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Model Evaluation of the Effect of Size, Shape and Surface State of Apatite Nanocrystals on the Deviation of Ca / P ratio from stoichiometric value

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The causes of Ca / P ratio deviation in biological apatites from stoichiometric one were discussed. By the simple model evaluation Ca / P ratio was shown to deviate from stoichiometric one because of small sizes of crystals, and peculiarities in chemical composition of their facets. Also size effect is noted to be unsufficient for explanation of wide variations of Ca / P ratio in biological apatites. It proves the significant contribution of lattice isovalent and heterovalent substitution into variability of Ca / P ratio. The problem of revealing the predominant causes of Ca / P deviation from stoichiometric one and estimation of their relative contribution is related to determination of the functional role of the structural imperfections in biological apatites of different origin.

Keywords: Apatite, Ca / P ratio, Stoichiometry, Surface density, Crystal size.

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1. INTRODUCTION

Ca / P ratio in calcium apatites of natural (geological), biological and synthetic origin is a very important factor of structure imperfection which significantly determines the physical and chemical properties of the mineral. According to the idealized formula of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, atomic ratio Ca / P is equal to 1.67. In real biological apatites this ratio deviates from the stoichiometric value to both the larger and smaller sides; and spread of Ca / P values can be in the range from 1.37 to 1.79 and more [1-4]. Calcium deficiency can be conditioned by both its vacancies and isovalent (Mg^{2+}, for example) and / or heterovalent (for example, Na^+, K^+) substitutions in cation sublattice of apatite. Substitution of the phosphate tetrahedron PO_4^{3-} by the plane carbonate triangle CO_3^{2-}, which is rather widespread in biological apatites and leads to the formation of carbonate apatite of the B-type, belongs to the major causes of phosphorus deficiency [4, 5]. At that, electric charge compensation is achieved by vacancies Ca^{2+} or heterovalent substitutions leading to the formation of apatite of $Ca^{2+}_{10-x}X^+_x(PO_4^{3-})_{6-x}(CO_3^{2-})_x(OH^-)_2$ composition, where X^+, for example, Na^+. Indeed, if assume that one substitution $CO_3^{2-} \rightarrow PO_4^{3-}$ corresponds to one substitution $Na^+ \rightarrow Ca^{2+}$, then Ca / P ratio increases up to 9/5 = 1.8. At loss of one Ca^{2+} ion for the conservation of neutral charge two substitutions $CO_3^{2-} \rightarrow PO_4^{3-}$ are necessary that leads to Ca / P ratio of 9/4 = 2.25. However, existence of Ca^{2+} vacancies is improbable, especially taking into account rather high concentrations of Mg, Na, K in bioapatite which significantly compensate the existing level of carbonate substitutions [2, 4].

$Ca_{8,3}\square_{1,7}(PO_4)_{4,3}(CO_3)_1(HPO_4)_{0,7}(OH,CO_3)_{0,3}\square_{1,7}$ composition, where $\square$ are the vacancies, proposed in the work of Legros R., et al. [3], can be one of the variants of biogenic apatite composition; in this case Ca / P = 1.66. Decrease in the ratio Ca / P to the value of 1.5 and lower is caused by further substitution of the phosphate ion PO_4^{3-} by the hydrophosphate ion HPO_4^{2-} with the charge compensation by vacancies Ca^{2+} or substitutions $Na^+(K^+) \rightarrow Ca^{2+}$. Presence in the apatite lattice of HPO_4^{2-} ion is discovered by the data of the infrared spectroscopy [3, 4, 6], although here its concentration and localization are not defined sufficiently precisely. Moreover, theoretically, formation of vacancies Ca^{2+} for the compensation of vacancies in position OH^- can lead to the decrease in Ca / P.

As seen, the necessity of maintenance of electrical neutrality of the crystal volume of apatite considerably limits the oscillation range of Ca / P ratio that does not allow to explain significant deviations of this ratio from the stoichiometry obtained in the investigations of biological apatites [7, 8].

Both the surface exchange or adsorption reactions (for example, existence of the so-called non-apatite environment of crystals, in whose composition there are $CaCO_3$ complexes or Ca^{2+} and CO_3^{2-} ions) and high value of the specific surface of bioapatite can be the reasons of wider variations of Ca / P. It is natural that for the crystal thickness, which does not exceed 2-4 unit cells, Ca / P ratio will be defined not only by volume but also by surface; and any changes in the surface composition can substantially influence the composition of the whole material.

