

IN VITRO AND IN VIVO EFFECTS OF FLUORIDE SOLUTIONS ON HUMAN SALIVARY AMYLASE

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SUMMARY: The effect of various concentrations of NaF on the activity of human salivary amylase was examined *in vitro* and *in vivo*. No statistically significant differences in the *in vitro* experiments were observed in amylase activity after one hour of incubation with fluoride concentrations up to 500 mM. In the *in vivo* study, the effect of 0.05% NaF solutions was studied on the amylase of the human saliva collected at different periods after mouthrinsing. Again, no statistically significant differences, were observed in the amylase activity of all samples examined.

Keywords: Human saliva, Fluoride effects, Fluoride mouthrinsing, Salivary amylase.

INTRODUCTION

Salivary amylase may play an important role in the colonization and metabolism of streptococcus, leading to the formation of dental plaque and caries. It has been identified as a constituent of the acquired pellicle^{1,2} and could, therefore, be available to act as a receptor for microorganism adhesion on tooth surfaces.³ Amylase was detected in plaque by immunochemical,⁴ enzymatic, and electrophoretic⁵ procedures. Another feature is its ability to bind on the bacterial surfaces and to hydrolyze starch, giving rise to products that are transformed into acids.⁶

Amylase activity has been the focus of many studies on the development of dental caries. There are conflicting reports on this aspect, however. Some of them show a positive or a negative relationship, whereas others show no correlation.⁷⁻¹³ In an *in vitro* study, using slices of parotid glands as well as an *in vivo* study injecting NaF solution into rats, an accumulation of cAMP and stimulation of the amylase secretion occurred.¹⁴ With rats drinking water containing 25 or 50 ppm F⁻ for four weeks, not only the flow rate but also the activity of amylase in the parotid secretion was stimulated.¹⁵ On the other hand, human salivary amylase was not affected by fluoride in the range of 5.25×10^{-7} to 2.63×10^{-4} M.¹⁶ More recently, in an *in vitro* study, concentrations equal to or higher than 5×10^{-2} M NaF inhibited human salivary amylase.¹⁷

Because exposure to fluoride is widespread through drinking water, toothpaste, mouthrinses, and other formulations for professional use, and in view of the conflicting results in the literature, we decided to investigate the influence of fluoride on human salivary amylase by using NaF solutions *in vitro* and as a mouthrinse as normally used in caries prevention.

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MATERIALS AND METHODS

Subjects: Ten healthy volunteers (5 men and 5 women) aged 23-40 years, with no caries or gingivitis and neither prosthetic nor orthodontic apparatus, volunteered to participate in this investigation. After verbal and written information on the aim of the study, they gave written consent to participate. The protocol was approved by the Ethical Committee, Faculty of Dentistry, University of São Paulo, Brazil.

Collection of saliva

In vitro experiments: Whole saliva was collected from two volunteers in the morning (between 9.00 and 9.30) after fasting overnight. Before collection of saliva, participants were asked to rinse their mouths with distilled water and chew a piece of parafilm. The saliva produced during the first 2 min after the water rinse was deglutated or expectorated. Saliva was then collected during 10 min and centrifuged at 12,500 x g for 10 min in a Sorvall RC2-B refrigerated centrifuge. The supernatant was used for the determination of amylase activity in the presence or absence of fluoride solutions.

In vivo experiments: Similar to the *in vitro* study, whole saliva was collected in the morning (between 9.00 and 9.30) after overnight fasting. Before the collection, participants were asked to rinse their mouths with 15 mL of distilled water or 0.05% NaF solution as directed. However, before the series of collections that followed the mouthrinses, saliva was collected for 5 min to serve as reference. Saliva for this *in vivo* study was collected on two different days of consecutive weeks. On the first day the individual rinsed his or her mouth with distilled water only, while on the second day a 0.05% NaF solution was used. Immediately after the rinse and then at 5, 10, 15, 30, and 60 min after the rinse, whole stimulated saliva was collected for 2.5 min.

After the collection of saliva, the pH of the collected samples was measured and the samples were centrifuged at 12,500 x g for 10 min in a Sorvall RC2-B refrigerated centrifuge. The supernatant was diluted in 0.1 M tris buffer pH 7.0 and the resulting solution was used for determination of protein (1:10) and enzyme activity (1:50).

Analysis

The pH of freshly-collected saliva samples was measured with a Metrohm Herisau, pH meter standardized against pH 4.0 and pH 7.0 solutions.

Protein was determined by the method of Lowry *et al*¹⁸ using bovine serum albumin as standard.

