Crystallographic properties of heterogeneous Mg-containing fluoridated apatites synthesized with a two-step supply system

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Mg-containing fluoridated hydroxyapatites (Mg–MgFAp and MgF–MgAp) were synthesized heterogeneously with two different modes of fluoride supply. The expanded (300) reflections of the X-ray diffraction of both apatites were broader than and different from those of the mechanical mixture of the homogeneous apatites (MgAp and MgFAp). Scanning electron microscopy (SEM) showed that the crystal size and shape of Mg–MgFAp and MgF–MgAp were different. Energy dispersive spectroscopy (EDS) indicated that the fluoride intensity of Mg–MgFAp synthesized with the fluoride supply in the latter half of the experimental period was higher than that of MgF–MgAp. The apparent solubility of Mg–MgFAp was lower than that of MgF–MgAp. These results suggested that two different types of Mg-containing fluoridated hydroxyapatites might be formed: surface fluoride-rich apatites (Mg–MgFAp) and inner fluoride-rich apatites (MgF–MgAp).

Keywords: Heterogeneous synthesis, apatites, magnesium, fluoride

Received 10 August 1994; accepted 19 October 1994

Heterogeneous formation of apatites seems to occur in nature. To investigate such heterogeneous precipitation phenomena in biological systems, we are continuing our synthesis experiments using a simple two-step supply system. Our previous study on the heterogeneous synthesis of fluoridated hydroxyapatites with two different fluoride supplies, and by means of wavelength dispersive spectroscopy (WDS) analysis and solubility experiments, suggested that hydroxyapatite covered with fluorapatite and fluorapatite covered with hydroxyapatite might be formed. Heterogeneous fluoridated CO₃ apatites also showed two different types of crystals, including a large crystal covered with many small crystals in the case of synthesis with the fluoride supplied during the initial half of the experimental period. Fluoride is known to act as a caries-preventive agent and to increase the crystallinity of apatites. On the other hand, magnesium significantly affects the morphology ofapatite crystals by retarding crystal growth and promoting dissolution in addition, magnesium uptake accelerates in the presence of fluoride. Mg-containing fluoridated hydroxyapatites showed unique crystallinity behaviour in various series with different magnesium contents.

Therefore, in this study Mg-containing fluoridated hydroxyapatites were synthesized heterogeneously using a two-step fluoride supply system, which was carried out as in the previous studies, and their crystallographic properties were examined.

MATERIALS AND METHODS

Two types of Mg-containing fluoridated hydroxyapatites (Mg–MgFAp and MgF–MgAp) were synthesized at 80 ± 1°C in the presence or absence of fluoride. Mg–MgFAp-type apatites were made by feeding 0.5 l of 100 mmol l⁻¹ Ca(CH₃COO)₂H₂O solution containing 10 mmol l⁻¹ Mg(CH₃COO)₂H₂O and 0.25 l of 60 mmol l⁻¹ NH₄H₂PO₄ solution during the initial half of the experimental period, and then 0.25 l of 60 mmol l⁻¹ NH₄H₂PO₄ solution containing 20 mmol l⁻¹ HF during the remainder of the experiment. The other MgF–MgAp-type apatite was synthesized under the reverse conditions. The two solutions were supplied at 250 ml h⁻¹ with a Tokyo Rikakikai microtube pump into 1 l of mechanically stirred 1.3 mol l⁻¹ CH₃COONH₄ solution. The pH was maintained at 7.4 ± 0.2 with occasional addition of concentrated NH₄OH solution. The suspension was stirred for 3 h, then kept at 25°C for 24 h. Slurries were filtered, washed with distilled water, then dried at 80°C. For comparison, Mg containing hydroxyapatite, fluoridated hydroxyapatite with the degree of fluoridation X = 0.5, and fluorapatite were also synthesized homogeneously using a constant fluoride supply.
X-ray diffraction was employed to identify precipitates and estimate crystallinity. Measurements were made on the Rigaku Denki X-ray diffractometer with graphite-monochromatized CuKα radiation at 35 kV and 23 mA. Photomicrographs of crystals were obtained with a Nihon Denshi JSM-5400 scanning electron microscope, and the fluoride content of each crystal was analysed by energy dispersive spectroscopy (EDS). Apatite pellets (10 mm in diameter) were prepared from 300 mg of powder by compression at 0.5 ton and heating at 500 °C. Calcium and magnesium concentrations (analytical error ±3%) were determined by atomic absorption spectrophotometry. Total phosphate concentrations (analytical error ±3%) were determined by the ultraviolet spectrophotometric method of Eastoe. Fluoride concentrations (analytical error ±2%) were determined with a fluoride electrode. To estimate solubility, samples were left to stand in 50 ml of 0.51 mol l−1 acetate buffer solution (pH 4.0) at 37 °C for 1 d or 1 month, and then the calcium and phosphate concentrations in the solution were determined.

