Surface adsorption of fluoride and ionic exchange reactions on hydroxyapatite

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The experiments showed that a part of the fluoride which was taken up by hydroxyapatite or teeth at low concentrations of fluoride at neutral pH was soluble in alkali (IN KOH, 24 h) and was thus not fluoroapatite. Calcium fluoride could not form under the present conditions because the solubility of this compound was not exceeded. It is suggested that the alkali soluble fluoride is adsorbed to calcium ions bound as counterions in the hydration layer of the hydroxyapatite or the enamel. This is consistent with previous findings in this laboratory, showing that fluoride displaced acidic proteins adsorbed to calcium receptors on the hydroxyapatite surface in the same way as other anions displace proteins.

It could also be shown that polyanions and polycations adsorbed to hydroxyapatite surfaces by ionic exchange, displacing phosphate or phosphate and calcium respectively in the process. A model is suggested which differs slightly from that proposed by Bernardi.

Key-words: Fluoride; enamel; protein adsorption

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The current concept of fluoride uptake by hydroxyapatite at low concentrations of fluoride and at neutral pH, is that an exchange with hydroxyl groups on the apatite surface takes place (3). However, certain data indicate that a surface adsorption of fluoride occurs; that fluoride interacts with the calcium ions in the hydration layer and that they form a -CaF phase even if the solubility of calcium fluoride is not exceeded (5).

This is also in agreement with the observation that fluoride causes desorption of acidic proteins adsorbed to hydroxyapatite like other anions which are known to interact with the surface of hydroxyapatite, i.e. phosphate (4).

The present paper describes experiments which lend further support to the concept of surface adsorption of fluoride to hydroxyapatite and tooth enamel, and is also concerned with the mechanisms of protein adsorption to hydroxyapatite.

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MATERIALS AND METHODS

Hydroxyapatite and teeth

Hydroxyapatite HTP (Biogel) of a Ca/P ratio of 1.5 and a sample with a Ca/P ratio of 1.61 (received from Dr. G.S. Ingram) were used in the study. Intact premolar teeth cut by the collum were used for the study of F^- uptake by tooth enamel. The exposed dentine was covered with nail varnish. Roots were treated in the same way and used for measurements of uptake of fluoride by cementum. The teeth were thoroughly scrubbed with a brush to remove organic material.

Uptake of F^- by hydroxyapatite or teeth

To each of a series of 100 mg samples of HA was added 3 ml of distilled water containing 1, 2 or 3 ppm of fluoride. The uptake of fluoride was measured after 10 minutes by measuring the loss of fluoride in the supernatant after centrifugation, with an Orion electrode (Orion Ionalyzer Model 407A).

The uptake of 5 toothcrowns or 5 roots was measured in the same way, but no centrifugation was necessary.

The apatite samples or the teeth were then rinsed in distilled water and treated with 1 M KOH for 24 hours (2). The supernatant was neutralized with 6 N HCL and released fluoride calculated by measuring the concentration of fluoride in the supernatant.

Mechanism of adsorption of protein to hydroxyapatite

Two different systems were used; either adsorption of Ca^{45} to hydroxyapatite from a 1 mM solution of $CaCl_2$, or from a 1 mM phosphate buffer pH 7 containing P^{32} phosphate. In both systems the hydroxyapatite samples were treated with both solutions (in the sequence calcium-phosphate), but only one of them contained radioactivity (Fig. 1). Both treatments lasted for 1 hour.

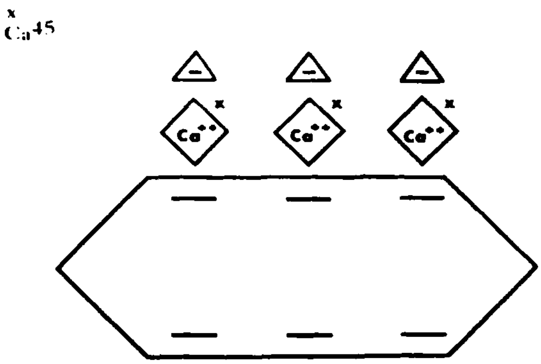

Fig. 1. A diagramatic illustration of the model used for ionic exchange experiments. Two layers of counterions were adsorbed to hydroxyapatite crystals. Either calcium (as in the figure) or phosphate (triangles) were labelled. Displacement of counterions by macromolecules could be demonstrated by release of radio-activity from the apatite samples during adsorption of macromolecules.

The proteins tested for ionic displacements on tooth surfaces were the strongly anionic salivary bloodgroup substance (6) and the strongly cationic protamine (Sigma).

The proteins were dissolved in distilled water pH 6.5–7 and were allowed to interact with the apatite samples for one hour. The displacement of small molecular ions in the hydration layer was estimated by scintillation counting.

