EFFECT OF FLUORIDE ON HUMAN SALIVARY AMYLASE ACTIVITY

K Hara[a] and M-H Yu[b]
Bellingham, Washington, USA

SUMMARY: The effect of various concentrations of NaF on human salivary amylase was studied. Sodium fluoride was found to inhibit the enzyme when the fluoride concentration was at and above 5 x 10^{-2}M.

Key words: Human salivary amylase; NaF inhibition.

Introduction

α-Amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) catalyzes the breakdown of α-1,4 glucosidic bonds of glucan in a random manner. Thus, when starches undergo amylolytic digestion, the end products are maltose, some glucose, and "limit dextrins" that contain α-1,6 glucosidic linkages, in addition to α-1,4 linkages. In humans, α-amylase is present in saliva and pancreatic juice, with the pancreatic amylase playing a major role in dietary starch digestion.

There has been much interest among many investigators in relating the starch-splitting properties of salivary amylase to the development of dental caries. Some investigators reported a positive correlation between dental caries and high salivary amylase activity,[1-3] whereas others showed inverse relationship,[4,5] or no correlation.[6,7] Based on *in vitro* experiments, Jacobsen *et al*[8] concluded that salivary amylase did not contribute to the formation or accumulation of dental soft deposits. By contrast, Mundorff-Shrestha *et al*[9] recently demonstrated that salivary amylase content was related to the promotion of the number of sulcal lesions in rats.

Salivary amylase and the effects of various salts on its activity were reported as early as in 1919.[10] However, studies on the effect of fluoride on amylase activity are limited. Furthermore, discrepancies often exist among reported results. Boros *et al*[11] reported a significant increase of salivary amylase activity in rats exposed to 25 and 50 ppm fluoride in drinking water. Schmidt and Gocke[12] investigated the human salivary amylase and showed that the activity was not influenced by fluoride at concentrations ranging from 5.26 x 10^{-7} to 2.63 x 10^{-4}M. In this paper, we report that human salivary amylase activity was inhibited by NaF when the enzyme was exposed to fluoride at and above 5 x 10^{-2}M.

---

[a] On sabbatical leave from Kanagawa Dental College, Yokosuka, Japan.
[b] Center for Environmental Sciences, Huxley College of Environmental Studies, Western Washington University, Bellingham WA 98225-9181, USA. Correspondence to Professor Yu. Part of this work was presented at the XVIIIth ISFR Conference in Arcata, California, USA.

Materials and Methods

Collection and preparation of saliva samples:

Human saliva samples were collected from 3 to 5 invididuals and centrifuged at 13,000 x g for 20 min. An aliquot of the supernatant was diluted 100 times with 0.1 M Tris buffer (pH 7.0). The diluted solution was mixed with water or NaF to yield extracts containing 0, 5, 50, 100, and 500 mM NaF, respectively. These extracts were then used in the assay.

Enzyme assay:

Salivary amylase was assayed by the method reported previously,[13] with a slight modification. An assay mixture consisted of 3.0 ml of 0.2% starch solution, 3.0 ml of 0.1 M Tris buffer (pH 7.0), and 1.0 ml of the diluted salivary extract. The assay mixture was incubated at 37°C for 1 h and, at the end of the experimental period, a 1.0 ml aliquot was pipetted into a test tube containing 1.0 ml Nelson-Somogyi solution.[14] The mixture was heated in boiling water for 10 min. One ml of arsenomolybdate solution was added to the cooled mixture. The absorbance of the resultant solution, after suitably diluted, was read in a spectrophotometer at 520 nm.

Enzyme activity:

Protein of the enzyme was determined by the method of Lowry *et al.*[15] Enzyme activity was defined as μg glucose produced/mg protein/10 min.

Results and Discussion

Tables 1a and 1b show the influence of NaF at different concentrations on salivary amylase activity for assay mixtures incubated for 1 h and 3 h, respectively. Sodium fluoride at and above 50 mM was found to inhibit the enzyme significantly, and the inhibitory effect was increased with increase in F concentration. The decrease in amylase activity was 7% and 11% for 50 mM and 500 mM NaF, respectively (Table 1a). This pattern of inhibitory effect remained unchanged when assay time was 3 h. In separate experiments where lower concentrations of NaF were used, slight increases and decreases in enzyme activity were also observed, but the changes were found to be insignificant (data not shown). Our results from treatment with low levels of NaF are consistent with those reported by Schmidt and Gocke.[12]

McClure[16] attributed the decrease in salivary amylase activity in the presence of fluoride to F-induced increase in pH. As shown in Tables 1a and 1b, the pH values at the end of the experiments were increased in reaction mixtures containing 100 and 500 mM NaF. Such changes in pH were not observed in assay mixtures containing NaF up to 50 mM, where inhibitory effect was manifested. Again, similar results were obtained when the incubation time was extended to 3 h (Table 1b). These results lead us to conclude that the observed inhibition of amylase activity in the presence of NaF is probably due solely to the action of F^- ions. On the other hand, a steady increase in pH and a concomitant decrease in enzyme activity were

TABLE 1a. Fluoride effect on salivary amylase activity[a]

NaF mM	n	pH	Amylase activity (μg/mg/10 min)	Percent of control
0	3	7.00	2048 ± 27.1[b]	-
5	3	6.99	1931 ± 78.4	94
50	3	6.99	1905 ± 57.3*	93
100	3	7.05	1931 ± 36.4*	94
500	3	7.23	1820 ± 50.9*	89

[a] Assay mixture was incubated for 1 h.
[b] Values are means ± S.D.
* $p < 0.05$.