RESULTS

X-ray diffraction patterns of the precipitates synthesized homogeneously and heterogeneously are shown in Figure 1. All patterns were typically apatitic and relatively well-crystallized.

The expanded (300) reflection peaks of both heterogeneous Mg-containing fluoridated apatites (MgMgFAp and MgF-MgAp) were broader than those of the homogeneous Mg-containing apatite (MgAp) and fluoridated apatite (MgFAp) as shown in Figure 2A. However, those peaks were not as less broad as those of a mechanical mixture of MgAp and MgFAp and quite different from the sharp peak of homogeneous fluoridated apatites synthesized with the degree of fluoridation X = 0.5 (Figure 2B).

The chemical compositions of the heterogeneous Mg-containing fluoridated apatites are shown in Table 1. The supplied fluoride was almost completely taken up into the apatite crystals, and the fluoride contents of both Mg–MgFAp and MgF–MgAp were half that of homogeneous MgFAp, which is almost the same as that of fluorapatite (2 mmol g−1). The magnesium content of Mg–MgFAp was slightly higher than that of MgF–MgAp, probably because magnesium uptake accelerates in the presence of fluoride. In fact, the fluoride content of homogeneous MgAp and MgFAp was much higher than that of fluoride-free MgAp. The calcium and phosphate contents of the heterogeneous and homogeneousapatites were not significantly different.

Scanning electron photomicrographs of both heterogeneous Mg–MgFAp and MgF–MgAp, together with those of homogeneous MgAp and MgFAp, are shown in Figure 2.

Table 1 Chemical compositions of homogeneous and heterogeneous Mg-containing fluoridated hydroxyapatites (n = 5)

<table>
<thead>
<tr>
<th>Apatite Type</th>
<th>Ca (mmol g⁻¹)</th>
<th>Mg (mmol g⁻¹)</th>
<th>P (mmol g⁻¹)</th>
<th>F (mmol g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg–MgFAp</td>
<td>9.21±0.09</td>
<td>0.24±0.008</td>
<td>5.57±0.03</td>
<td>0.872±0.010</td>
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<tr>
<td>MgF–MgAp</td>
<td>9.45±0.03</td>
<td>0.22±0.005</td>
<td>5.67±0.04</td>
<td>0.998±0.006</td>
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<tr>
<td>MgAp</td>
<td>9.20±0.08</td>
<td>0.19±0.001</td>
<td>5.73±0.03</td>
<td>0</td>
</tr>
<tr>
<td>MgFAp</td>
<td>9.40±0.08</td>
<td>0.26±0.008</td>
<td>5.41±0.05</td>
<td>1.87±0.02</td>
</tr>
</tbody>
</table>

Figure 1 X-ray diffraction patterns of heterogeneous Mg-containing fluoridated hydroxyapatites (Mg–MgFAp and MgF–MgAp), and of homogeneous Mg-containing hydroxyapatite (MgAp) and fluorapatite (MgFAp).
in Figure 3. The crystal size of MgF–MgAp was larger than that of Mg–MgFAp, possibly due to the effect of MgFAp as an inner core, reflecting the later crystal growth.

