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Features of Calcium Phosphate Crystallization in the Presence of Amino Acids

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Abstract

Thermodynamic and experimental studies were performed concerning phase formation process in the solution compositionally close to the oral fluid of humans with respect to the mineral and amino acid content. It has been established that amino acids to a considerable extent contribute into the ionic strength of the system promoting an increase in the electrostatic repulsion between the ions of the like charge and inhibiting the deposition of low-soluble calcium phosphates. In this process, the complexation of amino acids with Ca^{2+} does not result in a decrease of the solution supersaturation level and in changing the sequence of salt precipitation. The experimental simulation results for hydroxyapatite crystallization process have demonstrated that the inhibiting effect of amino acids is caused by the adsorption interaction between ionized species and the surface of the solid phase.

Key words: hydroxyapatite, amino acids, inhibition, crystallization

INTRODUCTION

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] represents an important physiogenous mineral, the main inorganic component of solid tissues in a human organism. Moreover, hydroxyapatite is more frequently occurring in the structure of pathogenic organomineral aggregates (OMA, including dental and salivary stones) than other compounds. The process of basic calcium phosphate biocrystallization occurs in the medium of complex composition. Important information concerning the formation of low-soluble phases in multicomponent biological systems could be obtained on the basis of thermodynamic calculations thereby conditions for precipitating the compounds would be determined. In this case there is a number of the difficulties connected, first of all, with a complicated structure of the system under simulation as well as with a variety of simultaneous processes. In this connection, studies concerning the features of phase formation processes in biological liquids is of currently central importance.

Earlier [1] we had carried out thermodynamic calculation of precipitation possibility for low-soluble calcium and magnesium salts from the solution compositionally close to human saliva with respect to inorganic components. However, in this case we did not take into account any influence of amino acids contained in significant amounts in the oral liquid of humans.

The role of amino acids in the conditions determining the formation of low-soluble calcium phosphates is not clear up till now. The authors of [2, 3] hypothesized that amino acids could inhibit the precipitation of hydroxyapatite. This fact could be caused, firstly, by an adsorption interaction resulting in the change of crystal growth rate; secondly, by a decrease of the system supersaturationя owing to the formation of some soluble complex compounds of amino acids with calcium ions.

Thus, establishment the role and character of the influence of amino acids upon processes of hydroxyapatite formation is of a considerable importance in order to understand of

the mechanism responsible for the formation of both biogenic and pathogenic OMA.

The goal of the present work consisted in carrying out thermodynamic calculation of phase formation possibility in the solution modeling the mineral and amino-acid composition of the oral liquid as well as experimental simulation of hydroxyapatite crystallization process *in vitro* in the presence of a number of amino acids.

EXPERIMENTAL

Thermodynamic calculation

Performing the thermodynamic calculation, as a prototype of the biological liquid we have chosen the modelling solution (Table 1) whose mineral composition (the content of inorganic macro components) temperature, ionic strength and the acid-base status of medium (pH 4.5–8.0) corresponds to the oral liquid of a healthy adult human [4, 5]. The influence of microelements, in particular of heavy metal ions, in this case was not taken into account.

For the calculation, we have chosen amino acids occurring in the oral liquid with a high values of stability constant for the complex compounds with calcium ions (it was supposed, only compounds with 1 : 1 structure are formed) [6]. We also took into account the contribution of ionized species of amino acids present in the solution at prescribed pH value. The values of thermodynamic solubility product (pK_s^0) for low-soluble compounds those could be formed in the solution under investigation [$CaHPO_4 \cdot 2H_2O$, $Ca_8H(PO_4)_6 \cdot 5H_2O$, $Ca_{10}(PO_4)_6(OH)_2$, $Ca_{10}(PO_4)_6F_2$, $CaCO_3$, $Ca(OH)_2$, $MgHPO_4 \cdot 3H_2O$, $MgNH_4PO_4 \cdot 6H_2O$, $Mg_3(PO_4)_2$, $MgCO_3 \cdot 3H_2O$, $Mg(OH)_2$], correspond to data available from the database for instability constants complex compounds (SC database, SC-QUERY Version 1.37 (1993)).

