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Effect of the Components of Biological Environment on the Formation of Hydroxyapatite

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Abstract

Using the modified procedure of the synthesis of non-stoichiometric hydroxyapatite species at the physiological pH and solution temperature, we obtained the solid phases with the Ca/P atomic ratios within the range from 1.58±0.01 to 1.67±0.01. According to the data of X-ray phase analysis, these samples are composed of hydroxyapatite. The effect of inorganic additives (phosphate, oxalate, carbonate anions, magnesium cations) and organic ones (amino acids) on the crystallization of hydroxyapatite was studied. The electrokinetic properties of the sol of synthesized hydroxyapatites of different stoichiometric composition were studied. It was established that the charge and the value of o potential of hydroxyapatite particles depend on the Ca/P atomic ratio. It was discovered that the character of amino acid interaction with non-stoichiometric hydroxyapatite differs from that with the stoichiometric one. It was shown that adsorption observed on the samples of synthesized hydroxyapatite is described within Langmuir and Freundlich models.

Key words: synthesis, hydroxyapatite, crystallization, electrokinetic properties, ξ potential, additives, adsorption, Langmuir and Freundlich models

INTRODUCTION

Attention to the apatites of biogenic origin has grown substantially by present. This is due to the fact that hydroxyapatite is close in chemical composition to the inorganic component of bones and therefore it has similar physicochemical and mechanical characteristics. Due to this fact, hydroxyapatite may be used as implants in the form of ceramics, cements and composites. In addition, apatites are components of the majority of pathogenic biominerals that are formed due to unknown reasons, and the mechanisms of their formation are still unknown [1, 2].

One of the goals of investigations of that kind is to establish a connection between the composition of biominerals (of pathogenic and physiogenic origin) and the conditions of their formation that are determined by anomalous changes of the state of an organism and the environment. The applied significance of these investigations is determined by the development of methods that will allow diagnostics of diseases connected with pathogenic biomineral formation, dental and bone diseases, at early stages, and their prophylactics. It is known that a special place in the investigation of pathogenic biominerals is occupied by establishment of the role of protein substances in the processes of formation, growth of calculi, and the formation of their structure [3–6]. The primary structure of proteins is represented by a sequence of the amino acids of different structures, so investigation of the effect of amino acids on the process of biomineral formation is urgent and important.

The goal of the present work was to establish the effect of inorganic and organic additives on the composition of the solid phase composed of the synthesized calcium hydroxyapatite and to study the features of its interaction with amino acids.

EXPERIMENTAL

Selection of the conditions of hydroxyapatite synthesis from solution by means of initial addition [7] at the temperature and pH modelling the conditions of the formation of nephroliths in human organism (in the absence of amino acids) was carried out. The synthesis of the stoichiometric hydroxyapatite may be represented by the reaction:

$$10Ca^{2+}(aq.) + 6HPO_4^{2-}(aq.) + 10H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2(s.) + 8H_3O^+$$

To study the specific features of the process of hydroxyapatite formation under the conditions clo9se to the physiological ones, we carried out a series of experiments at different pH values (6.5 and 7.6) and the ionic force of solution (0.1 and 0.3 mol/L). Temperature of solution was (37.0±0.5) °C in both cases.

The concentration of additives during hydroxyapatite synthesis corresponded to that in urine [8] (for magnesium ions: starting from 8.2 mmol/L, for carbonate ions: from 50 mmol/L, for oxalate ions: from 0.31 mmol/L), then it was increased by a factor of 2 and 3.

The composition of precipitates was determined using X-ray phase analysis (XPA) and IR spectroscopy. When studying the electrokinetic properties of the sols of synthesized hydroxyapatites, we investigated the effect of the stoichiometry of the phase, in particular the Ca/P atomic ratio, and adsorption of amino acids ($C = 0.004$ mol/L) on the value of o potential and particle charge. Electrophoresis was carried out using Rabinovich–Fodiman device according to a standard procedure described in [9]. To study adsorption interactions, the experiment was carried out within the concentration range of glutamic acid 0.002–0.20 mol/L, at pH 6.5±0.1 and contact time 24 h. Adsorption was performed on hydroxyapatite samples with different Ca/P ratios: stoichiometric (Ca/P = 1.67) and non-stoichiometric (Ca/P = 1.57).