Figure 4 shows the energy dispersive spectra of both heterogeneous Mg-containing fluoridated apatites. Mg–MgFAp, which was synthesized with the fluoride supply during the second half of the experimental period, showed a higher F Ka peak than MgF–MgAp.

After incubation in 0.5 M acetate buffer solution (37 °C, pH 4.0) for one month, the (300) reflection peaks (Figure 5) in the X-ray diffraction patterns of both Mg-MgFAp and MgF–MgAp were sharper than those of the original apatites (Figure 2A). In particular, the (300) reflection peak of MgF–MgAp approached that of homogeneous MgFAp. After incubation for one day, the calcium concentration as a measure of the apparent solubility of MgF–MgAp was higher than that in Mg–MgFAp as shown in Table 2. However, after incubation for one month, both calcium concentrations were close to each other, although they were lower than in the fluoride-free homogeneous Mg-containing apatite MgAp. Calcium and phosphate concentrations in the solution of homogeneous MgFAp after incubation for one month were lower than those after incubation for one day. This may be due to the use of Ca^{2+} and PO_4^{3-} ions for recrystallization. In fact, the magnesium concentration in the solution after incubation for one month was much higher than that after incubation for one day.

DISCUSSION

At the weak alkaline pH of 7.4, the relatively high calcium concentration of 100 mmol l^{-1} and the temperature of 80 °C used for the synthesis in this study, non-apatitic substances and/or amorphous calcium phosphates (ACP) are difficult to precipitate even in the presence of Mg^{2+} ions as a crystal growth inhibitor, and especially in the presence of fluoride ions, although a high concentration of Mg^{2+} ions induces the formation of amorphous apatites. Therefore, the two-step supply system is simpler and easier to use to investigate heterogeneous precipitation phenomena compared with those observed in general spontaneous precipitation and in other biological
systems. For heterogeneous precipitations with the two-step supply system such as in this study, several different types of product can be predicted: a mixture of crystals synthesized independently in the first and second halves of the experiment, crystals formed in the presence of fluoride both early and late in the experiment, and complex crystals with an inner and outer layer.

The (300) expanded reflection peaks of both heterogeneous Mg-containing fluoridated apatites, Mg-MgFAp and MgF-MgAp, were broader than those of the Mg-free fluoridated apatites, H-FAp and F-HAP, reported previously. However, they did not split like those of the heterogeneous fluoridated CO₃ apatites, CO₃-FCO₃Ap and FC0₃-C0₃Ap, reported previously. On the other hand, the (300) peak of the mechanical mixture of homogeneous MgAp and MgFAp was broader, and was split (Figure 2B). Furthermore, homogeneously synthesized fluoridated hydroxyapatite with the degree of fluoridation X = 0.5 showed a much sharper (300) reflection peak. Therefore, it can be speculated that both Mg-MgFAp and MgF-MgAp have crystal features quite different from those of the mixture and/or homogeneous Mg-containing fluoridated hydroxyapatite with X = 0.5. In general, crystallinity, or broadening of the X-ray diffraction peak, is affected by crystal size and lattice imperfection. In this study, the apparent broadening observed in the X-ray diffraction spectra of heterogeneous Mg-containing fluoridated hydroxyapatites may be due to the overlap of the peaks of outer and inner crystals rather than to both above-mentioned factors, because the X-ray diffraction beam could partially penetrate the inner core.

Although SEM observation did not clearly show a double-layered crystal structure such as that observed in heterogeneous fluoridated CO₃ apatites, the crystal shapes were quite different from each other (Figure 3). In addition, surface analysis (EDS) indicated that fluoride peak intensity was different for both heterogeneous apatites Mg-MgFAp and MgF-MgAp (Figure 4). Therefore, these results suggest that two different types of heterogeneous Mg-containing fluoridated apatites were formed, and support the speculation proposed previously from the results of heterogeneous fluoridated CO₃ apatites and heterogeneous fluoridated CO₃ apatites. The results of the apparent solubility experiments (Table 2) also support these findings.

