Published in final edited form as:

J Dent Res. 2008 January ; 87(1): 39–44.

Effects of Fluoride on the Interactions between Amelogenin and Apatite Crystals

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Abstract

Fluorosed enamel is more porous and less mineralized, possibly related to altered amelogenin-modulated crystal growth. The purpose of this study was to examine the role of fluoride in interactions between amelogenin and apatite crystals. Recombinant human amelogenin (rh174) was bound to carbonated hydroxyapatite containing various amounts of fluoride, and analyzed by protein assay, SDS PAGE, and AFM. Interactions between rh174 and fluoride were assayed by isothermal titration calorimetry (ITC). The initial binding rate of rh174, as well as total amount of rh174 bound to fluoride-containing carbonated hydroxyapatite, was greater than that in the control carbonated hydroxyapatite. Fluoride in solution at physiologic (5.3 micromolar, or 0.1 ppm) concentrations showed no significant effect on binding, but higher fluoride levels significantly decreased protein binding. ITC showed no interactions between fluoride and rh174. These results suggest that fluoride incorporation into the crystal lattice alters the crystal surface to enhance amelogenin binding, with no direct interactions between fluoride and amelogenin.

Keywords

amelogenin; hydroxyapatite; fluoride; enamel; fluorosis

INTRODUCTION

Fluoride participates in various aspects of enamel formation and has substantial effects on the properties of the enamel crystals, even at relatively low (micromolar in plasma) concentrations (DenBesten *et al.*, 2002; Robinson *et al.*, 2004). Excess intake of fluoride during enamel development causes enamel defects, which become more severe with increasing fluoride intake and exposure time (DenBesten, 1999; Aoba and Fejerskov, 2002). The mechanisms by which fluoride affects enamel formation to result in fluorosis are not fully understood. However, effects of fluoride on mineralization of the enamel matrix, resulting in enhanced adsorption of matrix proteins to growing crystals, have been proposed (Aoba *et al.*, 1990; Robinson and Kirkham, 1990).

The enamel matrix is secreted by epithelial-derived ameloblasts, as a protein matrix that self-assembles to promote mineral growth (Sasaki and Shimokawa, 1979; Fincham *et al.*, 1995; Fincham and Simmer, 1997; Paine and Snead, 2005). Amelogenin, which is the

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A supplemental appendix to this article is published electronically only at <http://jdr.iadrjournals.org/cgi/content/full/87/1/39/DC1>.

dominant protein in the developing enamel matrix, plays an essential role in the control and modulation of enamel crystal growth (Fincham *et al.*, 1999; Gibson *et al.*, 2001).

When amelogenin binds to crystal surfaces, it inhibits apatite crystal growth (Aoba and Moreno, 1987). Presumably, controlled hydrolysis of amelogenins by the proteinases in the enamel matrix, including MMP-20 and KLK-4 (Hu *et al.*, 2002), results in directed growth of the enamel matrix crystals. Any disruption of this process is likely to alter enamel formation.

The question that we sought to address in this study is whether fluoride can alter the surface properties of apatites, similar to those found in tooth enamel, to affect amelogenin binding to the apatites. Changes in amelogenin binding to apatites could contribute to the formation of fluorosed enamel.

MATERIALS & METHODS

Synthesis and Characterization of Apatites

Hydroxyapatite, carbonated hydroxyapatite, and fluoride-containing carbonated apatites were synthesized as previously described (Featherstone *et al.*, 1983; Ellies *et al.*, 1988), and characterized by x-ray diffraction and FTIR (see APPENDIX for details). The specific surface areas of the apatite particles were measured by the Brunauer-Emmet-Teller (BET) method with a Micrometrics TriStar 3000 (Micrometrics Instrument Corp., Norcross, GA, USA). The surface areas for the various synthetic apatites were similar, ranging between 72 and 80 m²/g.

The fluoride concentrations of the apatite particles were measured by means of an ion specific electrode, following diffusion into an alkaline trap (Zero *et al.*, 1992). Fluoride-containing carbonated apatites that we synthesized contained either 98, 943, or 2021 ppm fluoride.