A Packard Tri-carb liquid scintillation counter using 10 ml of Packard "Insta-Gel" was employed. The isotopes were received from Norsk Atominstitutt, Kjeller, Norway.

RESULTS

Uptake and release of fluoride from hydroxyapatite samples

Both hydroxyapatite preparations took up increasing amounts of fluoride at increasing concentrations of fluoride, but the HTP apatite having a Ca/P ratio of 1.5 took up

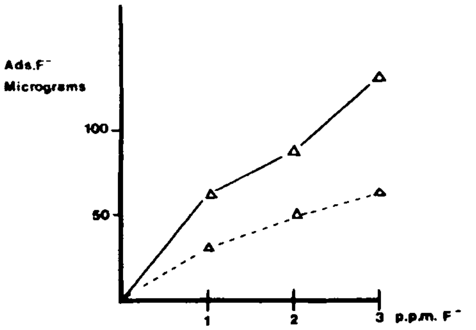

Fig. 2. The figure shows uptake of fluoride by hydroxyapatite (Biogel HTP) from neutral solutions of sodium fluoride containing 1, 2 or 3 p.p.m. of fluoride (solid line) and the amounts which could be recovered after alkali treatment of the apatite (dotted line). (100 mg hydroxyapatite, 100 ml fluoride solution, pH 7, reaction time 10 min.)

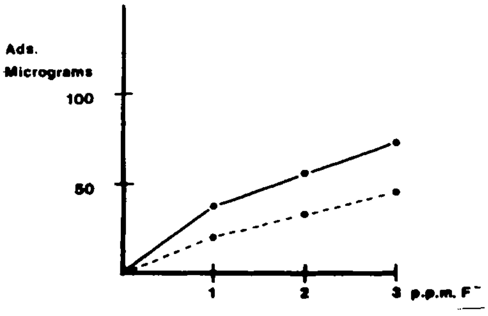

Fig. 3. Uptake of fluoride by a hydroxyapatite sample Ca/P 1.61, (solid line) and the alkali soluble fraction of this uptake (dotted line). Experimental conditions as described in Fig. 2.

more than the preparation with a ratio of 1.61. About 50 per cent of the fluoride originally lost to the apatite samples was regained after alkali treatment of the samples (24 h, 1 M KOH). The results are given in Figs. 2 and 3.

Uptake and release of fluoride from dental enamel and cementum

A similar pattern was seen in teeth and roots. Several groups of teeth were tested. Figures 4 and 5 give representative results. They show that the tooth crowns took up much less fluoride than the hydroxyapatite samples and that almost all the fluoride could be accounted for after alkali treatment. The roots behaved more like the apatite samples, and took up more fluoride than the enamel.

Ionic exchange on hydroxyapatite surfaces during adsorption of proteins

Adsorption of polyanionic salivary blood group substance caused displacement of phosphate from the apatite surface, but no displacement of calcium.

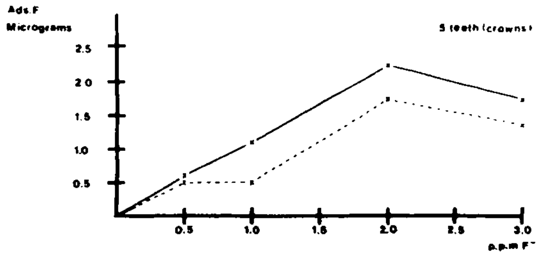

Fig. 4. Uptake of fluoride by 5 tooth crowns from fluoride solutions containing 0.5–3 p.p.m. of fluoride (solid line). Release of fluoride after alkali treatment (dotted line). (100 ml, NaF pH 7, 10 min. reaction time.)

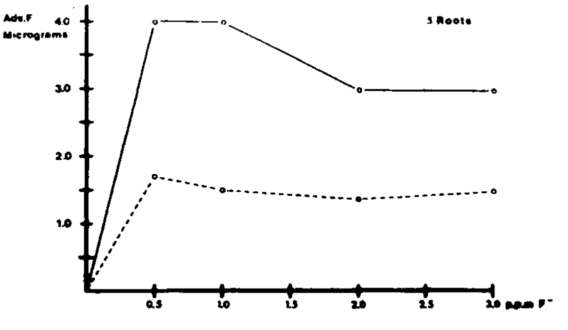

Fig. 5. Uptake of fluoride by 5 roots (solid line) and release of fluoride after alkali treatment (dotted line). (Experimental conditions as in Fig. 4.)

Table 1. *Displacement of adsorbed $P^{32}O_4$ from hydroxyapatite by proteins*

100 mg HA took up 880 000 c.p.m. $P^{32}O_4$	
2 mg/ml Protamine released	154 640 c.p.m. 179 325 c.p.m.
Salivary blood group substance 1 mg/ml	169 992 c.p.m. 177 906 c.p.m.
Distilled water	23 680 c.p.m. 22 632 c.p.m.