RESULTS AND DISCUSSION

Previous research and the analysis of literature data showed that the atomic ratio Ca/P in hydroxyapatite of pathogenic minerals varies within the range from 1.55 to 1.62 (stoichiometric hydroxyapatite Ca/P = 1.67) [2].

Synthetic hydroxyapatite

Model synthesis of hydroxyapatite samples with the initial Ca/P ratios equal to 1.67, 1.80 and 1.90 gave after 24 h the solid phases with the Ca/P atomic ratios equal to 1.57, 1.63 and 1.67, respectively. The XPA investigation of the solid phases formed during crystallization demonstrated that all the precipitates are represented by hydroxyapatite.

Synthesis in the presence of magnesium ions

According to the XPA data, the major phase of all the solid samples obtained is represented by struvite ($NH_4MgPO_4 \cdot 6H_2O$); in the case of the minimal added amount of magnesium ions (8.2 mmol/L) hydroxyapatite is partially present. It is known [5] that magnesium ions serve as the inhibitor of hydroxyapatite crystallization from amorphous calcium phosphate, proceeding according to dissolution-precipitation mechanism. Struvite is precipitated from neutral and alkaline media containing also calcium ions in addition to magnesium at the molar ratio $Mg/Ca > 0.6$. Under the synthesis conditions, this inequality is true for magnesium concentrations in the initial solution higher than 0.01 mol/L (0.0164 and 0.0246 mol/L); it is these solutions in which struvite precipitation occurs along with the formation of amorphous calcium phosphate.

Synthesis in the presence of carbonate ions

One of the features of biogenic apatite is the presence of carbonate ions in it, which is due to the high concentration of these ions in the medium from which biogenic hydroxyapatite gets crystallized (50 mmol/L). According to the data of XPA of the solid sample synthesized from the solution with the concentration of carbonate ions corresponding to their concentration in the hypothetic solution, the major reflections characteristic of the hydroxyapatite phase are present in the diffraction patterns. At the same time, broadening of X-ray bands in comparison with pure hydroxyapatite is observed, which is an evidence of worsening of he crystallinity of the compound. It should be noted that the calcite phase ($CaCO_3$) is also formed under the chosen synthesis conditions, in addition to the hydroxyapatite phase.

Synthesis in the presence of oxalate ions

According to the data reported in [10], in the hypothetic solution with the mineral composition similar to that of human urine, the formation of calcium oxalate is the next (after hydroxyapatite) most probable. According to the data of the XPA of the solid sample synthesized from the solution containing oxalate ions in the concentration corresponding to that of the hypothetic solution, the diffraction patterns contain the major reflections characteristic of the hydroxyapatite phase. The evidence of the presence of the oxalate group in the solid phase is the band at 1640 cm^{-1} in the IR spectra of the synthesized solid precipitates. The spectrum of the hydroxyapatite sample synthesized from the solution with the maximal concentration of oxalate ions (0.93 mmol/L) exhibits the appearance of a band at 1316 cm^{-1}, which allows us to identify oxalates in mixture with other minerals. In addition, we detected a band at 780 cm^{-1}, which is characteristic of the vibrations of CO group.

At the next stage of work, we determined the effect of the concentrations of amino acids (glycine, lysine, glutamic and asparaginic acids) on the formation and properties of hydroxyapatite samples with different compositions; the concentrations of amino acids were close to those in the biological liquid. The choice of these amino acids was due to their relatively high content both in the nephroliths of phosphate type and in the biological liquid [11]. It may be concluded on the basis of the data obtained (Table 1) that the charge and the value ξ potential of hydroxyapatite particles depend on the atomic ratio Ca/P. A regular shift of the o potential of the particles to the positive region is observed while this atomic ratio approaches the stoichiometric value. As the non-stoichiometric hydroxyapatite gets transformed into the stoichiometric one, due to specific sorption of ions Ca^{2+}, $CaOH^+$ and $CaH_2PO_4^+$ [8] on the negatively charged surface and their embedding into the apatite lattice, compensation and change of the surface charge occur.