Synthesis of Recombinant Human Amelogenin (rh174)

rh174 was expressed and purified as described previously (DenBesten *et al.*, 2002; Li *et al.*, 2003). The purified protein was characterized by Western blot, and by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Effects of Fluoride Substitution in Apatite on rh174 Binding to Apatites

To measure the effect of fluoride incorporation into apatite on the initial rate of protein binding, we incubated 150 µg fluoride-containing carbonated hydroxyapatite (950 ppm) and a carbonated hydroxyapatite control with from 0 to 8 µg of rh174 in 200 mM Tris buffer, pH 8.0, at room temperature for 0–240 min. The amount of rh174 remaining in solution was measured by Bradford protein assay.

To determine the effect of fluoride in apatite on the total protein bound to the apatite, we washed hydroxyapatite, carbonated hydroxyapatite, and fluoride-containing hydroxyapatites (98 and 2021 ppm F⁻) (0.5 mg each separately), and equilibrated them in 20 mM Tris-HCl, pH 7.5, in 1.7-mL siliconized tubes (Corning, Corning, NY, USA). Different amounts of rh174 (20, 35, 50, 65, 80, 95, and 110 µg) were incubated with the washed apatite crystals on a shaking incubator for 60 min at 25°C. The samples were centrifuged, and the pellets were collected, washed with 20 mM Tris buffer, and dissolved in 50 µL of 1.0% formic acid, neutralized by 1.5 M Tris-HCl, pH 8.8. The bound protein was visualized by SDS PAGE and quantified by the Bradford assay (Bio-Rad, Hercules, CA, USA). The value was calibrated to the specific surface area of each form of apatite (m²/g).

Effects of Fluoride and Calcium in Solution on rh174 Binding to Apatites

To determine whether F^- in solution would compete with rh174 binding to apatites, we added NaF, and NaCl as a control, at concentrations of 0, 0.1 (or 5.3 μM at a physiological concentration), 500, 1000, and 2000 ppm, to the binding buffer as described above. We added $CaCl_2$ and $MgCl_2$ at 0, 1, 5, and 25 mM, to determine the effect on positively charged ions in blocking rh174 binding to apatite. The bound protein was detected after SDS PAGE and confirmed, by Bradford protein assay, following dissolution of the mineral in acid.

Effect of Fluoride Pre-treatment of Apatite Crystals on rh174 Binding

In separate sets of experiments, 0.5 mg of carbonated hydroxyapatite and fluoride-containing carbonated hydroxyapatite (98 ppm F^-) were pre-treated with 500 μL of NaF solution (500, 1000, 2000, and 5000 ppm F^-) at 25°C for 1 hr. The samples were washed 3 times with 20 mM Tris-HCl, pH 7.5, and analyzed for binding to rh174 as described above.

Atomic Force Microscopy (AFM) Observation of Amelogenin Bound to a Fluorapatite Surface

To determine further the nature of amelogenin binding to fluoride-containing apatite, we used a polished fluorapatite-ceramic disc with oriented rod-like fluorapatite crystals (0.7–1.0 μm wide) embedded in a glass ceramic (Habelitz *et al.*, 2004). These discs were first treated with 200 μL of binding buffer (20 mM Tris-HCl, pH 7.5) containing NaF/NaCl (1000 ppm F^-), allowing either the fluoride or chloride in the solutions to bind to positive sites on the apatite crystal surface. The samples were thoroughly washed, and a 40- μg quantity of rh174 was then added to each sample tube, incubated for 60 min at 25°C in a shaking incubator. The bound protein on the disk surface was observed by AFM (Nanoscope III, Digital Instruments, Santa Barbara, CA, USA) in tapping mode with silicon tips (SSS-NCH-20, Nanosensors, Neuchatel, Switzerland).

Interactions between Amelogenin and Fluoride, Detected by Isothermal Titration Calorimetry (ITC)

After optimization, the interaction between fluoride ions and rh174 was analyzed by computer-controlled incremental (10 μL /injection) injection of 10 mM of NaF (prepared in 10 mM HEPES, pH 7.5, 25°C) into 1.8 mL of 0.05 mM rh174 dissolved in the same HEPES buffer. NaCl (10 mM) was used as a negative control, while calcium (5 mM) was used as a positive control. The resulting titration data were analyzed and fitted with the use of Origin 7.0 for ITC software (OriginLab, Northampton, MA, USA).

