Radiation induced paramagnetic radicals in synthetic octacalcium phosphate

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Abstract. Created at room temperature by X-ray irradiation at the dose of about 5 kGy radiation induced centres in stable octacalcium phosphate (OCP) obtained by double transformation of α-tricalcium phosphate (α-TCP) are studied by means of the conventional and pulsed electron paramagnetic resonance (EPR). No EPR signals within the sensitivity of the used equipment in the non-irradiated samples were detected. In the irradiated species complex EPR spectrum signals due to H0 and CO-like radicals appear. The obtained results could be used for the tracing of the mineralization processes from its initiation to the completion of the final product and for the in situ identification of the OCP phase.

1. Introduction

Among the non-destructive methods which can give an invaluable information about the content and structure of various materials is electron paramagnetic resonance (EPR) which detects paramagnetic impurities, trapped free radicals or hole centres [1]. EPR is also used for dating minerals and rocks containing paramagnetic defects, cave deposits with defects in calcite, biocarbonate defects in fossils and sediments as shells, corals or mollusks, apatite related defects in bones, tooth enamels and dentine. Human and animals bones and teeth are suitable for dating, for an estimation of absorbed dose of irradiation, for detection of irradiated foodstuffs containing bones or fish-bones (see papers [2-4] and references therein). A number of precursor minerals have been suggested to be important in the biomineral evolution of Earth’s mineralogy, including amorphous calcium phosphate – hydroxyapatite (HA), tricalcium phosphate (TCP), octacalcium phosphate (OCP), and brushite [5].

Synthetic and sintered calcium phosphates, first of all hydroxyapatites, irradiated with γ-rays and X-rays or doped with paramagnetic impurities have been intensively studied by EPR and by electron-nuclear resonance (ENDOR) techniques giving a background for interpretation of EPR spectra of natural materials [3]. Several paramagnetic species located at hydroxy or phosphate sites have been identified in synthetic HA: oxygen radical O•, trapped atomic hydrogen, and hole trapped on OH− and PO43−. Carbonate radicals CO2−, CO3−, and CO33− are often observed as well as additional organic radicals (alanine-like) signals and color centres signals appears. In burnt bones the coal-type C• radicals were identified [2, 3]. In HA synthesized by the solid state phase methods or wet-synthesis technique from the nitrate containing substances stable H0 and/or NO3− radicals, correspondingly, are often detected [5-10].
Even for HA despite of decades of investigation, some problems of EPR spectra separation and unambiguous assignment exist, especially in samples of biogenic origin [11]. Already for the secondly frequently used CaP - tricalcium phosphate amount of the EPR studies is practically negligible [10, 12]. Since recently, octacalcium phosphate as one of the minerals formed by organisms has attracted increasing attention because of good osteoconductivity, biocompatibility, and biodegradability [13-15]. In particular, OCP is considered to be a better scaffold for bone regeneration in comparison to hydroxyapatite or to β-tricalcium phosphate. To the knowledge of the authors, the only OCP related research of the radiation induced paramagnetic centres is paper [16] in which OCP was used as a precursor for HA while EPR in HA was investigated. We present the preliminary results of our study of the X-ray created paramagnetic centres in stable OCP at conventional for EPR frequency of 9 GHz (X-band).

2. Materials and Method

The crystal structure of OCP with the chemical formulae Ca$_8$H$_2$(PO$_4$)$_6$ • 5H$_2$O is triclinic with a space group of P1 (the center of inversion is located at (½,½, ½), the unit cell contains 110 atoms) and exhibits the much longer a-axis dimension than the b. OCP has the lattice constants $a \approx 19.8$ Å, $b \approx 9.6$ Å, $c \approx 6.87$ Å, $\alpha = \beta = 90^\circ$ and $\gamma = 109^\circ$. It resembles the structure of HA, Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ with the corresponding hydroxyapatite constants for P6$_3$/m hexagonal group $a = 18.84$ Å, $a = b \approx 9.42$ Å, $c \approx 6.885$ Å, $\alpha = \beta = 90^\circ$ and $\gamma \approx 120^\circ$ [17]. In ref. [18] it was first shown that OCP consists of apatite and hydrated layers. It has been suggested that a progressive evolution from OCP to apatite can occur by a topotactic reaction corresponding to the elimination of the hydrated layer.