A comparative analysis of the data on the effect of the sorption of amino acids on the electrokinetic properties of the sol particles (Ca/P = 1.57 and Ca/P = 1.67) showed that the interaction of amino acids with the surface of the crystals of non-stoichiometric hydroxyapatite increases with a decrease in the negative charge of the amino acid dominating in solution. A reverse dependence is observed for the

TABLE 1

Electrokinetic properties of the sol particles of hydroxyapatite having different stoichiometric composition in the presence of amino acids (I = 0.3, pH 6.5, C = 0.004 mol/L)

Atomic ratio Ca/P in the solid phase	Amino acid	Particle charge	Electrophoretic mobility, 10^{-4} cm	ξ potential*, mV
1.57±0.01	–	–	2.1	−28.6
	Glycine (Gly)	+	1.2	+16.3
	Asparagic (Asp)	–	1.0	−22.2
	Glutamic (Glu)	–	1.6	−13.8
	Lysine (Lys)	+	2.1	+28.3
1.67±0.01	–	+	0.54	+7.4
	Glycine (Gly)	–	0.87	−11.7
	Asparagic (Asp)	–	1.2	−17.0
	Glutamic (Glu)	–	1.2	−16.5
	Lysine (Lys)	+	0.54	+7.2

*$HCP_{0.5}$ = 4.7.

stoichiometric hydroxyapatite. It was established that under the experimental conditions the amino acids under investigation are present in the aqueous solution mainly as charged particles ($^{+}NH_3CH_2COO^{-}$, $^{-}OOCCH(NH_3)^{+}CH_2COO^{-}$, $^{-}OOCCH(NH_3)^{+}(CH_2)_2COO^{-}$, $^{+}H_3N(CH_2)_4CH(^{+}NH_3)COO^{-}$) that are able to get sorbed both on the positive regions of the surface of hydroxyapatite crystals and on the negative ones. It is also necessary to stress that amino acids exist in solution within a broad pH range in the form of zwitter ions. This promotes stabilization of the forms of amino acid and carboxyl groups, the interaction of which with the crystal surface proceeds without changes of molecular conformation, that is, more favourably with respect to the energy [12]. Additional stabilization of the surface compounds may be due to the ability of amino acids to form polydentate chelate complexes with calcium ions.

The amino acids under test may be arranged in the following row in which the strength of the electrostatic interaction increases: lysine > glycine > glutamic acid > asparagic acid. A reverse regularity is observed for the stoichiometric apatite.

It follows from the obtained regularities that amino acids must cause a substantial effect on the formation of hydroxyapatite from biological solutions.

To confirm the results obtained, we studied the adsorption of glutamic acid on hydroxyapatite samples differing from each other in the stoichiometry. One can see in the data presented in Fig. 1 that adsorption on the samples with different Ca/P ratios has complicated character; for the sample of the stoichiometric hydroxyapatite (Ca/P = 1.67) adsorption is higher in comparison with adsorption for the sample of non-stoichiometric hydroxyapatite (Ca/P = 1.57). This difference may be explained within the framework of the electrostatic model of the interaction of glutamic acid with hydroxyapatite. It is known that glutamic acid in the solution under investigation (pH 6.5) is in the form of negatively charged zwitter ion [5], and the surface of hydroxyapatite with the ratio Ca/P = 1.67 is charged positively due to the adsorption of positively charged calcium ions on it (see Table 1). Therefore, the higher degree of adsorption of glutamic acid is characteristic of hydroxyapatite with the high calcium content.

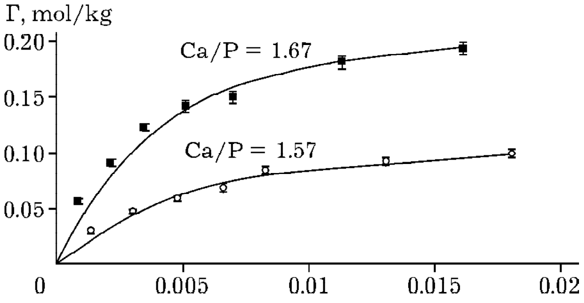

Fig. 1. Isotherms of the adsorption of glutamic acid (GA) on hydroxyapatite samples of different stoichiometry.