Statistical Analysis

All assays were done in triplicate and repeated with 3 different samples. The data were compared by one-way analysis of variance (ANOVA).

RESULTS

Fluoride Substitutions in Apatite Crystal Lattice Increased rh174 Binding

The initial rate of rh174 binding to fluoride-containing hydroxyapatite was greater than that to carbonated hydroxyapatite (Figs. 1A, 1B). The saturated amount of bound rh174 to fluoride-containing hydroxyapatite (2021 ppm F^-) was significantly higher than that of fluoride-containing hydroxyapatite (98 ppm F^-) (1253.6 and 1101.6 $\mu g/m^2$, respectively) (Fig. 1C). Similar amounts of rh174 bound to hydroxyapatite as to carbonated hydroxyapatite (843.3 and 858.5 $\mu g/m^2$, respectively), with no significant difference between the 2 samples.

Fluoride Effects on rh174 Binding to Apatite Crystals were Reduced with Increased Fluoride in Solution

The addition of a physiological concentration of F^- (0.1 ppm) to the assay buffer did not affect the binding of rh174 to carbonated hydroxyapatite and fluoride-containing hydroxyapatite (Fig. 2A). However, the addition of 1000 and 2000 ppm F^- to the buffer solution resulted in significantly decreasing binding to carbonated hydroxyapatite, with a 11.1, 17.1, and 27.6% decrease of bound rh174, respectively (Fig. 2B). rh174 bound to fluoride-containing hydroxyapatite (98 ppm F^-) resulted in 8.6, 41.5, and 58.6% decreases, respectively. There was no effect of NaCl on binding at the same concentrations (Fig. 2C).

Pre-treatment of the apatite with 2000 ppm NaF resulted in significant decreases (30.2 and 42.8%) of rh174 binding on carbonated hydroxyapatite and fluoride-containing carbonated hydroxyapatite (98 ppm F^-) (Fig. 3), as compared with 2000 ppm NaCl, with no further changes at higher fluoride concentrations.

The AFM observation on fluorapatite ceramic disks showed that the (001) face of the typical hexagonal fluorapatite crystal (Fig. 4A) bound rh174 nanospheres (30–60 nm) in control samples pre-treated with NaCl (Fig. 4B). Fewer rh174 nanospheres were detected by AFM on the crystal surfaces pre-treated with NaF (Fig. 4C).

rh174 binding was not affected by pre-treatment of apatite with the divalent ions, Ca^{+2} or Mg^{+2} (APPENDIX).

Fluoride Binding to rh174 Detected by ITC

There was no binding isotherm between F^- and rh174, indicating no apparent detectable interactions between rh174 and fluoride, as measured by ITC (APPENDIX).

DISCUSSION

In these studies, we first sought to determine whether fluoride incorporation into carbonated hydroxyapatites could alter amelogenin binding to apatite. Second, we sought to improve our understanding of the nature of amelogenin/apatite interaction by examining the effects of fluoride ions in solution on protein binding. For these studies, we synthesized carbonated apatites, shown to be similar to the 3–5% carbonated hydroxyapatites found in dental enamel (Nelson and Featherstone, 1982). The fluoride levels chosen for these fluoride-containing carbonated hydroxyapatites were based on fluoride levels in human fluorosed enamel (Richards *et al.*, 1989). The amount of fluoride in the lower-fluoride-containing apatite (approximately 100 ppm F^-) is similar to that found in the inner enamel (300 μm from the surface) of human teeth with minimal fluorosis. The amount in the higher-fluoride-containing apatite (approximately 2000 ppm F^-) is similar to that found in the mid-layer of enamel (150 μm from the surface) in severely fluorosed teeth.