The purpose of the present work was to perform the model evaluations of the influence of size and shape of apatite crystals as well as of state of facing crystallographic planes on the Ca / P ratio deviations from the stoichiometric value for idealized defectless hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$.

2. INFLUENCES ON THE Ca / P RATIO IN APATITES

2.1 Structure and surface of apatite crystals

The unit cell of hydroxyapatite corresponding to the formula $Ca_{10}(PO_4)_6(OH)_2$ is presented in Fig. 1. As seen, six phosphate tetrahedrons and four Ca(1) ions binding

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them are located inside the cell of the shape of rhombic prism. Only two of other Ca(2) ions are inside the cell, and four ions consist of halves of Ca(2) conventionally divided by the boundaries of adjacent cells. Thus, facing planes of real crystals, which coincide with lateral faces of rhombic or hexagonal prisms {100}, can contain excess or deficiency of Ca depending on the presence or absence on them of adjacent Ca ions. It is not improbable that alternation of the excess and deficiency regions of Ca ions on the surface can take place in real crystals; then, in whole, such crystals can correspond to the apatite stoichiometry. Obviously, these surface features of apatite crystals have functional value in biological systems, where nanocrystalline bioapatite is in close contact with organic macromolecules forming the mineral-organic complexes or natural biocomposites. Chemical bonds dangling on the crystal boundaries and locally ordered electrical charge are the factors of interaction of organic and mineral components. Naturally that in this case sizes and shape (morphology) of bioapatite crystals play the key role, since field of this interaction is determined by the "surface/volume" ratio of biocrystals.

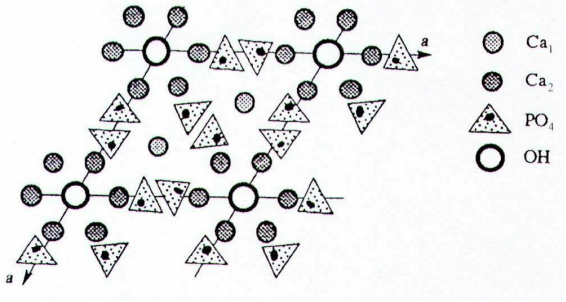

Fig. 1 – Structure of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ projected on the basal plane of hexagonal unit cell (according to the works [1, 5, et al.])

However, the case that crystallographic planes {100} are the faceting planes of real apatite crystals is an unproven fact, and, possibly, mistaken one. It is shown in the work [9] by the surface charge measurement techniques that apatite crystal surface in the physiological conditions is represented by phosphate ions; and planes {200} passing through the center of the unit cell shown in Fig. 1 are the faceting planes of real crystals. In this case, for one unit cell plane (200) with area of 0.65 nm^2 contains four phosphate ions and does not contain anions OH^-. The data of many publications [6, 10] implies that surface phosphate ions of apatite PO_4^{3-} because of the hydration become hydrophosphate HPO_4^{2-} that is energetically justified, since it decreases the surface charge. An increased HPO_4/PO_4 ratio in the apatite of bones of young animals [6] characterized by comparatively larger specific crystal surface is the indirect proof of the preferential surface localization of HPO_4^{2-}.

According to the work [9], at pH = 7 and higher, apatite crystals surface enriched by hydrophosphate ions adsorbs well Ca^{2+} ions from the surrounding solution. Adsorption mechanism is connected with substitution of two H^+ ions of neighboring surface HPO_4^{2-} by one Ca^{2+} ion. The performed quantitative evaluations give surface concentration of Ca^{2+} which slightly exceeds two ions by 1 nm^2. Here, adsorption / desorbtion of Ca^{2+} is regulated by the pH index of the environment.

The theory of the apatite crystal growth from ionic $Ca_9(PO_4)_6$ groups, called the Posner clusters, developed by Japanese scientists [11] is in accordance with the foregoing. This growth model also leads to the preferential concentration on the surface of phosphate or hydrophosphate ions with the maintaining of stoichiometry and electrical neutrality of the crystal volume.