Experimental simulation

The process of mineral phase crystallization in the presence of amino acids was investigated *in vitro* within the medium being compositionally close to human saliva in the content of electrolytes (see by Table 1, pH 6.93±0.05). As initial reagents we used analytical and chemical purity grade salts such as $CaCl_2 \cdot 2H_2O$, $(NH_4)_2HPO_4$, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $NaHCO_3$, NaCl qualifications and distilled water. The concentration of Ca^{2+} and PO_4^{3-} ions in model solutions was created to be equal to 4.78 and 23.7 mmol/L, respectively. In this case the supersaturation level with respect to hydroxyapatite in the system was equal to 50. In separate experiments, we introduced glutamic acid into model solutions, with the concentration C equal to 0.107 g/L (norma), 0.396 g/L (lithogenesis in an oral cavity) and 1.070 g/L (10-fold norma)), serine (C = 0.128, 0.215, 1.28 g/L, respectively), lysine (C = 0.080, 0.440, 0.880 g/L, respectively) or proline (C = 0.009, 0.046, 0.093 g/L, respectively). The choice of these amino acids as additives was connected with the fact that they are present in the biological solution (the oral liquid) in free state and

TABLE 1

Data on the concentration of components (ionic and molecular species) in the model crystallization medium

Components	Concentration, mmol/L	Components	Concentration, mmol/L
Calcium ions	1.5	Fluoride ions	0.00106
Sodium ions	13.9	Tyrosine	0.524
Potassium ions	26.2	Arginine	0.620
Magnesium ions	0.65	Glycine	1.93
Ammonium ions	3.6	Alanine	1.54
Chloride ions	12.7	Serine	1.67
Carbonate ions	7.4	Asparaginic acid	1.28
Phosphate ions	5.6	Glutamic acid	1.88

are invoplved in the composition of some proteins, as well as since these compounds are prevailing in the structure of pathogenic OMA (dental and salivary calculae). Ready solutions were left to crystallize at a room temperature for 30–120 days. Precipitates were analysed using XRD technique (DRON-3), IR spectroscopy (Perkin Elmer) and AES-ICP (OPTIMA 2000 DV). For the liquid phase, we determined residual concentration of calcium, magnesium, phosphate ions and amino acids using the methods of chemical analysis.

RESULTS AND DISCUSSION

Thermodynamic calculation of phase formation possibilities in the prototype of human oral liquid

At the first stage, we have calculated the parameters of conditional solubility product constant pK for low-soluble compounds under investigation taking into account the complex formation with amino acids, ionic strength and the acid-base status (pH) of the solution medium. The possibility of the formation of almost insoluble compound in the solution was estimated using data concerning the supersaturation index value SI:

$$\Omega = \left(\frac{\mathrm{IAP}}{K_{\mathrm{s}}^{0}}\right)^{\frac{1}{p+q}} = \mathrm{SI} = \lg \Omega \qquad (1)$$

and the Gibbs energy for crystallization:

$$\Delta G = -\frac{RT}{\nu} \ln \frac{\mathrm{IAP}}{K_{\mathrm{s}}^{0}} \qquad (2)$$

Here Ω is the supersaturation level, in the solution for the given salt; IAP is the activity product for precipitate-forming, ions to the power of stoichiometric coefficients; K_{s}^{0} is the thermodynamic solubility product constant for a low-soluble compound; ΔG is the Gibbs energy for the crystallization of a low-soluble compound from solution, J/mol; R is the absolute gas constant, J/(mol · K); T is the temperature, K; n is the quantity ions in the structure of the low-soluble compound.

In this case when SI > 0, and $\Delta G < 0$ the precipitation of the solid phase from the solution is thermodynamically probable.