Salivary amylase activity was determined in an assay mixture containing 1.5 mL of 0.2% starch solution, 1.5 mL of 0.1 M tris buffer (pH 7.0), 0.1 mL of diluted supernatant of the centrifuged saliva and 0.4 mL of distilled water. After incubation for 60 min at 37°C, an aliquot was taken and mixed with Somogyi-Nelson reagent. The intensity of the developed color was meas-

ured at 520 nm in a Beckman DU-68 spectrophotometer. A standard curve was constructed using maltose solutions.

For the *in vitro* experiments, Student's t test was used to determine whether there were any significant differences between the mean of each group and the control ($p < 0.05$). For the *in vivo* experiments, the data were processed with the ANOVA analysis ($p < 0.05$).

RESULTS AND DISCUSSION

Preliminary analysis of the data did not reveal significant differences in amylase activity between male and female subjects relative to any of the parameters studied. The results were thus processed with the sexes combined.

Table 1 shows the results of the *in vitro* study. Varying the NaF concentration from 0 to 500 mM in the incubation medium did not cause any statistically significant differences in amylase activity. In absolute values up to 100 mM NaF the activity was greater than 100% of the control. At 500 mM NaF, however, the activity of salivary amylase was 92.8% of the control.

Table 1. Effect of various concentrations of NaF solution on human salivary amylase activity after incubation for 60 min.

NaF (mM)	n	mg maltose/ mg protein/min	Percent of control
0 (control)	7	8.31 ± 1.27	
10	7	8.70 ± 1.78	104.7
50	7	8.36 ± 1.70	100.6
75	7	9.06 ± 1.38	109.0
100	7	8.73 ± 1.19	105.0
500	7	7.71 ± 1.48	92.8

Values are mean ± SD. Each sample represents the mean of three determinations.

Table 2 shows the data for the *in vivo* study. The flow rate, protein concentration, and the amylase activity were determined either after a rinse with distilled water or with 0.05% NaF solution for 30 sec. In this experiment, as in the *in vitro* study, no statistically significant differences were observed.

Salivary amylase has been considered significant for oral health in view of its intraoral activity. It is the principal digestive enzyme produced by the salivary glands and is present in the parotid gland and at lower concentrations in the submandibular gland. It is a glycoprotein with a molecular weight of 62-67 kDa and plays a central role in the digestion of polysaccha-

rides by hydrolyzing the α -1,4 glucosidic linkages of starch, glycogen, and related polysaccharides.

Table 2. Flow rate, protein concentration, and human salivary amylase activity from parafilm-stimulated whole saliva before (C) and after mouthrinse with distilled water (W) or 0.05% NaF solution (F), collected at different times (in min) after rinsing

Group (min)	Flow Rate		Protein		Amylase	
	W	F	W	F	W	F
C	1.19 ± 0.40	1.05 ± 0.24	1.88 ± 0.37	1.56 ± 0.38	5.53 ± 1.81	7.13 ± 2.56
(0)	1.05 ± 0.33	1.30 ± 0.48	1.69 ± 0.36	1.44 ± 0.42	6.26 ± 2.00	8.28 ± 3.53
(5)	1.04 ± 0.41	1.02 ± 0.37	1.72 ± 0.36	1.55 ± 0.42	6.65 ± 2.08	8.12 ± 2.87
(10)	0.84 ± 0.30	0.89 ± 0.29	1.76 ± 0.34	1.56 ± 0.40	7.05 ± 1.85	7.90 ± 2.85
(15)	0.94 ± 0.36	0.94 ± 0.28	1.80 ± 0.37	1.58 ± 0.41	6.16 ± 1.68	7.51 ± 2.86
(30)	0.87 ± 0.35	0.94 ± 0.35	1.84 ± 0.39	1.66 ± 0.37	5.95 ± 1.80	7.35 ± 2.81
(60)	1.11 ± 0.39	1.03 ± 0.40	2.05 ± 0.41	1.81 ± 0.38	5.04 ± 1.37	6.35 ± 2.11

Values are mean ± SD for 10 samples. Each sample represents the mean of three determinations.

In our *in vivo* experiments we employed a 0.05% NaF solution, the same as currently used in caries prevention. This concentration, corresponding to 11.9 mM, similar to that found *in vitro* with a final concentration of 10 mM, did not exert any appreciable effect on the activity of salivary amylase. Although it has been reported that F ions have no apparent effect on the activity of salivary amylase,¹⁹ subsequent studies indicated that salivary amylase was inhibited by NaF.^{17,20}

Using nearly the same experimental conditions as recorded in the literature, in which inhibition of salivary amylase with NaF concentrations equal to or higher than 50 mM was reported,¹⁷ we did not observe this effect. This discrepancy may be due, *inter alia*, to: (1) preparation of our standard curve with maltose instead of glucose; (2) dilution of our saliva samples 1:50 instead of 1:100; (3) individual differences between the two study groups.

