Feasibility Study for the Detection of Lead Substitution Sites in the Hydroxyapatite Crystal Structure using High-Resolution Electron Microscopy (HREM) at Optimum Focus

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Abstract

The theoretical feasibility of differentiating high-resolution electron microscope images obtained at Sherzer focus for two crystals of the same chemical composition [Pb6Ca4(PO4)6(OH)2] has been investigated for two commercially available electron microscopes: the Jeol 4000 EX microscope (Cs = 0.48 mm, V = 400 kV) and the Jeol 200 CX microscope (Cs = 1.2 mm, V = 200 kV) and at the following resolutions: 1.4, 2.0 and 2.5 Å. In these crystals the lead atoms are distributed either preferentially in the Ca(2) sites [Pb6(2)Ca4(1)(PO4)6(OH)2] or evenly in both Ca sites [Pb3.6(2)Ca2.4(2)Pb2.4(1)Ca1.6(1)(PO4)6(OH)2]. Crystals of various thicknesses oriented along the [2110] crystallographic direction have been investigated. The results show differences between images of the two structures, which are more marked when the crystal thickness is increased. A compromise between medium and ultra-high resolution had to be reached for interpretation of the images.

Introduction

Lead as well as other metals (Sr, Cd, Na, K, Mn, Zn, Mg, Fe, Ti, V, U) can be readily incorporated into the hydroxyapatite unit cell [chemical formula: Ca6(2)Ca4(1)(PO4)6(OH)2] where they replace the calcium ions (Dallemagne, 1964; Akhavan-Niaki & Wallaeys, 1958; Lacout, Assarane & Trombe, 1984). The study of the mechanism of substitution of these metals into the hydroxyapatite structure is important for understanding their incorporation in the calcified tissues of vertebrates in different environmental situations (lead pollution, strontium radioactive fall out etc.). This problem is also relevant to the industrial purification of precious metals such as Ti, V and U.

Exposure to lead in soils, in the atmosphere or in food can be harmful to health. Although not much is known about the effects of relatively low doses (1 µg of Pb per dl of blood), exposure to large doses of lead can cause anemia (Klander & Petering, 1977), renal insufficiency (Campbell, Beattie, Moore, Goldberg & Reid, 1977; Wedeen, Maesaka, Weiner, Lipat, Lyons, Vitale & Joselow, 1975) and encephalopathy (Goldstein, 1977). As stated by Rabinowitz, Wetherill & Kopple (1973) the calcified tissues (bones and teeth) and more specifically their inorganic phases act as sinks for the lead absorbed by the human body where it is ultimately incorporated into the apatite structure.

A step towards understanding the mechanisms of substitution of Pb for Ca in mineral and biological apatites is the determination of the detailed structural location of the Pb-substituted atoms in the simpler systems provided by synthetic apatites.

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Ge atoms in the crystals analyzed. Since the images only show a projection of the crystal structure, a unique atomic structure could not be determined, however the concentration of metal atoms at given sites, accounting for a given image contrast, was determined by varying the occupancy factor in the computed images until a match was obtained with the micrographs.

Very recently, with the use of high-resolution electron microscopes, electron micrographs of synthetic and enamel apatite showing Ca, P and OH atomic sites were obtained. These micrographs were matched for several directions, defocus values and crystal thicknesses to images, computed for the hydroxyapatite atomic positions and microscope parameters (Brès, Barry & Hutchison, 1983, 1985; Ichijo, Yamashita, Akhori, Kanaya & Baba, 1983; Ichijo & Yamashita, 1983; Kanaya, Baba, Shinohara & Ichijo, 1984; McLean & Nelson, 1982, 1983).

However, in these studies the variation of stoichiometry due to the substitution of Ca atoms by other metal atoms has not been investigated.