According to the results obtained, for the solutions under investigation the formation is thermodynamically probable of the following low-soluble compounds: $Ca_{10}(PO_4)_6(OH)_2$, $Ca_{10}(PO_4)_6F_2$, $CaHPO_4 \cdot 2H_2O$, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ and $CaCO_3$. In comparing the supersaturation indices calculated for data concerning low-soluble calcium salts, it should be noted that in the conditions under investigation (at pH 4.5–8.0) the greatest supersaturation level is exhibited by fluoroapatite and hydroxyapatite. At pH < 7.0 according to a decrease of SI values, calcium phosphates under investigation could be arranged as it follows: $Ca_{10}(PO_4)_6F_2 > Ca_{10}(PO_4)_6(OH)_2 > CaHPO_4 \cdot 2H_2O > Ca_8H_2(PO_4)_6 \cdot 5H_2O$. At pH > 7.0 the sequence would be the following: $Ca_{10}(PO_4)_6F_2 > Ca_{10}(PO_4)_6(OH)_2 > Ca_8H_2(PO_4)_6 \cdot 5H_2O > CaHPO_4 \cdot 2H_2O$. It is known [5] that octacalcium phosphate and brushite represent metastable phases with respect to hydroxyapatite. The data of thermodynamic calculation have demonstrated that $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ and $CaHPO_4 \cdot 2H_2O$ exhibit much lower SI and ΔG values as compared to hydroxyapatite. This indicates that the given phases are less stable in the conditions under investigation. Similar laws have been established for the model system whose composition involved only inorganic components [1].

Under the conditions of complex formation with amino acids the concentration of free calcium ions those participate in the processes of phase formation decreases. However, changing the amount of Ca^{2+} ions at the average total physiological concentration of amino acids is insignificant (the initial concentration of calcium ions is equal to 1.50 mmol/L, the final being of 1.49(9) mmol/L) thus it does not influence upon the conditional solubility product value, the sequence and pH of crystal phase precipitation beginning.

Thus, the presence of amino acids in the oral liquid does not exert any influence upon the thermodynamic stability of calcium phosphates with different stoichiometric composition with respect to each other.

At the same time, the charged amino acid species rather significantly contribute to the ionic strength of the solution which ionic strength exhibits an almost 1.5-fold increase (from 0.039 up to 0.051 mol/L) already at the physiological concentration. As the ionic strength of a hypothetical solution increases, an increase

TABLE 2

Data concerning the precipitation beginning pH values for solid phases in model solutions with amino acids

Compounds	C_{AA}*, mol/L				
	0	0.0159	0.159	0.318	1.160
Fluoroapatite	4.8	4.9	5.5	5.9	7.4
Hydroxyapatite	5.6	5.6	6.2	6.5	–
Брушит???	6.4	6.5	7.4	–	–
Octacalcium phosphate	6.5	6.6	7.5	–	–
Calcite	7.6	7.7	–	–	–

*C_{AA} is the total concentration of seven amino acids under investigation in the solution (see Table 1).

is observed in the conditional solubility product values for calcium phosphates and carbonates, whereas the Gibbs energy values for the crystallization of each compound become more positive. In this case, the ranges of pH values wherein the conditions are reached for the precipitation of low-soluble compounds, differ from each other to a considerable extent depending on the concentration of amino acids in the model system (Table 2).

At the biological value of total amino acid concentration (C_{AA} = 0.0159 mol/L), shifting the pH values of precipitation beginning for almost all compounds is observed. The further increase in the amount of organic substances in the solution (up to C_{AA} = 0.159 mol/L) results in the fact that within the range of medium acidity under investigation the crystallization of calcite becomes impossible, whereas at $C_{AA} > 0.3$ mol/L the compounds such as $CaHPO_4 \cdot 2H_2O$ and $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ are unstable. Thus, the increase in amino acid concentration in the solution results in growing the ionic strength, whereas the precipitation beginning pH value for low-soluble compounds is shifted towards the alkaline area, with the growth of ionic strength.

