only by acting as a weak-acid carrier of protons across the cell membrane but also by inhibiting the ATPase which acts to move protons out of the cell. An initial review of the data presented here would suggest that direct inhibition of ATPases would not be very important because it does not show a strong dependence on pH as does the antimicrobial action of fluoride for whole cells. However, the hydrolytic portion of the ATPase is on the cytoplasmic face of the membrane. Therefore, when cells in acid media concentrate fluoride, the ATPase would be exposed to levels of fluoride higher than those in the environment, and so inhibition would be greater at lower pH values. In essence, the weak-acid property of fluoride would be important for inhibition of membrane ATPases primarily because of concentration of fluoride into the cytoplasm of cells in acid media.

The mechanisms of inhibition of ATPases by fluoride are unexpectedly complicated and appear to involve reversible, noncompetitive inhibition over short periods and irreversible inhibition over long periods. The latter type of inhibition was not entirely unexpected, and similar inhibition occurs with other phosphatase enzymes, for example, the pyrophosphatase of yeasts (2) or mitochondria (3). Baikov et al. (3) analyzed fluoride inhibition of mitochondrial pyrophosphorylase in terms of rapid and reversible binding of F^- to the enzyme-substrate complex, with subsequent slow isomerization of the enzyme to an entirely inactive form. The kinetics of inhibition of membrane ATPases by fluoride are very similar to those for pyrophosphorylases. The picture is somewhat more complicated for the ATPases because they are inhibited by the product ADP, and so there is an apparent loss of activity even for the control enzyme with time. However, removal of the ADP restores full activity. In contrast, full activity could not be restored after the enzyme was incubated with fluoride at concentrations of 10 or 20 mM. Whether or not irreversible inhibition occurs in vivo is an open question. Although the ATPases could be exposed to high concentrations of fluoride when it is concentrated by cells in acid media, still, once ΔpH across the membrane is dissipated, the fluoride would be released from the cells, and the cytoplasmic level would then drop. This release might then protect the enzyme from irreversible inactivation.

The finding that the F_1 enzyme of *S. mutans* is less

TABLE 3. Fluoride inhibition of F_1 ATPases

Fluoride concn (mM)	% Inhibition					
	<i>S. mutans</i>		<i>S. sanguis</i>		<i>L. casei</i>	
	Initial rate	Extent of hydrolysis ^a	Initial rate	Extent of hydrolysis	Initial rate	Extent of hydrolysis
5	4	16	0	34	0	25
10	11	41	23	45	25	42
20	-9	25	8	42	40	58
50	-15	6	-15	13	45	29

^a The extent of hydrolysis was assessed after 30 min of incubation at 37°C.

sensitive to fluoride than the F_1F_0 holoenzyme associated with the membrane was unexpected. In essence, it seems that the coupled reaction involving both ATP hydrolysis and proton translocation is more sensitive to fluoride inhibition than the uncoupled reaction. Possibly, fluoride affects the proton pore or interacts in some other way with the F_0 subunit. Clearly, the reaction is a novel one and deserves further study.

ACKNOWLEDGMENTS

This investigation was supported by grants R01 DE06127 and P50-DE07003 from the National Institute of Dental Research.

LITERATURE CITED

- Abrams, A., P. McNamara, and F. B. Johnson. 1960. Adenosine triphosphatase in isolated bacterial cells. *J. Biol. Chem.* **235**:3659-3662.
- Baikov, A. A., A. A. Artjukov, and S. M. Awaeva. 1976. Fluoride inhibition of inorganic pyrophosphatase. I. Kinetic studies in a Mg^{2+} -PP system using a continuous enzyme assay. *Biochim. Biophys. Acta* **429**:982-992.
- Baikov, A. A., I. N. Smirnova, and S. E. Volk. 1984. Membrane inorganic phosphatase. "Syncatalytic" inactivation by the fluoride ion. *Biokhimiya* **49**:696-702. (English translation.)
- Bender, G. R., S. V. W. Sutton, and R. E. Marquis. 1986. Acid tolerance, proton permeability, and membrane ATPases of oral streptococci. *Infect. Immun.* **53**:331-338.
- Bibby, B., and M. van Kesteren. 1940. The effect of fluorine on mouth bacteria. *J. Dent. Res.* **19**:391-402.
- Corner, T. R., and R. E. Marquis. 1969. Why do bacterial protoplasts burst in hypotonic solutions? *Biochim. Biophys. Acta* **183**:544-558.
- Dawes, C., G. N. Jenkins, J. L. Hardwick, and S. A. Leach. 1965. The relation between fluoride concentrations in the dental plaque and in drinking water. *Br. Dent. J.* **119**:164-167.
- Eisenberg, A. D., G. R. Bender, and R. E. Marquis. 1980. Reduction in the aciduric properties of *Streptococcus mutans* GS-5 due to membrane active agents and fluoride. *Arch. Oral Biol.* **25**:133-135.
- Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**:375-400.
- Gutknecht, J., and A. Walter. 1981. Hydrofluoric and nitric acid transport through lipid bilayer membranes. *Biochim. Biophys. Acta* **644**:153-156.
- Hamilton, I. R. 1977. Effects of fluoride on enzymatic regulation of bacterial carbohydrate metabolism. *Caries Res.* **11**(Suppl. 1):262-291.
- Hamilton, I. R., and D. C. Ellwood. 1978. Effects of fluoride on carbohydrate metabolism by washed cells of *Streptococcus mutans* grown at various pH values in a chemostat. *Infect. Immun.* **19**:434-442.
- Kashket, S., and E. R. Kashket. 1985. Dissipation of the proton motive force in oral streptococci by fluoride. *Infect. Immun.* **48**:19-22.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
- Luoma, H., A-R. Luoma, and L. Seppä. 1984. Exchange of fluoride between bovine enamel and the surface-related cells of the oral bacterium *Streptococcus mutans*. *Arch. Oral Biol.* **29**:343-348.
- Marquis, R. E. 1977. Inhibition of streptococcal adenosine triphosphatase by fluoride. *J. Dent. Res.* **56**:704.
- Ophaug, R. H., G. N. Jenkins, L. Singer, and P. H. Krebsbach. 1987. Acid diffusion of different forms of fluoride in human dental plaque. *Arch. Oral Biol.* **32**:459-461.
- Schöller, M., J. P. Klein, P. Sommer, and R. Frank. 1983. Protoplast and cytoplasmic membrane preparations from *Streptococcus sanguis* and *Streptococcus mutans*. *J. Gen. Microbiol.* **129**:3271-3279.
- Senior, A. E., J. A. Downie, G. B. Cox, F. Gibson, L. Langman, and D. R. H. Fayle. 1979. The *uncA* gene codes for the α -subunit of the adenosine triphosphatase of *Escherichia coli*. *Biochem. J.* **180**:103-109.
- Sutton, S. V. W., and R. E. Marquis. 1987. Membrane-associated and solubilized ATPases of *Streptococcus mutans* and *Streptococcus sanguis*. *J. Dent. Res.* **66**:1095-1098.
- Tatevossian, A. 1978. Distribution and kinetics of fluoride ions in the free aqueous and residual phases of human dental plaque. *Arch. Oral Biol.* **23**:893-898.
- Thibodeau, E. A., W. H. Bowen, and R. E. Marquis. 1985. pH-dependent fluoride inhibition of peroxidase activity. *J. Dent. Res.* **64**:1211-1213.
- Vicaretti, J., E. Thibodeau, G. Bender, and R. E. Marquis. 1984. Reversible fluoride uptake and release by *Streptococcus mutans* GS-5 and FA-1. *Curr. Microbiol.* **10**:317-322.