With the help of the plotted experimental dependencies in the coordinates $1/C$ (concentration)−$1/\Gamma$ (adsorption), we obtained Langmuir adsorption isotherms in the linear form (Fig. 2); they are described by the following equations:

$y = 0.036x + 8.70$ for Ca/P = 1.57 (1)

$y = 0.012x + 4.81$ for Ca/P = 1.67 (2)

Equations (1) and (2) are characterized by the high linear correlation coefficient: $R^2 = 0.99$. Having calculated the values of Γ_∞ and K in equation $1/\Gamma = 1/\Gamma_\infty + 1/(\Gamma_\infty KC)$ (Table 2) we obtain Langmiur equations for hydroxyapatite with Ca/P = 1.57:

$$\Gamma = 0.11\frac{245C}{1+245C} \quad (3)$$

and for hydroxyapatite with Ca/P = 1.67:

$$\Gamma = 0.21\frac{411C}{1+411C} \quad (4)$$

The calculated Γ values are evidence that adsorption does not reach saturation under the experimental conditions, which is likely to be due to the limitation for the concentration of glutamic acid in solution. The fact that the ob-

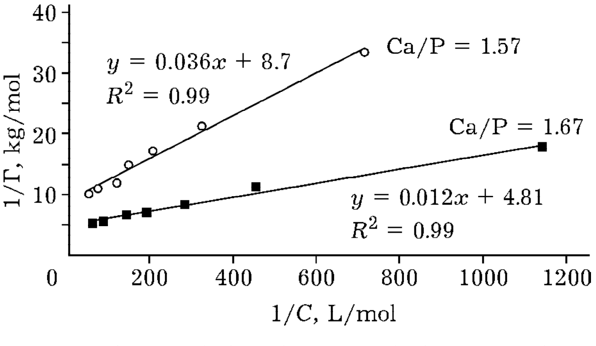

Fig. 2. Isotherms of the adsorption of glutamic acid on hydroxyapatite samples of different stoichiometry in the linear coordinates of Langmuir model.

TABLE 2

Calculation of the coefficients of Langmuir equation

Ca/P	Equation	Limiting adsorption (Γ_∞), mol/kg	$1/\Gamma_\infty$, kg/mol	Adsorption equilibrium constant (K)	$1/(K\Gamma_\infty)$, kg/mol
1.57	$y = 0.036x + 8.70$	0.11	8.70	245	0.04
1.67	$y = 0.012x + 4.81$	0.21	4.81	410	0.01

TABLE 3

Calculation of coefficients in Freindlich equation

Ca/P	Equation	Adsorption index (n)	$1/n$	K, L/kg	ln K
1.57	$y = 0.48x + 0.30$	2.08	0.48	0.74	−0.30
1.67	$y = 0.43x - 0.22$	2.34	0.43	1.25	0.22

served adsorption does not have purely physical character also should be taken into account. Thus, according to the data reported in [12], glutamic acid may enter chemical interaction with calcium ions incorporated into hydroxyapatite or adsorbed on its surface. The close values of the experimental and calculated Γ values are the evidence of the formation of a monolayer on the adsorbent.

Having changed the coordinates for −ln C and − ln Γ one may obtain Freindlich adsorption isotherms in the linear form (Fig. 3). The obtained straight lines are described by the following equations:

for hydroxyapatite with Ca/P = 1.57:

$$Y = 0.48X + 0.3 \quad (5)$$

for hydroxyapatite with Ca/P = 1.67:

$$Y = 0.43X - 0.22 \quad (6)$$

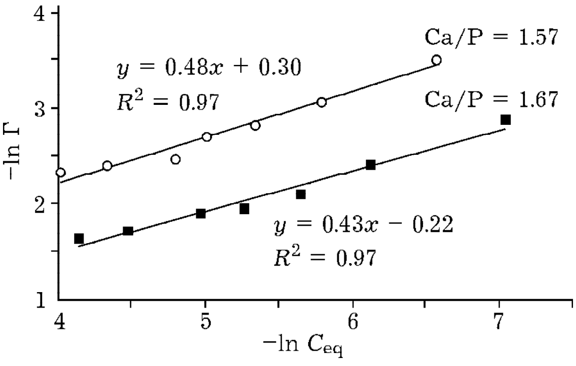

Fig. 3. Isotherms of the adsorption of glutamic acid on hydroxyapatite with different stoichiometry in the linear coordinates of Freindlich model.