We found that carbonate did not affect amelogenin binding to apatite, suggesting that while carbonate is known to destabilize the crystal lattice (LeGeros and Tung, 1983), the substitution of approximately 3% (overall w/w) carbonate in place of the phosphate ions does not significantly affect the affinity of amelogenin for mineral. However, increased fluoride incorporation into the crystalline lattice resulted in increased amelogenin binding to the apatite crystals. These results support those from a previous study by Tanabe and co-workers (Tanabe *et al.*, 1988), who found that commercially available fluorapatites with increasing levels of fluoride substitutions had increased binding of porcine enamel matrix proteins.

There are several possible explanations for this effect of fluoride. The first, as described by Tanabe and co-workers (Tanabe *et al.*, 1988) is that the fluoride-containing apatite crystal lattice has higher stability, which implies a lower free energy on crystal surfaces. Protein adsorption on the crystal surfaces would then displace more water from fluoride-containing carbonated apatite surfaces, increasing the entropy gain and the intensity of electrostatic interactions between the mineral and protein surfaces. This mechanism may be important in studies with fluorapatite; however, it is not certain whether fluoride at the lower levels incorporated into fluorosed enamel results in a significantly more stable crystal lattice.

A second possibility is that a linear decrease in the magnitude of the unit apatite cell with the addition of fluoride may result in a reduction of the volume of the unit cell when fluoride is incorporated (Moreno *et al.*, 1974; Elliott, 1994). If the fluoride alters the packing of a unit apatite cell, it is possible that relatively more amelogenin binding sites will exist *per* surface area of apatite, thus explaining an increased amount of rh174 bound to fluoride-containing apatite. However, Elliott (1994) showed that there are no measurable changes in the unit cell parameters for enamel if fluoride content is 70–670 ppm (levels that would be found in fluorosed enamel).

A third possibility is that there is a specific interaction between fluoride in the apatite crystals and amelogenin proteins. Kawaski and co-workers suggested that hydroxyapatite binds proteins through both calcium (positive) and phosphate (negative) sites on the surface (Kawasaki *et al.*, 1986, 1987). We used fluoride in solution and also pre-treated the apatite crystals with fluoride to block positive charges on the apatite surfaces. At high fluoride concentrations, amelogenin binding to apatites was partially blocked. ITC showed that this could not be due to a direct interaction between fluoride and amelogenin. These results suggest that fluoride (high levels in solution) can block positively charged apatite surfaces, resulting in reduced amelogenin binding.

Our studies showed that the effects of fluoride pre-treatment on amelogenin binding to surfaces are specific to apatites. When apatite crystals and their surrounding glass surface were pre-treated with fluoride, AFM imaging showed that amelogenin binding was specifically inhibited on the apatite crystal face, but not on the glass surface. It is likely that pre-treatment with the highly electronegative fluoride ion would block positive sites. Therefore, the decreased binding of rh174 to fluorapatite suggests a specific interaction between amelogenin and positive charges in the apatite crystal.

Robinson and co-workers (Robinson *et al.*, 2006) used AFM to show a decreased pKa of the surfaces of apatite crystals dissected from rats ingesting high levels of fluoride as compared with control rats, suggesting that more negatively charged proteins were retained on the apatite crystal surfaces of fluorosed tooth enamel. An explanation for these results could be that fluoride incorporation into apatite alters the overall surface charge of the apatite crystals, to result in a more positively charged surface.

However, our results, showing only partial inhibition of protein binding after the pre-treatment of apatites with high fluoride levels, suggest that amelogenin binding to apatite is due to more than just positive charges on the apatite crystals. Other amelogenin/apatite interactions may include electrostatic interactions between positively charged groups in the protein and negatively charged groups on the apatite surface, as well as hydrophobic interactions and van der Waals forces.

Clearly, further studies are needed, either to predict or to measure the effect of fluoride incorporation on apatite surface charge. It should also be noted that the recombinant amelogenin lacks a single phosphorylation of serine 16. The effect of this phosphorylated amino acid on amelogenin binding to apatite is not known.

Altogether, these results suggest that a primary effect of fluoride incorporation into developing enamel mineral is to enhance binding of amelogenin to fluoride-containing apatite. It is possible that enhanced amelogenin binding to fluoride-containing carbonated apatite in enamel could delay enamel crystal growth during enamel formation, contributing to enamel fluorosis. Further investigations into the effects of fluoride incorporation on the surface charge of apatite crystals, including the formation of amorphous stages of apatite synthesis, are needed to improve our understanding of this interaction.