The results obtained indicate a significant contribution of amino acids to the ionic strength of the solution, which is expressed in an increase in the electrostatic repulsion between the like ions and in a hindrance in the phase formation process. In this case, amino acids exhibit a rather weak ability of binding calcium ions to produce complex compounds, as it was noted above. It is so since the formation of chelate compounds from dissolved amino acids and Ca^{2+} does not result in a considerable decrease of the solution supersaturation level, which would result in some changes in the course of mineral phase crystallization observed. Thus, the amino acid complex formation with calcium ions cannot be any basic cause of the inhibiting action in the process of calcium phosphate precipitation. In our opinion, the delay of growth and the size reduction of hydroxyapatite crystals formed in the presence of amino acids in the crystallization medium (see Table 1) could be caused at a greater extent by the adsorption interaction between the ionized forms of organic molecules with charged sites on the solid phase surface. The results of the experimental simulation confirm this assumption.

Modelling the phase formation in the prototype of human oral liquid

The XRD and IR spectroscopic analysis of solid phases obtained by the precipitation from the solutions, simulating the composition of the oral liquid of a healthy human has demonstrated, that all the samples are composed of hydroxyapatite (according to the thermodynamic calculations, hydroxyapatite exhibits one of the maximal values of the supersaturation index).

The crystallinity level of the compounds varies in the presence of additives and depends on the latter concentration. Worsening the crystallinity of precipitates as compared to the ref-

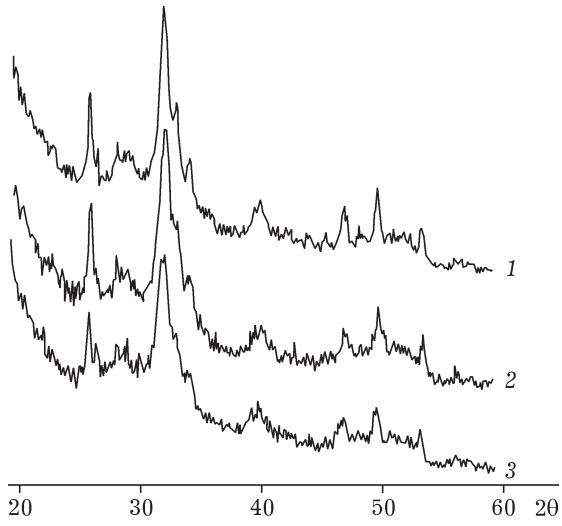

Fig. 1. XRD profiles for the samples resulting from the solutions: *1* – without any additives; *2, 3* – with the content of serine amounting to 0.128 and 1.28 g/L, respectively.

TABLE 3

Characteristics of solid phases from the solutions with amino acids

Amino acids	C_{AA}, g/L	Precipitate mass, g	Content, mass %		Ca/P	K^*
			Ca	P		
Glutamic acid						
[$HOOCCH(NH_2)CH_2CH_2COOH$]	0.107	0.071	35.83	18.82	1.77	4.75
	0.396	0.080	34.86	18.36	1.80	4.43
	1.073	0.081	33.10	17.27	1.85	4.18
Serine						
[$HOCH_2CH(NH_2)COOH$]	0.128	0.088	28.56	14.85	1.79	4.67
	0.215	0.092	30.07	16.18	1.83	4.41
	1.282	0.094	30.23	16.36	1.81	4.27
Proline						
[HNC_4H_7COOH]	0.009	0.089	40.09	19.66	2.02	4.29
	0.046	0.098	42.27	18.86	1.94	3.77
	0.093	0.101	46.08	22.86	1.96	3.13
Lysine						
[$H_2N(CH_2)_4CH(NH_2)COOH$]	0.088	0.103	42.85	19.16	1.92	4.18
	0.440	0.117	39.57	19.31	1.91	3.61
	0.880	0.123	37.12	18.47	1.89	3.02
Without any additives	—	0.493	31.94	18.74	1.70	5.35