Thus, adsorbed Ca^{2+} layer, which compensates negative charge of surface PO_4^{3-} ions is, probably, one of the sources of excess calcium of biological apatite. Moreover, both in the conditions of complex biosystems and in synthetic model materials apatite crystals are surrounded by a structured hydrate layer enriched by different ions including Ca^{2+} [10].

2.2 Sizes and shape of bioapatite nanocrystals

Sizes and shape of the crystals of biological apatite according to numerous published data have a rather wide spread of values. This spread, first of all, is conditioned by the belonging of biomineral to different physiological or pathological tissues. Physiological apatites considerably differ in their functionality. In accordance with the results of many works, length of bioapatite crystals of bone tissue is about equal to 15-40 nm, and width (thickness) – 2-4 nm [1, 2]. There is a data that bioapatite crystals of a bone can be of the size of 110 nm in one direction and 5-6 nm in another [2]. Tooth enamel crystals are characterized by the largest size [2]. Systematized information about sizes and shape of apatite crystals of pathological deposits is absent in the literature. This is conditioned, first of all, by a wide variety of biogenic apatites of pathological nature. Some authors imply the significantly larger sizes of ectopic bioapatite crystals in comparison with physiological ones. Thus, for example, according to [8], for deposit apatite crystals of mitral valve (Fig. 2), longitudinal sizes are equal to 60-90 nm and the transverse ones – 20-30 nm.

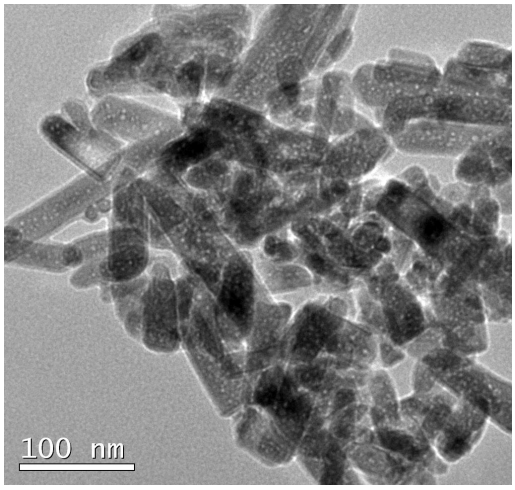

Fig. 2 – Electron microscopic image of the bioapatite crystals of pathological deposit of human mitral valve leaflet [8]

We should note that all experimental methods indicate elongation of biological apatite crystals along the hexagonal crystallographic axis. I.e. irrespective of the fact if crystals are columnar, acicular or laminar, substantially larger part of their surface is represented by lateral faces of hexagonal prisms, and not by basal planes.

3. MODELING OF VARIATIONS OF THE Ca / P RATIO DEPENDING ON THE SIZE OF HYPOTHETICAL APATITE CRYSTALS

In model calculations performed in the given work we have used hypothetical apatite crystals, whose sizes, in whole, corresponded to the investigation results of different authors with application of various instrumental techniques to different materials (bioapatites of bone tissues, tooth enamel, dentin, pathological calcificates). Internal volume of the crystal was considered to be the corresponding in composition to defectless hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$.

Surface area S, volume V and number of unit cells n enclosed in the crystal were calculated for each variant of hypothetical crystal (Table 1). Area S was determined as a total area of lateral hexagonal faces of the crystal. The values of n were found by division of V by the volume of unit cell equal to 0.53 nm^3 [1]. Specific surface of apatite crystals was calculated from the ratio $\sigma = S_{total}/V\rho$, where S_{total} is the overall area of the crystal surface and ρ is the specific weight of the apatite ($\rho = 3.18$ mg/mm^3 [1, 2]); and it was used only for the comparison with the literature data.

If suppose that lateral face of the apatite unit cell with the sizes of $a = 0.943$ nm and $c = 0.688$ nm [2] has one excess Ca^{2+} ion, then amount of these ions on the unit area of the crystal surface will be equal to $\eta = 1/0.943 \cdot 0.688 = 1.541$ [1/nm^2]. Product of the surface area of hypothetical crystal and calcium surface density $S \cdot \eta$ gives the total amount of excess calcium ions on the surface. Then, for the calculation of Ca / P ratio of hypothetical crystal one should use the formula

$$Ca / P = \frac{10n + S \cdot \eta}{6n}.$$

The value of η can be chosen taking into account one or another feature of the crystal surface (calcium excess or deficiency). In the case of one missing ion, one should take $\eta = 1.541$ with sign "minus". If suppose, according to the work [9], that surface concentration of adsorbed Ca^{2+} ions accounts two ions per 1 nm^2, then we take $\eta = 2.0$ [1/nm^2]. We have to note that surface area of lateral face of the apatite unit cell does not depend on the fact if it is represented by the planes {100} or {200}.