It is the purpose of this work to determine the theoretical feasibility of a subsequent study concerning the detection of preferential Pb-substitution sites inside the hydroxyapatite unit cell using HREM. The chemical formula of the crystal under investigation is \( \text{Pb}_6\text{Ca}_4(\text{PO}_4)_6(\text{OH})_2 \). Two cases have been considered. Case 1: 'Preferentially distributed lead apatite' (PDLA) where all Pb atoms occupy all Ca(2) sites and all Ca atoms occupy all Ca(1) sites. Case 2: 'Evenly distributed lead apatite' (EDLA) where the lead and the calcium atoms are evenly distributed over all Ca(1) and Ca(2) sites. Images of PDLA and EDLA have been computed for different microscope resolutions and crystal thicknesses.

**Structure of lead-substituted hydroxyapatite**

Hydroxyapatite crystallizes in the hexagonal system and has \( P6_3/m \) for space group. Since lead-substituted hydroxyapatite is isomorphous with calcium hydroxyapatite (Bhatnagar, 1971), the atomic coordinates of calcium hydroxyapatite were used (Kay, Young & Posner, 1964). The two possible sites for the hydroxy-group oxygen atoms were accounted for by setting the occupancy factor to 0.5 for these sites. In this way the centrosymmetry of the structure is preserved because of the statistical distribution of the hydroxy groups above and below the mirror plane. The lattice parameters used have been determined by Verbeeck, Lassuyt, Heijligers, Driessens & Vrolijk (1981) for a 60% lead substitution (\( a_1 = a_2 = a_3 = 9.668, c = 7.096 \) Å). For EDLA the occupancy factor was set to 0.6 for Pb atoms and 0.4 for Ca atoms in all Ca sites. For PDLA all Pb atoms were assigned to the Ca(2) sites and all Ca atoms to the Ca(1) sites. In both cases the Debye–Waller factors determined by Kay, Young & Posner (1964) were used, Fig. 1.

**Procedure for computation**

The images shown in this paper were calculated by the multislice method (Cowley & Moodie, 1957) using the SHRLI suite of computer programs described by O'Keefe & Buseck (1979).

The values used for the microscope parameters correspond to the specifications of two existing microscopes: (i) a Jeol 4000 EX microscope such as the one to be installed at Oxford, which shows a Sherzer resolution of 1.4 Å, an information resolution limit of 0.83 Å⁻¹ and a voltage of 400 kV; (ii) a Jeol 200 CX microscope showing a Sherzer resolution of 2.4 Å, an information resolution limit of 0.6 Å⁻¹ and a voltage of 200 kV (Spence, 1981). (i) In the case of the 4000 EX microscope the sizes of the apertures were 0.71 and 0.5 Å⁻¹, these values correspond to resolution values at optimum focus of 1.4 and 2.0 Å, respectively; (ii) in the case of the 200 CX microscope an aperture of 0.4 Å⁻¹ corresponding to the cut-off value of the contrast transfer curve at optimum focus was chosen, this value corresponds to a resolution of 2.5 Å, which is currently obtained on the Jeol 200 CX microscope.

The \( \sin(\chi) \) curves for both microscopes set at optimum focus are shown in Figs. 2 and 3.

The other parameters used in the calculation were the following. Direction of observation: [2110]; slice thickness: 4.834 Å; thickness values of the structures imaged: \( T_1 = 9.668, T_2 = 19.336, T_3 = 38.672, T_4 = 77.344, T_5 = 154.688 \) and \( T_6 = 241.70 \) Å. In the case of the Jeol 4000 EX microscope, the operating param-
eters used were: halfwidth of Gaussian spread of focus: 30-0 Å; semi-angle of beam convergence: 0-2 mrad; in the case of the 200 CX microscope these values were 50-0 Å for the halfwidth of Gaussian spread of focus and 0-8 mrad for the semi-angle of beam convergence. No vibration of the microscope was assumed. The number of beams used in the multislice calculations was 1165 and in all cases the convolution check gave a value of 0-99999(1) (≈ 1).