Recently,²¹ it was found that a decoction of tea containing high concentrations of fluoride exhibited no inhibition on amylase, and, even after adding more NaF, no effect on the enzyme activity was detected. One of the explanations given for the inhibiting effect of NaF was based upon the variation of the pH of the incubation medium.¹⁷ In our experiments, after one hour of incubation, it was not possible to detect variation greater than 0.01 pH unit in the incubation medium. However, comparing the combined results obtained for the experiment with water with those obtained from the

rinsing with 0.05% NaF solution, the difference is statistically significant. It is possible that the large individual variations obtained for each group may have influenced the analyses.

In the *in vivo* experiments, the results obtained for amylase activity were submitted to the Kolmogorov-Smirnov test ($p < 0.05$), yielding the value for $d = 0.06868$, $p = ns$, indicating that the data fit a normal distribution. Although the mean values obtained for amylase activity after rinsing with 0.05% NaF solution were higher than those obtained after rinsing with distilled water, the differences were not statistically significant by ANOVA analysis.

Overall, our results are in agreement with those reported by several other authors.^{16,19,21} Under the conditions used in the present investigation, either *in vivo* rinsing the mouth with 0.05% (11.9 mM) NaF or *in vitro* with up to 500 mM of NaF in the incubation medium, it was not possible to observe any significant effect of fluoride on the activity of human salivary amylase.

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REFERENCES

- 1 Orstavik D, Kraus FW. The acquired pellicle immunofluorescent demonstration of specific proteins. *J Oral Pathol* 1973;2:68-76.
- 2 Al-Hashimi I, Levine MJ. Characterization of the *in vivo* salivary-derived enamel pellicle. *Arch Oral Biol* 1989;34:289-95.
- 3 Scanniepo FA, Torres GI, Levine MJ. Salivary amylase promotes adhesion of oral streptococci to hydroxyapatite. *J Dent Res* 1995;74:1360-6.
- 4 Di-Paola C, Herrera MS, Mandel ID. Immunochemical study of host proteins in human supragingival compared with denture plaque. *Arch Oral Biol* 1984;29:161-3.
- 5 Birkhed D, Skude G. Relation of amylase to starch and lysozyme metabolism in human dental plaque *in vitro*. *Scand J Dent Res* 1978;86:248-58.
- 6 Scanniepo FA, Bhandary K, Ramasubbu N, Levine MJ. Structural relationship between the enzymatic and streptococcal binding sites of human salivary alpha amylase. *Biochem Biophys Res Commun* 1990;173:1109-15.
- 7 Turner NC, Crane EM. A relationship between dental caries and saliva. *J Dent Res* 1944;23:413-6.
- 8 Sullivan JH, Storvik CA. Correlation of saliva analysis with dental examination of 574 freshmen at Oregon State College. *J Dent Res* 1950;29:165-72.
- 9 Turner NC. A biochemical pattern basic to tooth decay. *J Dent Res* 1960;61:20-31.
- 10 Day CDM. The amylolytic enzyme of the saliva in relation to dental caries. *Dental Cosmos* 1934;76:683-9.
- 11 Svejda J, Budejovice C. Decoloration of blue starch solution as a test for caries susceptibility. *J Dent Res* 1950;29:698.

- 12 Hubbell RB. The composition of saliva and blood serum of children in relation to dental caries. *Am J Physiol* 1933;105:436-42.
- 13 Bergheim O, Barnfield WF. Lack of correlation between dental caries and salivary amylase. *J Dent Res* 1945, 24:141-2.
- 14 Shahed AR, Biondi J, Allmann DE. Effect of NaF on rat parotid gland amylase activity and cAMP concentration in vitro and in vivo. *J Dent Res* 1985;64:1126-9.
- 15 Boros I, Mozsik Gy, Keszler P. Effect of F⁻ on major salivary glands. The amylase activity, stimulated salivary flow response and cAMP levels in parotid gland of rats consuming F⁻ via drinking water. *Fluoride* 1984;17:217-23.
- 16 Schmidt H, Gocke R. Die beeinflussung der amylase-aktivitat (EC3.2.1.1) des menschlichen speichels durch fluor. *Zahn, Mund-, und Kieferheilkunde mit Zentralblatt* 1976;64:377-84.
- 17 Hara K, Yu MH. Effect of fluoride on human salivary amylase activity. *Fluoride* 1995;28:71-74.
- 18 Lowry DH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
- 19 McClure FJ. Effect of fluorides on salivary amylase. *Public Health Rep* 1939; 54:2165-71.
- 20 Pase V, Dalla-Venezia S. Attivita amilasica salivare e sua sensibilita al fluoro. *G Stomatol Ortognatodonzia* 1984;3:730-2.
- 21 Zhang J, Kashket S. Inhibition of salivary amylase by black and green teas and their effects on the intraoral hydrolysis of starch. *Caries Res* 1998. 32:233-8.