Synthesis of α-TCP was carried out by precipitation from solutions of calcium nitrate and ammonium hydrogen phosphate:

$$3Ca(NO_3)_2 + 2(NH_4)_2HPO_4 + 2NH_4OH \rightarrow Ca_3(PO_4)_2 + 6NHNO_3 + 2H_2O. \quad (1)$$

A solution of ammonium hydrogen phosphate (0.5 mol/l) was gradually added to a solution of calcium nitrate (0.5 mol/l) with constant stirring. The resulting suspension was allowed to age in the air during the 24 hours. The precipitate was then filtered off and dried at 120 °C.

For the production of α-TCP ceramic granules, the technology of impregnating a cellular polymeric template with ceramic slip was used [19], followed by sintering at 1300 °C with quenching. The quenching was carried out by cooling the ceramics at room temperature. After that, the ceramic was crushed and granules of 500-100 μm were sifted.

α-TCP ceramic granules were transformed to OCP according to the route described in ref [20]. Briefly, 10 g of α-TCP ceramic granules were immersed to 1000 ml of 1.5 M sodium acetate aqueous solution and 0.15 ± 0.02 M glutamic amino acid, adjusted with orthophosphoric acid to a pH value of 5.5 ± 0.1. The granules were kept in for 168 h with shaking at 35 °C. The granules were thoroughly washed in distilled water at least five times and dried overnight at 37 °C. Then, the granules were immersed in an aqueous 1.5 M sodium acetate solution with a pH value of 8.7 ± 0.1 for 168 h with shaking at 35 °C.

The phase composition was analyzed by conventional X-ray diffraction (XRD) technique (Shimadzu XRD-6000 (Japan), Ni-filtered CuKa1 target, $\lambda=1.54183$ Å). Samples were scanned from 20 ranging from 3° to 40° with a step size of 0.02° and a preset time of 5 second.

Continuous wave (CW) EPR spectra of the samples were obtained using table-top Labrador (Ekaterinburg, Russia) and ESP-300 (Bruker) spectrometers. operating at 9.4 – 9.9 GHz (X-band) microwave frequency. PC concentration was estimated at room temperature in the double cavity ER4105DR of ESP-300 by comparing the integrated intensities of the spectra of a test sample and a reference samples (Cu-DETC solution and Mn$^{2+}$ in MgO powder). Pulsed EPR measurements were done exploiting the X-band abilities of Bruker Elexsys 580/680 spectrometer. X-ray irradiation of the synthesized powders was provided by using URS-55 tube (U = 55kV, I = 16mA, W anticathode) at room temperature with the estimated dose of 5 kGy to create stable paramagnetic centers in the nominal pure material.
3. Results and Discussion

The XRD pattern of the product obtained after soaking in standard buffer solution for 168 h is shown in Figure 1. XRD of the investigated samples confirmed OCP phase with following characteristic: (010) main reflection at $2\Theta = 4.9^\circ$ and peaks (2 6 0), (151) and (150), (0 7 0), (0 0 2).

![Figure 1. XRD pattern of OCP](image)

Pure, non-substituted OCP (as well as the mentioned HA and TCP) is supposed to be EPR silent. Consequently, EPR might be used for the purity check of CaP materials [10]. As Figure 2 demonstrates, in the investigated samples no EPR signal was detected at room temperature (i.e. concentration of the paramagnetic impurities $C < 10^{15}$ spin/g) while after the irradiation a signal in the central part of the spectrum appears with the total concentration of $C \approx 8(1) \cdot 10^{16}$ spin/g.

![Figure 2. Conventional room-temperature X–band EPR of the initial sample, empty tube (glass holder) and X–irradiated sample of OCP.](image)
The central part of the spectrum (in the magnetic field range of 300-400 mT) for the irradiated species was investigated in details. From Figure 3 it follows that a pair of weak lines with the splitting of about 500 mT due to the $^{1}H_{0}$ (nuclear spin $I = 1/2$) could be observed. This feature is also characteristic for the HA and TCP materials synthesized by solid state reaction [10]. But the part of the spectrum in the range of the magnetic fields of 340-350 mT resembles those for HA and TCP synthesized by the wet precipitation techniques [9]. The remarkable differences for CaP produced by various methods could be exploited for the fairy simple identification of the synthesizing route of the commercial CaP materials, for example which is often a proprietary information.

