Journal of Molecular Structure 1119 (2016) 484–489

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Synthesis and characterization of nanocrystalline apatites from solution modeling human blood

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ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form
14 April 2016
Accepted 25 April 2016
Available online 27 April 2016

Keywords:
Pathological cardiovascular deposits
Blood plasma
Biological apatite
Crystal chemistry
Model experiment
Biomimetic synthesis

ABSTRACT

Present paper is devoted to the research of the calcification processes in the blood plasma of human body. Spontaneous crystallization from the solution modeling the inorganic part of the blood plasma has been carried out. Obtained precipitates were studied by the various instrumental methods (X-ray powder diffraction, Fourier-transformed infrared spectroscopy, scanning electron microscopy, electron probe microanalysis and gas-volumetric method). All gathered data allow to summarize that non-stoichiometric carbonated hydroxyapatite with low crystallinity (CSD lengths 18–28 nm), high water content and small amount of chlorine ion was obtained throughout the syntheses. Part of vacancies at the Ca sites varies from 0.17 to 0.87; the value of the Cat/(P + C) ratio—from 1.52 to 1.64 (where Cat = Ca^{2+} + Na^{+} + K^{+} + Mg^{2+}).

The poor crystallized synthetic apatites with high carbonate ion content (from 4.34 to 5.54 wt%) and *c* parameter (6.888–6.894 Å) are analogues of the apatites of the pathological cardiovascular deposits. They can be obtained from the solution modeling human blood plasma by the inorganic components with calcium phosphate supersaturation 25 and 50 and with 10 and 12 weeks experiment time.

1. Introduction

Apatite is one of the main mineral in the human body, regardless whether they occur in a desired way (like teeth and bone) or in an undesired (pathologic) way [1–3]. Pathological masses can be formed in a wide variety of sites, and the cardiovascular system is one of them. Pathological cardiovascular calcification is, probably, the deadliest among other types of the pathological mineral formation. It plays an important role in atherosclerosis and arteriosclerosis progression, and these diseases are the major cause of death in the industrial countries [4–7]. That's why researches of the Pathological Cardiovascular Deposits (PCD) possess high priority nowadays.

Structure and chemical composition of PCD themselves have been already reported in a considerable amount of papers. It is common knowledge now that PCD consist of mineral and organic components [8–10]. Main mineral of the PCD is carbonated hydroxyapatite [10–16], but other minerals, like Mg-containing whitlockite (β-TCMP) or calcite, can also be presented [17]. PCD apatites, as other biological apatites, are non-stoichiometric, with high amount of Na^{+}, Mg^{2+} and vacancies at Ca sites, carbonate- and hydrophosphate ions at PO_4^{3-} sites and with water at OH^{-} sites [9]. Their crystallite lengths are about 20–60 nm [10,17].

Theories of the PCD formation [18,19] are really tricky to interpret and prove because of very complicated human blood composition with high amount of inorganic ions (H^{+}, K^{+}, Na^{+}, Ca^{2+}, $H_2PO_4^{-}$, HPO_4^{2-}, PO_4^{3-}, HCO_3^{-}, CO_3^{2-}, SO_4^{2-}) and organic components (like proteins or chelates) [20–22] and due to the fact that pathological cardiovascular calcification often is connected with other diseases [6,23,24].

So, despite the fact that cardiovascular deposits are intensively investigated, their formation mechanism and crystal chemistry peculiarities are still unclear which leads to the absence of the effective medical treatments to delay valve calcification course progression by far [7].

The most promising prospective in this field which can be seen today is in the model experiments. That is confirmed by the results of the numerous researches of the apatite crystallization from the analogues of physiological fluids of the human body [25–27] including the several studies dedicated to the synthesis of the analogues of the PCD apatite [6,21,28–30].

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http://dx.doi.org/10.1016/j.molstruc.2016.04.080
0022-2860/

The present work continues the research of the calcification in the blood plasma of human body on the base of simulation experiments. Our interests were focused on: (a) spontaneous crystallization from the solution modeling human blood plasma; (b) characterization of the obtained precipitates by the complex variety of instrumental methods; (c) comparison of the synthesized apatite with other biological apatites including the apatite of pathological cardiovascular deposits; (d) determining the conditions of the biomimetic synthesis for the PCD apatites.

2. Experimental section

2.1. Synthesis

For the matching to the characteristics of the human blood plasma (Table 1), solution modeling it has been prepared as follows.

Solution containing (Na_2SO_4, $NaHCO_3$, K_2HPO_4, $(NH_4)_2HPO_4$) in a volume of 500 ml was quickly poured into the solution containing (NaCl, $CaCl_2 \cdot H_2O$, KCl, $MgCl_2 \cdot 6H_2O$) in the volume of 500 ml. Total volume of the resulting solution was 1000 ml. After the mixing of the solutions pH of the system was adjusted to the value 7.40 ± 0.05 with NaOH (10%) and/or HCl (10%) solutions. Starting reagents and their ratios in the solution were adjusted to make the model solution match the human blood plasma in ionic strength (0.146 M). All the reagents for the synthesis were 98% purity and were used without additional purification as purchased at "Himreaktiv" (Omsk, Russia).