Acknowledgments

We acknowledge helpful discussions with Drs. Antonius Bronckers and Don Lyaruu, Vrije University and University of Amsterdam (The Netherlands), in the preparation of this manuscript. We are also grateful for support and advice from Dr. Kazuo Tanne, Hiroshima University (Japan). This research was supported by R01-DE015821 to W.L. and R01-DE013508 to P.D.B. from the National Institute of Dental and Craniofacial Research, and by the Overseas Advanced Educational Research Practice Support Program of the Ministry of Education, Culture, Sports, Science and Technology, No. 16-325, Japan. We gratefully acknowledge Dr. Ewa Witkowska for the MALDI-TOF MS analysis of rh174, and Dr. Sally Marshall for x-ray diffraction analysis of the apatites.

REFERENCES

- Aoba T, Fejerskov O. Dental fluorosis: chemistry and biology. *Crit Rev Oral Biol Med*. 2002; 13:155–170. [PubMed: 12097358]
- Aoba T, Moreno EC. The enamel fluid in the early secretory stage of porcine amelogenesis: chemical composition and saturation with respect to enamel mineral. *Calcif Tissue Int*. 1987; 41:86–94. [PubMed: 3115550]
- Aoba T, Moreno EC, Tanabe T, Fukae M. Effects of fluoride on matrix proteins and their properties in rat secretory enamel. *J Dent Res*. 1990; 69:1248–1255. [PubMed: 2162362]
- DenBesten PK. Biological mechanisms of dental fluorosis relevant to the use of fluoride supplements. *Community Dent Oral Epidemiol*. 1999; 27:41–47. [PubMed: 10086925]
- DenBesten PK, Yan Y, Featherstone JD, Hilton JF, Smith CE, Li W. Effects of fluoride on rat dental enamel matrix proteinases. *Arch Oral Biol*. 2002; 47:763–770. [PubMed: 12446183]
- Ellies LG, Nelson DG, Featherstone JD. Crystallographic structure and surface morphology of sintered carbonated apatites. *J Biomed Mater Res*. 1988; 22:541–553. [PubMed: 3410872]
- Elliott, JC. *Studies in inorganic chemistry*. Elsevier; Amsterdam: 1994. Structure and chemistry of the apatites and other calcium orthophosphates.
- Featherstone JD, Mayer I, Driessens FC, Verbeeck RM, Heijligers HJ. Synthetic apatites containing Na, Mg, and CO₃ and their comparison with tooth enamel mineral. *Calcif Tissue Int*. 1983; 35:169–171. [PubMed: 6850399]
- Fincham AG, Simmer JP. Amelogenin proteins of developing dental enamel. *Ciba Found Symp*. 1997; 205:118–130. discussion 130–134. [PubMed: 9189621]
- Fincham AG, Moradian-Oldak J, Diekwisch TG, Lyaruu DM, Wright JT, Bringas P Jr, et al. Evidence for amelogenin “nanospheres” as functional components of secretory-stage enamel matrix. *J Struct Biol*. 1995; 115:50–59. [PubMed: 7577231]
- Fincham AG, Moradian-Oldak J, Simmer JP. The structural biology of the developing dental enamel matrix. *J Struct Biol*. 1999; 126:270–299. [PubMed: 10441532]
- Gibson CW, Yuan ZA, Hall B, Longenecker G, Chen E, Thyagarajan T, et al. Amelogenin-deficient mice display an amelogenesis imperfecta phenotype. *J Biol Chem*. 2001; 276:31871–31875. [PubMed: 11406633]
- Habelitz S, Kullar A, Marshall SJ, DenBesten PK, Balooch M, Marshall GW, et al. Amelogenin-guided crystal growth on fluoroapatite glass-ceramics. *J Dent Res*. 2004; 83:698–702. [PubMed: 15329375]
- Hu JC, Sun X, Zhang C, Liu S, Bartlett JD, Simmer JP. Enamelysin and kallikrein-4 mRNA expression in developing mouse molars. *Eur J Oral Sci*. 2002; 110:307–315. [PubMed: 12206593]