*K is the crystallinity level determined from the IR spectra of the samples using PeakFit_v 4.11 software.

erence sample is observed in the presence of amino acids. Thus, the higher the content of glutamic acid, serine, lysine or proline in the solution, the greater is the decrease of solid phase crystallinity (Fig. 1 and Table 3). To all appearance, this fact could be caused by inhibiting the process of hydroxyapatite precipitation due to the adsorption of amino acids on the active centres of crystal growth.

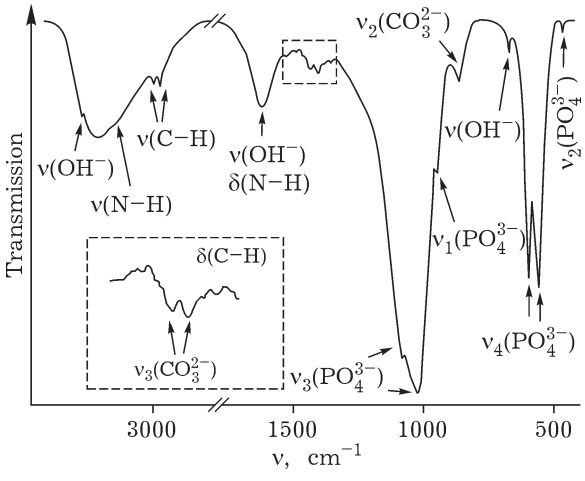

Fig. 2. IR spectrum for hydroxyapatite resulting from the solution with the content of serine equal to 1.282 g/L.

This hypothesis is confirmed by the results obtained from IR spectroscopic investigation (Fig. 2). The IR spectra of all the samples exhibit absorption bands for groups inherent in amino acid molecules: a wide band within the range of 3100–3600 cm^{-1} (the transmission minimum being cantered at 3200 cm^{-1}) whereas vibrations at 1650 cm^{-1} include overlapped absorption bands for the vibrations of N–H bonds in the ionized amino groups of the acids and the O–H bonds of water adsorbed onto apatite, and the bands with minima cantered at 2940, 2880 and 1380 cm^{-1} correspond to the vibrations of C–H bonds in methylene groups.

The presence of the mentioned types of vibrations indicates the adsorption of amino acids in the form of zwitterions (the ions carrying positive and negative charges simultaneously, spatially separate and located at different atoms) on hydroxyapatite surface and contradict any possible hypotheses concerning the formation of mechanical mixtures from individual components or embedding the molecules of amino acids into the crystal lattice of hydroxyapatite. The latter, moreover, it is improbable

owing to a greater size of amino acid molecules under investigation as compared to the size of OH^- and PO_4^{3-} positions in apatite.

The mass of the mineral component crystallizing from model solutions in all the cases in 5–7 times less than the mass of a sediment precipitating from the model system without additives. This indicates also the inhibiting influence of amino acids upon the crystallization of hydroxyapatite.

The studies on the structure of precipitates using the method AES has demonstrated that for the solid phases obtained molar ratio of Ca/P > 1.67 (see Table 3). Under the conditions of the experiment, the deviation the from the atomic ratio Ca/P = 1.67 for the hydroxyapatite with ideal formula $Ca_{10}(PO_4)_6(OH)_2$ could be connected with the formation of amorphous calcium phosphate $Ca_x(PO_4)_y \cdot zH_2O$ together with hydroxyapatite. This assumption is confirmed by the results obtained employing the XRD phase analysis within the range from 10 to 25° of 2θ (CuK_α). According to those results, a considerable increase in the background (see Fig. 1) is observed, which indicates the presence of a roentgen-amorphous component, whose Ca/P ratio can change over a wide range (1.2–2.5 at pH 6.6–11.7 and $[Ca] \cdot [P] = 25–(5 \cdot 10^5)$ mM^2; pH_{exp} 6.93±0.05, $[Ca] \cdot [P]_{init} = 113$ mM^2) [7].