Calcium Phosphate supersaturation (S) was calculated on the base of the classic thermodynamic approach using common equations from Todes et al. [31]. Three series of experiments have been carried out, each with the specific supersaturation (S = 25; 50; 100) on calcium phosphate (Table 1). Range of S values was selected to receive sufficient amount of the precipitate in each experiment for further study. All experiments were carried out at the room temperature without stirring. The exposition time was 2, 4 (S = 50, 100); 8, 10, 12 (S = 25, 50, 100) weeks. After the synthesis solution was filtered and precipitate was dried at 80°C until fully dry.

2.2. Analytical procedures

All the resulting precipitates were studied by various methods, including X-ray powder diffraction, FT-IR spectroscopy, scanning electron microscopy and different chemical analyses.

Powder diffraction investigations (phase identification, lattice constants and scattering domains (CSD) measurements) were carried out with Rigaku MiniFlexII diffractometer (CuKα radiation, graphite monochromator; 2Θ range 3–60° with a 0.05° step and speed equal to 2°/min). Lattice constants were calculated by the least squares method using 6–8 reflections and germanium powder as an internal standard. Lattice constants were also confirmed with the TOPAS software by the X-ray diffraction pattern fitting with Rietveld method. CSD lengths along [001] direction were calculated with the Debye Scherrer formula using the 002 reflex.

Infrared (FT-IR) spectroscopy was applied for the detection of CO_3^{2-}, OH^- and HPO_4^{2-} ions and molecular water. The spectra were collected with a Bruker Vertex IR-spectrometer in the range of 400–4000 cm^{-1} with a frequency resolution of 1 cm^{-1}. The samples were prepared as tablets in KBr. The results were interpreted according to the literature data [1,32–35]. The P-O band vibrations were detected using absorption bands at 1105, 1070, 1040, 975, 610, 570 and 480 cm^{-1}. The presence of OH^- ions was controlled by the presence of the absorption bands at 640 cm^{-1} (P–OH) and 3580 cm^{-1}, the presence of HPO_4^{2-} ions by a band at 870 cm^{-1}, and the presence of water by a group of bands in the range of 3300–3700 cm^{-1} (stretching vibrations) and a band at 1640 cm^{-1} (deformation vibrations). The carbonate-ion of the B type was localized using the C–O absorption bands at 1460 and 1420 cm^{-1}. The band at 870 cm^{-1}, also corresponding to B-type carbonate ion, wasn't used because it interferes with HPO_4^{2-} ion band [34].

Scanning electron microscopy was used to determine the shape of synthesized crystals and estimate their CSD lengths. It was carried out using Cross-beam SEM-FIB workstation Zeiss AURIGA Laser at the 20 kV acceleration voltage.

Chemical analysis data. Concentrations of Ca, P, Na, K, Mg and Cl (wt%) were determined by electron probe microanalysis using the scanning electron microscope Hitachi S-3400N equipped with X-ray energy microanalyser EDX-AzTec Energy 350 at a or 20 kV acceleration voltage and 1 μm electron beam diameter.

The concentrations of carbon in the samples were measured using the gas-volumetric method. Samples were heated at 1100-1300 °C in an oxygen flow (the analyst was S.N. Zimina).

Data of chemical analysis of the samples were used for calculation of the unit cell content. It was calculated assuming on the base of structure data [36] that phosphorous site is fully occupied (sum of P and C atoms = 6 apfu). Taking into account the maintenance of charge balance, the total amount of vacancies at OH^- sites ($Vac_{(OH)}$), water molecules and HPO_4^{2-} ions was estimated using the following formula: $HPO_4^{2-} + H_2O + Vac_{(OH)} = 3P + 2C_B + 2 - 2Me^{2+} - R^+$ (apfu).

The Cat/(P + C) ratio (where $Cat = Ca^{2+} + Na^+ + K^+ + Mg^{2+}$) also was used as a measure of the vacancy fraction at Ca sites. The higher deficit of Ca is in the apatite, the lower the observed ratio is in comparison with the value 1.67, corresponding to stoichiometric apatite.

Table 1
Elemental composition (mmol/l) of the simulating solutions and human's blood.

Ion	Model solution			Blood plasma [20]
	S = 25	S = 50	S = 100	
Ca^{2+}	11.65	16.46	23.2	2.35
Na^+	177	177	177	143
K^+	8.37	13.75	21.25	4.35
Mg^{2+}	0.95			0.95
NH_4^+	0.04			0.04
Cl^-	77.8	68.18	54.7	103
SO_4^{2-}	0.45			0.45
CO_3^{2-}	26			26
PO_4^{3-}	6.41	9.05	12.8	1.3

3. Results

3.1. X-ray powder diffraction

X-ray diffraction analyses showed that apatite was synthesized in all experiments (Fig. 1). Value of the apatite parameter *a* varies from 9.41 to 9.48 Å and value of the parameter *c* – from 6.874 to 6.894 Å (Table 2). In general, the value of the parameter a of the synthesized apatites is either close to the parameter a of the stoichiometric hydroxylapatite (a = 9.418 Å; c = 6.884 Å, JCPDS N 9-432) or larger than it. The value of the parameter c can be lower or higher than stoichiometric apatite value, but anyway close to it. All X-ray diffraction patterns have very wide peaks, that points on the low crystallinity of the apatite. The average CSD length along [001] of the synthesized apatites varies from 18 to 28 nm (Table 2).

3.2. FT-IR-spectroscopy

All FT-IR spectra of the obtained precipitates (Fig. 2) contain intensive bands corresponding to phosphate-groups (570, 610, 640,