- Kawasaki T, Ikeda K, Takahashi S, Kuboki Y. Further study of hydroxyapatite high-performance liquid chromatography using both proteins and nucleic acids, and a new technique to increase chromatographic efficiency. *Eur J Biochem.* 1986; 155:249–257. [PubMed: 3007123]
- Kawasaki T, Niikura M, Takahashi S, Kobayashi W. Strontium-phosphate hydroxyapatite high-performance liquid chromatography. *Biochem Int.* 1987; 15:1137–1149. [PubMed: 2830881]
- LeGeros RZ, Tung MS. Chemical stability of carbonate- and fluoride-containing apatites. *Caries Res.* 1983; 17:419–429. [PubMed: 6577956]
- Li W, Gao C, Yan Y, DenBesten P. X-linked amelogenesis imperfecta may result from decreased formation of tyrosine rich amelogenin peptide (TRAP). *Arch Oral Biol.* 2003; 48:177–183. [PubMed: 12648554]
- Moreno EC, Kresak M, Zahradnik RT. Fluoridated hydroxyapatite solubility and caries formation. *Nature.* 1974; 247:64–65. [PubMed: 4462607]
- Nelson DG, Featherstone JD. Preparation, analysis, and characterization of carbonated apatites. *Calcif Tissue Int.* 1982; 34(Suppl 2):S69–S81. [PubMed: 6293677]
- Paine ML, Snead ML. Tooth developmental biology: disruptions to enamel-matrix assembly and its impact on biomineralization. *Orthod Craniofac Res.* 2005; 8:239–251. [PubMed: 16238604]
- Richards A, Fejerskov O, Baelum V. Enamel fluoride in relation to severity of human dental fluorosis. *Adv Dent Res.* 1989; 3:147–153. [PubMed: 2640425]
- Robinson C, Kirkham J. The effect of fluoride on the developing mineralized tissues. *J Dent Res.* 1990; 69(Spec Iss):685–691. discussion 721. [PubMed: 2179330]
- Robinson C, Connell S, Kirkham J, Brookes SJ, Shore RC, Smith AM. The effect of fluoride on the developing tooth. *Caries Res.* 2004; 38:268–276. [PubMed: 15153700]
- Robinson C, Yamamoto K, Connell SD, Kirkham J, Nakagaki H, Smith AD. The effects of fluoride on the nanostructure and surface pK of enamel crystals: an atomic force microscopy study of human and rat enamel. *Eur J Oral Sci.* 2006; 114(Suppl 1):99–104. discussion 127–129, 380. [PubMed: 16674669]
- Sasaki S, Shimokawa H. Enamel proteins: biosynthesis and chemistry. *J Dent Res.* 1979; 58(Spec Issue B):765–772. [PubMed: 283117]
- Tanabe T, Aoba T, Moreno EC, Fukae M. Effect of fluoride in the apatitic lattice on adsorption of enamel proteins onto calcium apatites. *J Dent Res.* 1988; 67:536–542. [PubMed: 2844869]
- Zero DT, Raubertas RF, Pedersen AM, Fu J, Hayes AL, Featherstone JD. Studies of fluoride retention by oral soft tissues after the application of home-use topical fluorides. *J Dent Res.* 1992; 71:1546–1552. *erratum in J Dent Res* 72:87, 1993. [PubMed: 1522285]

Figure 1.

Affinity of rh174 to apatites. **(A)** Dissolution of mineral and measurement of the bound protein by Bradford assay showed a similar pattern of rh174 binding to carbonated hydroxyapatite (CHAP) as compared with that to fluoride-containing carbonated hydroxyapatite (FCHAP). **(B)** Within the initial 3 min, rh174 bound more rapidly to carbonated hydroxyapatite as compared with fluoride-containing hydroxyapatite. **(C)** Total rh174 bound to fluoride-containing hydroxyapatite (98 ppm fluoride, $1101.6 \pm 89.2 \mu\text{g}/\text{m}^2$; 2021 ppm fluoride, $1253.6 \pm 101.5 \mu\text{g}/\text{m}^2$) was significantly greater than rh174 bound to hydroxyapatite ($843.3 \pm 68.3 \mu\text{g}/\text{m}^2$) or carbonated hydroxyapatite ($858.5 \pm 69.5 \mu\text{g}/\text{m}^2$), as shown by the Bradford analysis. N (for each sample) = 3. Results represent the means $\pm$ SD. *Significance, $P < 0.01$.