The images were printed on a microfiche printer using an overwriting routine for producing the contrast. In order to take into account the variation of contrast between images care was taken to process all images simultaneously. A special routine was also used for making Pendellössung plots for a selection of beams.

**Results and discussion**

The results of the computations are shown in Figs. 4, 5 and 6. We have performed Pendellössung plots for a selection of beams at 200 and 400 kV; these beams are the ones with the highest intensity as well as the most dependent on the cation distribution. Several remarks can be made about these curves:

1. There is very little difference in the shape of the curves in the 200 and 400 kV cases. The difference in the overall intensity of the diffracted beams with respect to the central beam is higher in the 400 kV case, as is expected;
2. For both cases of cation distributions and for both voltages a direct comparison of the Pendellössung curves with the reference logarithmic curve shows, except for the 0330E reflection a linear behaviour of all beams up to a thickness of 77-34 Å. A rough estimate of the relative intensity dip of the 0330 beam of 1% in the linear range can be observed at that thickness value.

A direct comparison between images corresponding to one unit cell of crystals of the EDLA and the PDLA structures oriented along the [2110] direction is shown in Fig. 6. The choice of the direction of observation of the two crystal structures has been made because of the sensitivity of the observed images to the diffusion of the electrons from the electron gun in this particular direction. Furthermore, there are two directions equivalent to the [2110] direction in the hexagonal system (i.e. [110] and [1210]), which will

![Fig. 2. Plot of the sin(θ) curve for the following values: C_s = 0.485 mm, df = -325 Å and λ = 0.0164 Å.](image)

![Fig. 3. Plot of the sin(θ) curve for the following values: C_s = 1.2 mm, df = -650 Å and λ = 0.0251 Å.](image)

![Fig. 4. Pendellössung plots for a selection of beams at 400 kV for the PDLA (denoted by P) and EDLA (denoted by E) structures. The intensity curve is logarithmic, a check of the linearity of a curve can be made by comparison with the log_{10}(x) curve.](image)

![Fig. 5. Pendellössung plots for a selection of beams at 200 kV, the display parameters are the same as in Fig. 4.](image)
facilitate the orientation of the crystals under the microscope.

Several observations can be made about Fig. 6:
(1) no difference can be observed in the PCD (projected charge density) images;
(2) for all resolutions, no difference can be observed between the EDLA and the PDLA images up to a crystal thickness of 38.672 Å;
(3) for all resolutions, a clear difference between images of both structures can be observed at a thickness of 77.34 Å and above;
(4) in the 1.4 Å resolution case and for both

![Resolution Chart](image)

Fig. 6. Calculated images of the EDLA (denoted by E) and the PDLA (denoted by P) structures showing a variation of image contrast with thickness and resolution (PCD = projected charge density image). The 1.4 and 2.0 Å resolution values were obtained on the 4000 EX microscope at 400 kV and the 2.5 Å value was obtained on the 200 CX microscope at 200 kV. Each image shown corresponds to one unit cell, the c parameter corresponds to the vertical scale and the a parameter to the horizontal scale.
structures, a progressive variation of image contrast between the single-unit-cell-thick crystal (9-668 Å) up to the 77-34 Å thick crystal (= 16 unit cells) can be observed. Beyond this crystal thickness no correlation with the images produced at lower thickness values can be made;

(5) at a resolution of 2-0 Å, a similar observation can be made.

(6) at a resolution of 2-5 Å, the progressive variation of image contrast can only be observed up to a thickness of 38-67 Å (= 8 unit cells).

The inclusion of an increasing number of diffracted beams into the microscope objective aperture produces an increase in resolution of the images observed. This effect is shown in Figs. 2 and 3, where the sin(χ) curves are plotted for the two microscopes used and at optimum focus. The resolution corresponding to a series of reflections originating from the structures studied is shown in Table 1. At a resolution of 2-0 Å twelve more crystallographic reflections than at a resolution of 2-5 Å are included in the aperture. At a resolution of 1-4 Å thirty more reflections than at 2-0 Å are included in the aperture.