![Figure 3](image-url)

**Figure 3.** Conventional room-temperature X–band EPR of X–irradiated sample of OCP.

We have studied the behavior the signal in the range of the magnetic fields of 340-350 mT comprehensively. Figure 4 presents the dependence of the spectrum on microwave power. At least two different sorts of paramagnetic centres could be distinguished: while the intensity and amplitude of the broad line in the middle of the spectrum with the linewidth of 1 mT is growing with power (denoted as a paramagnetic centre 1, PC$_1$), while the second “structural” paramagnetic centre (PC$_2$) with the with the maximal splitting of 55 mT starts to saturate already at $P_{MW} = 10$ mW. Lineshape of PC$_1$ resembles those for the carbonate centered stable radicals in HA [3]. To study the nature of PC$_1$ in details we have measured transverse ($T_2$) and longitudinal ($T_1$) relaxation times at $T = 100$ K by pulsed methods ($\pi/2$-τ-π and $\pi$-T-τ/2-π pulse sequence, correspondingly) in the magnetic field marked by the blue dashed line in Figure 4. $T_2$ curve can be described by only one exponential decay with $T_2 = 1.8 \mu s$. $T_1$ curve is presented in Figure 5.

Experimental points on $T_1$ curve were approximated by one-, two- and three exponents with the corresponding $T_1$ times (1) $T_1 = 0.5$ ms; (2) $T_1^{(1)} = 0.06$ ms, $T_1^{(2)} = 0.7$ ms; (3) $T_1^{(3)} = 0.02$ ms, $T_1^{(2)} = 0.2$ ms, $T_1^{(3)} = 1.2$ ms. As it follows from Figure 5, three-exponential description is the best one. Assuming that the centre with the longest $T_1$ belongs to PC$_2$ (cf. Figure 4 and discussion below), there are two types of the fast relaxing centres in OCP.

EPR pattern of the slowly relaxing PC$_2$ resembles that in HA. In the irradiated HA samples EPR is mainly due to the stable NO$_3^-$ ions preferably substituting one of the PO$_4^{3-}$ position in the HA structure (substitution of B-type). Their powder EPR spectra can described by the spin-Hamiltonian of the axial symmetry

$$H = g_z \beta B_z S_z + g_s \beta \left( B_x S_x + B_y S_y \right) + A_z S_z I_z + A_s \left( S_x I_x + S_y I_y \right),$$

where $g_z$ and $g_s$ are the main components of the $g$ tensor, $A_z$ and $A_s$ are the main components of the hyperfine tensor, $B_z$, $S_z$ and $I_z$ are the projections of the external magnetic field strength, $S = \frac{1}{2}$ and
$I = 1$ are electronic and nuclear spin correspondingly onto the $i = \{x, y, z\}$ coordinate axis, $\beta$ is a Bohr magneton. The HA spectra could be simulated properly if we supposed a Gaussian (continuous) distribution of $A_i$ with a deviation of 0.40 mT around the mean value of 6.65(40) mT and a discrete distribution of $A_\perp$ around 3.37(5) mT, while the components of $g$-factors for all of the obtained radical’s modifications are the same ($g_\parallel = 2.0011(1), g_\perp = 2.0052(1)$) [6].

**Figure 4.** Conventional room-temperature X–band EPR of the X–irradiated sample of OCP at different microwave power levels. Lines with different microwave saturation behavior are marked.

**Figure 5.** $T_1$ curve (filled squares) with the corresponding approximations by one-, two- and three exponents.

In our paper [10] we have found that EPR spectrum of the wet synthesized TCP after radiation is also mainly due to the NO$_2^-$ with the same as for HA value of $A_i$ ($A_i$ is 2 times $A_\parallel$ of 6.65 mT) but the distribution of $A_\perp$ ($A_2, A_3$) is larger than that for the HA. We have suggested that it could be due to the
three different positions for PO$_4$ group substitution in TCP structure as well as due to the various schemes of the charge compensation. As it follows from the presented results, in OCP we have to suggest $A_J = 5.5/2 = 2.7$ mT. Such small value forces to suggest the presence of other nitrogen containing radicals such as NO$_2$, for example [6], or other paramagnetic centres [3].

4. Conclusion
Modern EPR techniques offer various opportunities for the rapid testing and comprehensive analysis of the condensed matter. The capabilities of even conventional EPR approaches for investigation and analysis of calcium phosphate materials are still not fully exploited and described in details. We hope that our paper would serve as a first step in understanding the nature and structure of various paramagnetic centres in octacalcium phosphates with the purpose of their usage as sensitive intrinsic probes to follow the changes under the doping or surface treatment.

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