The (300) reflection of heterogeneous and homogeneous Mg-containing fluoridated apatites shifted toward the high angle direction due to substitution of hydroxyl by fluoride ions which contracts the a-axis dimension. In heterogeneous systems, the crystallinity of Mg-containing fluoridated hydroxyapatites changes dramatically with the increase in Mg content. For a Mg supply of 10 mmol l⁻¹, the crystal size was the maximum (half of the fluoride content) at the degree of fluoridation X = 0.5.

Table 2: Apparent solubility of homogeneous and heterogeneous Mg-containing fluoridated hydroxyapatites after incubation in 0.5 mol l⁻¹ acetate buffer solution (37°C and pH 4.0) for one day and one month (n = 5)

<table>
<thead>
<tr>
<th></th>
<th>Ca (mmol l⁻¹)</th>
<th>Mg (mmol l⁻¹)</th>
<th>P (mmol l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>One day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg-MgFAp</td>
<td>8.02±0.20</td>
<td>0.603±0.003</td>
<td>3.90±0.11</td>
</tr>
<tr>
<td>MgF-MgAp</td>
<td>12.70±0.2</td>
<td>0.318±0.004</td>
<td>5.99±0.20</td>
</tr>
<tr>
<td>MgAp</td>
<td>32.30±0.3</td>
<td>0.643±0.006</td>
<td>17.00±0.10</td>
</tr>
<tr>
<td>MgFAp</td>
<td>6.70±0.02</td>
<td>0.395±0.004</td>
<td>2.64±0.05</td>
</tr>
<tr>
<td><strong>One month</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg-MgFAp</td>
<td>19.10±0.2</td>
<td>0.959±0.002</td>
<td>12.00±0.1</td>
</tr>
<tr>
<td>MgF-MgAp</td>
<td>20.60±0.1</td>
<td>0.706±0.002</td>
<td>13.00±0.1</td>
</tr>
<tr>
<td>MgAp</td>
<td>33.30±0.4</td>
<td>0.804±0.007</td>
<td>21.50±0.2</td>
</tr>
<tr>
<td>MgFAp</td>
<td>3.39±0.10</td>
<td>1.770±0.01</td>
<td>2.61±0.14</td>
</tr>
</tbody>
</table>

Biomaterials 1995, Vol. 16 No. 9
Homogeneous MgFAp crystals as shown in Figure 3D were large, while MgAp crystals were of a relatively small size (Figure 3C). Their crystal shapes seem to reflect those of the heterogeneous apatites. Mg-MgFAp (Figure 3A) is similar to homogeneous MgAp, and MgF–MgAp (Figure 3B) is similar to MgFAp. That is to say, the initial crystal shape as a core may have an effect upon the final crystal shape.

In the case of heterogeneous fluoridated CO₃ apatites, a double-layered crystal structure was observed because there was a large difference between the crystal size of the homogeneous CO₃ apatite and the fluoridated CO₃ apatite. However, in the heterogeneous fluoridated hydroxyapatites and Mg-containing fluoridated hydroxyapatites in this study, the size of the homogeneous hydroxyapatite and fluorapatite crystals were not significantly different, so that epitaxial formation of heterogeneous apatites might progress at the interface between inner core crystal and outer surface crystal.

Finally, data for the Mg containing fluoridated hydroxyapatites synthesized heterogeneously, using the present two-step fluoride supply system, supported the results concerning heterogeneous fluoridated hydroxyapatites reported previously.

ACKNOWLEDGEMENT

This study was supported in part by Research Grant No. 03670912 from the Ministry of Education of Japan.

REFERENCES