Earlier, in the course of experiments concerning the measurement of *Z*-potential for the particles of hydroxyapatite sol [8] it has been established that the higher the deviation from the ideal value of Ca/P ratio the higher positive charge is inherent in the solid phase surface in the solution. This causes the possibility of adsorption interaction between hydroxyapatite and amino acids [8, 9]. The authors of [9] have established that for the glutamic acid the mentioned process is described well basing on the Langmuir model, whereby a monomolecular layer is formed on the adsorbent surface. The coefficient *K* values determined by the authors of [9] for the Freundlich equations indicate the crystallinity of hydroxyapatite solid phases under segregation. The adsorption interaction between amino acids and the surface of $Ca_{10}(PO_4)_6(OH)_2$ is of electrostatic nature, and according to [10] the most active molecular fragments in the adsorption are ionized carboxylic and amino groups by means of those the molecules of organic substances can absorb both onto positive, and onto negative sites of solid phase surface. According to current concepts [11], at the initial stage of the interaction there is a spatial rapprochement between ionized carboxylic groups of amino acid and a surface site bearing a positive charge ($Ca^{\delta+}$). Further, a bond arises between the coordinated sites of the solid phase surface and the molecule of amino acid. As a result the loss of a proton by an ionized amino group becomes possible and thus the binding of one more calcium centre. Amino acids in this case act as polydentate ligands those are capable to block simultaneously several active growth centres due to the formation of strong chelate complexes with calcium as the result of adsorption.

The possibility of interaction between amino acids under investigation and hydroxyapatite has been confirmed by the results of molar fraction calculation for each amino acid species present in the model system under the experimental conditions (Table 4). It has been established that in an aqueous solution (pH_{exp} 6.93±0.05) the organic substances chosen are present mainly as zwitterions those are adsorbed on the surface of hydroxyapatite. In this case it should be noted that among all the amino acids in the conditions under investigation the negative charge is possessed only by the molecules of glutamic acid (in the solution they exist in the form of $^-OOC(CH_2)_2CH(^+NH_3)COO^-$), the positive charge is possessed by the molecules of lysine ($^+NH_3(CH_2)_4CH(^+NH_3)COO^-$), the total zero charge is possessed by proline ($H_3N^+(CH_2)_3CH_2COO^-$) and serine ($HOCH_2(^+NH_3)COO^-$). To all appearance, charged species at the initial stage of crystallization are adsorbed onto the active centres of the polar surface of hydroxyapatite crystal nuclei preventing them from further growth. In this case, nanometre- and micrometre-sized particles are formed, and as a consequence, the mass of the precipitate segregating from the system appears much less than the mass of a sample from the system without any additives.

Under the experimental conditions, positively charged areas prevail on the surface of hydroxyapatite (because the excess of calcium), therefore the interaction between amino acids and active centres grows with decreasing the positive charge of the amino acid species pre-

TABLE 4

Acidity constants and molar fractions of various amino acid species present in the solution (pH 7)

Amino acids	pK_a α-COOH	pK_a α-NH_3^+	pK_a −RH	α (H_3A)	α (H_2A)	α (HA)	α (A)
Glutamic acid	2.19	9.67	4.25	$2.74 \cdot 10^{-8}$	$1.77 \cdot 10^{-3}$	$9.96 \cdot 10^{-1}$	$2.13 \cdot 10^{-3}$
Serine	2.21	9.15	–	–	$1.61 \cdot 10^{-5}$	$9.93 \cdot 10^{-1}$	$7.03 \cdot 10^{-3}$
Proline	2.00	10.60	–	–	$1.00 \cdot 10^{-5}$	1.00	$2.51 \cdot 10^{-4}$
Lysine	2.18	8.95	10.53	$1.50 \cdot 10^{-5}$	$9.89 \cdot 10^{-1}$	$1.11 \cdot 10^{-2}$	$3.27 \cdot 10^{-6}$