Figure 2.

Effect of NaF on the affinity of rh174 to apatites. **(A)** Bradford assay showed that there was no significant difference in binding of rh174 to carbonated hydroxyapatite and fluoride-containing carbonated hydroxyapatite (98 ppm F⁻) in the presence of a physiological (0.1 ppm) concentration of fluoride. **(B)** Significantly less rh174 bound to carbonated hydroxyapatite in the presence of NaF ($329.9 \pm 32.2 \mu\text{g}/\text{m}^2$ and $274.2 \pm 35.3 \mu\text{g}/\text{m}^2$ at 1000 ppm F and 2000 ppm F, respectively) as compared with the NaCl control ($397.9 \pm 12.1 \mu\text{g}/\text{m}^2$ and $378.9 \pm 12.8 \mu\text{g}/\text{m}^2$ at 1000 ppm Cl and 2000 ppm Cl, respectively). **(C)** There was a similar reduction in the binding of rh174 to fluoride-containing carbonated hydroxyapatite (98 ppm F⁻) ($380.7 \pm 41.5 \mu\text{g}/\text{m}^2$ and $247.9 \pm 24.4 \mu\text{g}/\text{m}^2$ at 1000 ppm F and 2000 ppm F, respectively) in the presence of NaF in solution ($380.7 \pm 41.5 \mu\text{g}/\text{m}^2$ and $247.9 \pm 24.4 \mu\text{g}/\text{m}^2$ at 1000 ppm F and 2000 ppm F, respectively) as compared with the NaCl (control) at > 1000 ppm F in solution ($601.8 \pm 68.3 \mu\text{g}/\text{m}^2$ and $598.9 \pm 32.2 \mu\text{g}/\text{m}^2$ at 1000 ppm Cl and 2000 ppm Cl, respectively). N (for each sample) = 3. Results represent the means $\pm$ SD.

*Significance, $P < 0.01$. NS, no significance.

Figure 3.

Effect of NaF pre-treatment on the affinity of rh174 to apatites. **(A)** Pre-incubation of fluoride-containing carbonated hydroxyapatite (98 ppm F⁻) with NaF (500–5000 ppm) showed that NaF pre-treatment inhibited rh174 binding in dose-dependent patterns (369.7 ± 23.6 µg/m², 247.6 ± 5.3 µg/m², and 243.3 ± 38.2 µg/m² at 1000 ppm F⁻, 2000 ppm F⁻, and 5000 ppm F⁻, respectively) as compared with the NaCl-pre-treated controls (569.3 ± 45.2 µg/m², 538.4 ± 23.6 µg/m², and 564.7 ± 26.9 µg/m² at 1000 ppm Cl⁻, 2000 ppm Cl⁻, and 5000 ppm Cl⁻, respectively). **(B)** Binding of rh174 on carbonated hydroxyapatite or fluoride-containing carbonated hydroxyapatite (98 ppm F⁻) was inhibited by NaF (2000 ppm) pre-treatment (270.8 ± 27.4 µg/m² and 309.3 ± 31.9 µg/m², respectively) as compared with the NaCl-pre-treated controls (388.2 ± 30.2 µg/m² and 540.4 ± 33.2 µg/m², respectively). N (for each sample) = 3. Results represent the means ± SD. *Significance, P < 0.01.

Figure 4.

AFM images of rh174 on fluorapatite glass-ceramic substrates. **(A)** AFM imaging showed both ceramic glass and typical hexagonal (001) faces of fluorapatite crystals, which appear as a dark, hexagonal structure. **(B)** When the fluorapatite crystal embedded in glass ceramic was pre-treated with NaCl, rh174 bound to the glass surface as well as to the apatite. **(C)** When the disk was pre-treated with fluoride, less rh174 bound to the apatite surface (note appearance of the dark apatite shape, C), as compared with the NaCl-pre-treated disk.