In the right column of Table 1 we present the values of Ca / P for three variants of hypothetical crystals at one and two excess Ca^{2+} ions on one lateral face of the unit cell, as well as for the case when $\eta = 2.0$.

In the left column of Table 1 we give three variants of hypothetical apatite crystals. The first two cases (A and B) foresee the possibility of the existence of coalescent rod-like and laminar crystalline particles of apatite of bone tissue [1, 2]. The values of σ calculated for these cases agree well with the values of active surface of bioapatite crystals known from the literature [1, 2, 12]. The third of the considered variants ($70 \times 30 \times 30$ nm) corresponds to the data of our investigations of pathological apatites of the human cardiovascular system [8] and agrees with the results of other works. Clearly, variants of hypothetical crystals presented in Table 1 cannot overlap the whole spectrum of sizes and shapes of real crystals of biogenic apatite, although they are rather representative for this class of materials.

Obtained values of Ca / P represent well the general growth trend of deviations from stoichiometry at the decrease in the crystal sizes. However, the values of the calculated deviations are not so significant in order to explain by only size effects the wide experimental variations of Ca / P. This circumstance implies weighty contribution of other reasons of Ca / P deviations, and one the most important of them is the ability of apatites to isovalent and heterovalent substitutions.

Ascertainment of localization of excess calcium or cations, which substitute it, is an important moment in the interpretation of experimental results about calcium excess or deficiency in bioapatites. Besides the inherent volume of crystals with account of variants of their faceting, it is necessary to consider the ion adsorption on the surface by the mechanism of double electric layer formation [12]. Organic tissues and liquids surrounding biocrystals contain the same elements as the mineral component does, although with major variations of concentrations depending on the functional role of certain mineralized tissue. Separation of contributions into the total concentration of elements from the crystalline and non-crystalline components of the minerals of biological origin is a very complex experimental problem.

Table 1 – Results of the model evaluation of the Ca / P ratio deviations from the stoichiometric value depending on the sizes, shape and surface state of the apatite nanocrystals

Crystal sizes, nm	S, nm^2	V, nm^3	σ, m^2/g	n	η, 1/nm^2	$S \cdot \eta$*	(Ca / P), wt. % *
Variant A $20 \times 10 \times 4$	560	800	252	1509.4	1.541 2.0 3.082	862.96 1120.0 1725.92	1.762 1.790 1.857
Variant B $50 \times 20 \times 4$	2400	4000	201	7547.2	1.541 2.0 3.082	3698.4 4800.0 7396.8	1.748 1.773 1.830
Variant C $70 \times 30 \times 30$	8400	63000	51	118867.9	1.541 2.0 3.082	12944.4 16800.0 25888.8	1.685 1.690 1.703

* general amount of excess calcium ions on the crystal surface $S \cdot \eta$ and values of Ca / P are represented for the corresponding values of the surface density of Ca^{2+} η from the previous column

4. CONCLUSIONS

Effect of the sizes of apatite crystals on the Ca / P ratio deviations from the stoichiometric value under the conditions of limited and substantiated excess of adsorbed Ca^{2+} ions on the crystal surface is shown by simple model evaluations. It is established that size effect of Ca / P deviations is not enough for the explanation of wide variations of this parameter observed experimentally in biological apatites of different origin. It is true in a greater degree for sufficiently large crystals of bioapatite of calcined tissues than for bone tissue mineral. To that end, ability of apatites to isovalent and heterovalent substitutions in the crystal lattice should be considered as a weighty source of Ca / P deviations along with the size factor. In the majority of the cases, during the investigation of real biominerals one has to take into account also composition of non-apatite constituents including ionic components of a hydrate layer. The task of the detection of prevalent causes of the Ca / P ratio deviations from the stoichiometric value and evaluation of the relative contribution of each of them is connected with the clarification of functional role of structural imperfections of biogenic apatites of different origin.

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