The linear correlation coefficient is equal to 0.97 in this case. Having calculated the values of n and K in equation

$$\ln \Gamma = \ln K + (1/n)\ln C \quad (7)$$

(Table 3) we obtain Freindlich equation:

for hydroxyapatite with Ca/P = 1.57:

$$\Gamma = 0.74C^{0.48} \quad (8)$$

for hydroxyapatite with Ca/P = 1.67:

$$\Gamma = 1.25C^{0.43} \quad (9)$$

Not very high values of coefficient K in Freindlich equations connected indirectly with the specific surface of the adsorbent point to the crystallinity of the precipitated hydroxyapatite phases [9].

In order to test the adequacy of Langmiur and Freindlich models for the adsorption of glutamic acid on hydroxyapatite, we carried out calculation and comparative analysis of the obtained adsorption values with the practical values. It was established that the obtained experimental data may be described within both theories: $F_{\text{exp}} = 2.8 < F_{\text{tab}} = 4.3$ [13].

It is known that pH of solution has a strong effect on adsorption processes. In this connection, we obtained the data on the interaction of glutamic acid with hydroxyapatite (Ca/P = 1.57 and 1.67) within pH range 4−8 (Fig. 4). One can see that the effects of pH on the samples of the stoichiometric and non-stoichiometric hydroxyapatite are different. It is known that the fraction of negatively charged zwitter ions of glutamic acid increases with an increase in pH; the surface of hydroxyapatite with Ca/P = 1.57 is negatively charged, while the surface of hydroxyapatite with Ca/P = 1.67 is positively

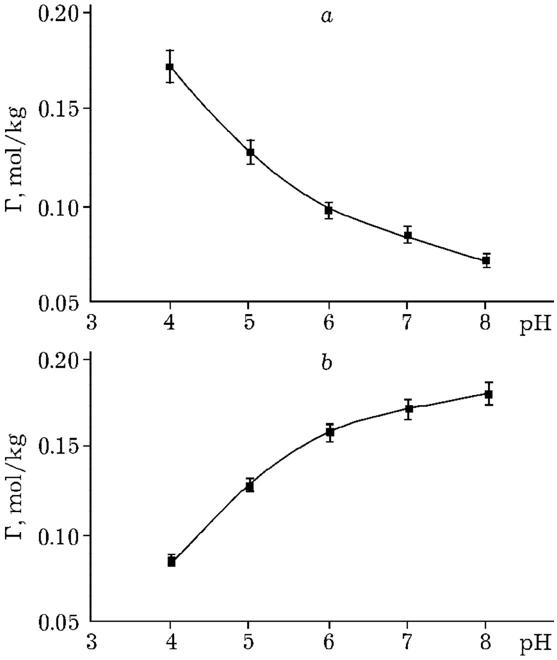

Fig. 4. Dependence of the adsorption of glutamic acid (0.01 mol/L) at hydroxyapatite on pH. Ratio Ca/P = 1.57 (*a*) and 1.67 (*b*).

charged. So, in the former case we observe a decrease in adsorption with an increase in pH, while in the latter case, quite contrary, we observe an increase in adsorption.

CONCLUSIONS

1. Hydroxyapatite samples with different stoichiometric composition were synthesized; the electrokientic properties of sols obtained from solutions in the presence and absence of amino acids were determined. The effect of magnesium, carbonate and oxalate ions on the synthesized hydroxyapatite was studied. It was established that an increase in the concentration of magnesium causes precipitation of struvite along with the formation of amorphous calcium phosphate; carbonate ions worsen the crystallinity of the formed hydroxyapatite, while their excess causes the formation of calcite; oxalate ions have an insignificant effect on the composition of the solid phase of hydroxyapatite.

2. It was established that the observed adsorption on the samples of synthesized calcium hydroxyapatite with different Ca/P ratios is described within Langmuir and Freindlich models. It was revealed that an increase in pH causes an increase in adsorption for the hydroxyapatite sample with the ratio Ca/P = 1.67 and a decrease in adsorption for the sample with the ratio Ca/P = 1.57.

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