TABLE 1b. Fluoride effect on salivary amylase activity[a]

NaF mM	n	pH	Amylase activity (μg/mg/10 min)	Percent of control
0	3	7.02	1942 ± 15.5[b]	-
5	3	7.02	1904 ± 29.7	98
50	3	7.02	1848 ± 16.2*	95
100	3	7.08	1800 ± 42.0*	93
500	3	7.25	1705 ± 50.2*	88

[a] Assay mixture was incubated for 3 h.
[b] Values are means ± SD.
* $p < 0.05$.

shown in mixtures containing 100 mM and 500 mM NaF (Tables 1a and 1b). Although α–amylase has been shown to remain exceptionally stable at high pH,[8] nevertheless it is possible that the observed decrease in activity in enzymes treated with 100 mM and 500 mM NaF was due to increased pH by F^- action as well.

The activity and stability of α-amylase have been shown to be dependent on the presence of Cl^- ions and Ca. Calcium, in particular, may form intramolecular cross-links with the enzyme protein, similar to disulfide linkages.[17] Many enzymes that require Ca for activity are inhibited by fluoride. The mechanism involved in the inhibition appears to be through F-induced removal of Ca, as suggested in our earlier studies using amylase from mung bean seedlings.[13] The observed inhibition of human salivary amylase by NaF may involve a similar mechanism.

References

1 Turner NC, Crane EM. A relationship between dental caries and saliva. *Journal of Dental Research 23* 413-416 1944.

2 Sullivan JH, Storvick CA. Correlation of saliva analyses with dental examination of 574 freshmen at Oregon State College. *Journal of Dental Research 29* 165-172 1950.

3 Turner NC. A biochemical pattern basic to tooth decay. *Journal of Dental Research 61* 20-31 1960.

4 Day CDM. The amylolytic enzyme of the saliva in relation to dental caries. *Dental Cosmos 76* 683-689 1934.

5 Svejda J, Budejoyice C. Decoloration of blue starch solution as a test for caries susceptibility. *Journal of Dental Research 29* 698 1950.

6 Hubbell RB. The chemical composition of saliva and blood serum of children in relation to dental caries. *American Journal of Physiology 105* 436-442 1933.

7 Bergheim O, Barnfield WF. Lack of correlation between dental caries and salivary amylase. *Journal of Dental Research 24* 141-142 1945.

8 Jacobsen N, Melvaer KL, Hensten-Pettersen A. Some properties of salivary amylase: A survey of the literature and some observations. *Journal of Dental Research 51* (2) 381-388 1972.

9 Mundorff-Shrestha SA, Featherstone JD, Eisenberg AD *et al.* Cariogenic potential of foods. II. Relationship of food composition, plaque microbial counts, and salivary parameters to caries in the rat model. *Caries Research 28* (2) 106-115 1994.

10 Rockwood EW. The effect of neutral salts upon the activity of ptyalin. *Journal of the American Chemical Society 41* 228-230 1919.

11 Boros I, Mozsik G, 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 17* (4) 217-223 1984.

12 Schmidt H, Gocke R. Die Beeinflussung der Amylaseaktivitat (EC 3.2.1.1.) des menschlichen Speichels durch Fluor. *Zahn-, Mund-, und Kieferheilkunde mit Zentralblatt 64* 377-384 1976.

13 Yu M, Sumway M, Brockbank A. Effects of NaF on amylase in mung bean seedlings. *Journal of Fluorine Chemistry 41* 95-100 1988.

14 Somogyi M. Notes on sugar determination. *Journal of Biological Chemistry 195* 19-23 1952.

15 Lowry DH, Rosebrough, NJ, Farr AL et al. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry 193* 265-275 1951.

16 MuClure FJ. Effect of fluorides on salivary amylase. In: *Fluoride Drinking Waters,* Public Health Service, Bethesda MD (USA) 1962 pp 505-507.

17 Hsiu J, Eishcer BH, Stein EA. Alpha-amylases as calcium-metalloenzymes. II. Calcium and the catalytic activity. *Biochemistry 3* 61-66 1964.

Published by the International Society for Fluoride Research
Editorial Office: 81A Landscape Road, Mount Eden, Auckland 4, New Zealand