dominating in the system. According to the charge of main species, the amino acids under investigation could be ranged according to the strength of interaction with apatite surface as it follows: lysine < proline < serine < glutamic acid. Hence, in the conditions under investigation the glutamic acid exhibits the strongest inhibition action in the course mineral phase crystallization, whereas lysine demonstrates the weakest action, which is confirmed by data concerning the mass of precipitates obtained in the presence of these amino acids (see Table 3).

It is important that the amino acid affinity with respect to calcium phosphate increases with the increase in the number of active (with respect to the adsorption) functional groups those are included in the structure of the molecule (COO^-, NH_3^+, OH^-) [2, 3, 10]. Two carboxylic groups and one amino group participate in the process of glutamic acid adsorption, which determines a much greater affinity of glutamic acid with respect to hydroxyapatite (K_{aff} = $30.21 \cdot 10^2$ L/mol [2]) as compared to other amino acids under investigation. As a consequence, already at a small concentration value in the solution the glutamic acid represents a much stronger inhibitor of crystallization and growth.

Serine has three functional groups in the structure (NH_3^+, COO^-, OH^-) active with respect to adsorption, as well as this amino acid exhibits a rather small size and a partial negative total charge of zwitterions. In this connection, under the experimental conditions demonstrates a high enough inhibition activity.

The molecules of lysine have total positive charge, therefore one could assume that owing to the like charges of the sorbate and the sorbent there is electrostatic repulsion between them. This results in the fact that the adsorption occurs only on the negatively charged sites of the hydroxyapatite surface (phosphate groups) being of low value under the experimental conditions.

Small value of the proline affinity constant with respect to hydroxyapatite (~$7.5 \cdot 10^2$ L/mol [2]) characterizes a low intensity of adsorption. However, the amino acid is capable to block a variety of active centres because the pyrrolidine ring could be arranged in parallel with respect to surface of the solid phase and thus to hinder the growth of hydroxyapatite crystals.

The factors of the interaction described result in the fact that the strongest inhibitors of hydroxyapatite crystallization under experimental conditions are glutamic acid and serine, therefore in their presence the mass of the precipitate is minimal, the content of calcium ions is lower, and crystallinity is higher than the for the phases obtained earlier.

Thus, in the course of the experimental simulation performed concerning the process of phase formation in a hypothetical solution, compositionally close to the oral liquid of a human it has been established that the inhibition of hydroxyapatite crystallization is caused by an adsorption interaction between ionized forms of amino acids and charged sites of the solid phase surface. In this case, according to the inhibiting action intensity the amino acids under investigation could be arranged in the following order: glutamic acid > serine > proline, lysine. The amino acid ability to hinder the hydroxyapatite crystallization process depends on the concentration of the substance in the system, molecular geometry, as well as on the form its existence in the solution.

CONCLUSION

$Ca_{10}(PO_4)_6F_2$ and $Ca_{10}(PO_4)_6(OH)_2$ exhibit the greatest supersaturation level and then they are the most stable calcium phosphates whose crystallization from the prototype of an the oral liquid of a human is possible. $CaHPO_4 \cdot 2H_2O$ and $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ manifest themselves as metastable phases under the conditions under investigation. The stability of salts with respect to each other does not change in the presence of amino acids in the solution under simulation. At the same time, an increase of the ionic strength of the medium (taking into account the contribution of the ionized forms of amino acids) promotes shifting the values of pH for beginning the precipitation of low-soluble calcium salts into a more alkaline pH range. From the results of the experimental simulation of hydroxyapatite crystallization *in vitro* in the presence of four amino acids involved in the biological liquid it has been established that amino acids hinder the formation of stoichiometric hydroxyapatite due to adsorption onto the surface.

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