OH^- (1 M KOH 24 h) and H^+ (0,1 M acetate buffer pH 4) released all the radioactivity from the hydro apatite.

Table 2. *Displacement of adsorbed Ca^{45} from hydroxyapatite by proteins*

100 mg HA took up 3 600 000 c.p.m. Ca^{45}	
distilled water released	28 046 c.p.m. 28 878 c.p.m.
2 mg/ml Protamine released	115 464 c.p.m. 122 187 c.p.m.
10 mg/ml Protamine released	136 446 c.p.m. 149 152 c.p.m.
20 mg/ml Protamine released	146 677 c.p.m. 143 151 c.p.m.
2 mg/ml acidic salivary glycoprotein	29 284 c.p.m. 29 897 c.p.m.

H^+ (0,1 M acetate buffer pH 4) released all the radioactivity.
OH^- had no effect.

Adsorption of the polycation protamine caused displacement of both calcium and phosphate.

A limited series where bacteria were used indicated that Strep. mutans (Strain Ingbritt) behaved like polyanions and displaced phosphate.

Representative results of experiments are given in Tables 1 and 2.

Alkali (1 M KOH) displaced phosphate but not calcium, whereas a 0.1 M acetate buffer (pH 4) displaced both ions in 1 hour.

DISCUSSION

The rapid uptake of fluoride from the solutions in the present experiment would normally have been interpreted in terms of fluoroapatite formation due to exchange of F^- with OH^- ions, according to the current concept of the mechanism of fluoride uptake (3). The interpretation would have been based on the argument that CaF_2 could not have been formed during the present conditions because the solubility of this substance was not exceeded in the supernatant. However, the fact that a major part of the fluoride was released by alkali treatment demonstrates clearly that this concept is not completely valid in this case. It seems likely that a part of the fluoride taken up is adsorbed to calcium ions in the hydration layer. It has been shown previously that a pretreatment of hydroxyapatite with calcium gives increased uptake of fluoride (5), and furthermore, that fluoride displaces acidic proteins adsorbed to hydroxyapatite surfaces in the same way as phosphate ions (4). All these observations are consistent with the concept that some fluoride binds to calcium ions present in the hydration shell, as mentioned above. The observation that anions in the hydration shell are displaced by hydroxyl ions (presumably by competition) further strengthens this view (Table 1).

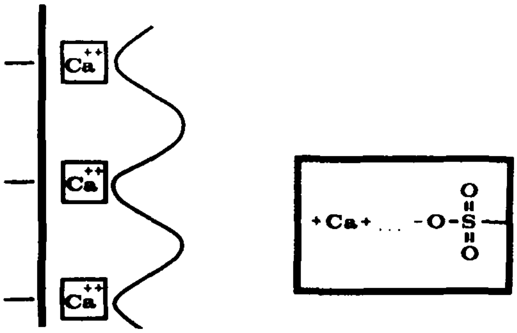

Fig. 6. A possible model explaining the results obtained in the present experiments. Acidic macromolecules are adsorbed to calcium ions present in the hydration layer of the hydroxyapatite.

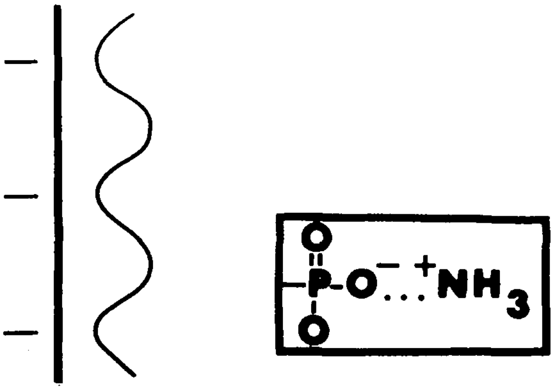

Fig. 7. A possible model explaining the results obtained in the present experiments. Basic proteins are adsorbed to the phosphate groups of the surface proper.

The relatively low uptake of tooth crowns compared with apatite samples is presumably related to the low surface area of the teeth. The high uptake of the cementum may have been influenced by the presence of traces of strongly ionic organic material or by the larger surface area of the cementum.

The amount of recovered fluoride after alkali treatment is probably on the conservative side in all cases reported.

The results concerning the ionic exchange caused by proteins adsorbed to apatite surfaces are consistent with the models illustrated in Figs. 6 and 7. These in general support the concept of Bernardi and coworkers (1), but indicate that a major part of the phosphate groups which bind polycations is covered by a layer of calcium counterions, which are displaced in the process. It seems likely that almost all the calcium groups binding polyanions are in fact present in the hydration layer, as counterions.

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