However, in this particular study our aim was to distinguish between cation distributions in two structures of identical chemical composition rather than identifying each ionic site separately. We can then be well served with a medium–high-resolution microscope of lower purchase price.

Also, the difficulty of interpreting images obtained at such a resolution would be reduced because of the smaller quantity of information present in the micrographs. But, as can be seen in Fig. 6, the use of a higher voltage permits the observation of thicker specimens, which facilitates the differentiation between the EDLA and the PDLA structures.

### Validity of the observations made

The most important constraint on high-resolution imaging of crystals is that the specimens should not exceed a maximum thickness of about one to two hundred ångströms, this thickness value being dependent on the specimen itself and on the accelerating voltage of the microscope used. As in this case it is not possible to use a practical test, which consists of observing negligible contrast of the specimen at Gaussian focus (d = 0), for checking the sample thickness, we have to use a direct interpretation of the Pendel-lösung plots (Figs. 4 and 5) and of the calculated images shown in Fig. 6. As we have shown on the Pendellösung plots, for both accelerating voltages and both structures a linear increase of intensity with thickness of every beam except for the 0330 beam can be observed up to a thickness of 77-34 Å. In the case of the 0330 beam, a relative intensity dip of 1% in the linear range can be observed at that thickness.

<p>| Table 1. Resolution corresponding to a set of diffracted beams originating from partially substituted lead hydroxyapatite (a = b = 9-668, c = 7-096 Å) |</p>
<table>
<thead>
<tr>
<th>Resolution (Å)</th>
<th>Reflection ( × nb)</th>
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</thead>
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<tr>
<td>3-548</td>
<td>0002(× 2)</td>
</tr>
<tr>
<td>2-790</td>
<td>3300(× 2)</td>
</tr>
<tr>
<td>2-600</td>
<td>3301(× 4)</td>
</tr>
<tr>
<td>2-365</td>
<td>0003(× 2)</td>
</tr>
<tr>
<td>2-194</td>
<td>3302(× 4)</td>
</tr>
<tr>
<td>2-093</td>
<td>4400(× 2)</td>
</tr>
<tr>
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<td>1-804</td>
<td>3303(× 4)</td>
</tr>
<tr>
<td>1-803</td>
<td>4402(× 4)</td>
</tr>
<tr>
<td>1-774</td>
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<td>1-675</td>
<td>5500(× 2)</td>
</tr>
<tr>
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<tr>
<td>1-568</td>
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</tbody>
</table>

Furthermore, in Fig. 6 a progressive variation of image contrast was observed for both structures up to a thickness of 77-34 Å in the 400 kV case and up to 38-67 Å in the 200 kV case.

It is therefore reasonable to say that the thickness limit to which crystals of both structures are still interpretable is 77-34 Å in the 400 kV case and 38-67 Å in the 200 kV case.

### Extension of this work to microscopes of different voltages

As we have seen above, the differentiation between the PDLA and the EDLA structures would require a microscope of optimum resolution better than 2-0 Å; however the use of a high voltage is necessary, since it permits the observation of thicker specimens. Several indications can be drawn from the present study concerning the use of microscopes of voltages different from the ones used here, but it should be borne in mind that for an accurate study a calculation with the specifications of each microscope should be made.

(1) For microscopes of accelerating voltages lower than 200 kV, it is reasonable to assume the maximum observable crystal thickness to be less than 77-34 Å.

(2) For microscopes of accelerating voltage between 200 and 400 kV, as for example the Philips EM430T and ST, it is reasonable to assume the maximum observable thickness to be between 38-67 (obtained for 200 kV) and 77-34 Å (obtained for 400 kV).

(3) Finally, for microscopes of accelerating voltage higher than 400 kV, one would expect a maximum observable thickness of more than 77-34 Å.
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References


