**Physicochemical regularities of the sorption of amino acids on the surface of hydroxylapatite**

Biogenic and pathogenic mineral formations contain calcium phosphates. It is known that hydroxylapatite is the main mineral component of bone tissue, tooth enamel, and dentin. The basis of the processes of mineralization is proposed to be in the adsorption interaction of free amino acids and associated protein molecules with inorganic components in body fluids and emerging phases. However, the mechanism of their interaction is not fully understood. In this paper the adsorption of amino acids on the surface of hydroxylapatite has been studied. The synthesis of calcium phosphates from solution has been carried out. According to the results of XRD and IR spectroscopy it has been established that precipitation is represented by the phase of hydroxylapatite. The adsorption of amino acids in a wide range of variation of their concentrations and the pH of the solution has been investigated. It has been established that adsorption of amino acids on the surface of hydroxylapatite reaches saturation. The effect of pH on the maximum adsorption of amino acids on hydroxylapatite has been considered. It has been established that adsorption of amino acids is described by the Langmuir model. The result of IR spectroscopy confirmed adsorption. It has been determined by the sign of the surface charge of the solid phase of hydroxylapatite. The values of the Gibbs energy have been calculated. It has been revealed that the interaction of amino acids with the surface of the hydroxylapatite is characterized by physical adsorption.

**Keywords:** hydroxylapatite, adsorption, amino acid, dissolution, surface charge, solution, Langmuir model, Freundlich model.

**Introduction**

Calcium phosphates are part of biogenic and pathogenic mineral formations. Biogenic formations are part of defined organs and have different functions; they are genetically determined, their place in the body is strictly defined. Pathogenic mineral formations occur in the disruption of the functioning of the whole organism or its separate organs. It is known that hydroxylapatite is the main mineral component of bone tissue, tooth enamel and dentin. The hydroxylapatite plays an important role in many physiological processes in the human body [1–4].

In the papers [5–13] the researchers described that calcium phosphates had an excellent biological activity and had been widely used in bone reconstruction or orthopedic supplements, as the primary inorganic material the composition of the hard tissues of the body.

Interaction of organic and mineral components is important in such processes of biogenic crystallization, as the formation of mammals bone matrix, as well as the emergence and growth of pathogenic entities.

Hydroxylapatite (HA) and dicalcium phosphate dihydrate (DCPD) CaHPO₄·2H₂O are of greatest interest from number of the biocompatible low-temperature calcium phosphates. In nature DCPD expressed in the mineral brushite is the most soluble of biocompatible calcium phosphates, whereas HA is less soluble [14]. In [9] it is examined the differences in stability of calcium phosphates, and also indicated the CA/P ratio, to confirm the differences between the calcium phosphates.
There are a number of assumptions, according to which the basis of the processes of mineralization, is in the adsorption interaction of free amino acids and associated protein molecules with inorganic components in body fluids and emerging phases [15, 16]. The mechanism of their interaction is not fully understood.

In this regard, recent studies aimed at studying the regularities of adsorption of amino acids on the inorganic component of physiognomic and pathogenic neoplasms.

The aim of this work is investigation of peculiarities of adsorption of amino acids on the hydroxylapatite by varying the pH of the solution.

**Materials and methods**

**The synthesis of hydroxylapatite.** It is carried out by deposition from aqueous solution at room temperature by the method of spontaneous crystallization in accordance with the following reaction:

\[
10\text{Ca(NO}_3\text{)}_2 + 6(\text{NH}_4\text{)}_2\text{HPO}_4 + 8\text{NH}_4\text{OH} \rightarrow \text{Ca}_{10}(\text{PO}_4\text{)}_6(\text{OH})_2 + 20\text{NH}_4\text{NO}_3 + 6\text{H}_2\text{O}
\]

The precipitate is obtained by mixing dilute solutions of calcium nitrate Ca(NO\(_3\))\(_2\).4H\(_2\)O, and ammonium hydrogen phosphate (NH\(_4\))\(_2\)HPO\(_4\), in the ratio of 1.67:1, and aqueous solution of ammonia NH\(_4\)OH at room temperature (22–25 °C). When the synthesis was carried out, to a 500 ml solution containing Ca(NO\(_3\))\(_2\), 5 ml of conc. NH\(_4\)OH solution, 500 ml of a solution (NH\(_4\))\(_2\)HPO\(_4\) with a concentration of (50 mmol/l) are rapidly poured. The total volume of the mixture is 1000 ml. After mixing the solutions, the pH of the system is corrected to 12.00 ± 0.05 with solutions of NaOH (20 %) and/or HNO\(_3\) (1:1).

After the heterogeneous system settling for two days, the solution was filtered using a water-jet pump, Bunsen flask and a Buchner funnel (using two filters, a blue ribbon). After filtration, the filter cake was washed with water (V=50 ml), dried in a drying box at a temperature of ~ 100 °C until complete removal of water. The dried precipitate is ground in a porcelain mortar into a powder, transferred into a special marked container and weighed on an analytical balance.

It is the determination of the sign of the charge of the particles of the sols of hydroxylapatite by the method of capillary analysis. When a piece of filter paper is immersed in water, thin wall paper is charged negatively. These allow determining the sign of the charge of the colloidal particles. Negatively charged particles rise through the capillaries.

We used the direct potentiometric method for pH measurements. The measurements were carried out in a glass beaker, which pre-processes. The measurement error is ±0.01 pH units.

Determination of the concentration of calcium ions in the experiment performed by direct potentiometry using ion-selective electrode and calibration schedule determined by the value of [Ca\(^{2+}\)].

Determination of phosphate ions was carried out by the molybdenum blue method (State Standard 18309–72) on the KFK-2 device, using the red filter (\(\lambda_{ef} = 690\) nm) and cuvettes with a layer thickness of 2 cm. The determination is repeated three times and the average values of optical densities to build a calibration curve: \(D = f(C(\text{PO}_4^{3-}))\), calculate the regression equation.

Error determinations are found within 2-4 Rel. %. X-ray diffraction (XRD) is used to study the mineral (phase) composition of synthesized solid phases of hydroxylapatite. A diffractogram was obtained by the «method of powder» for stationary x-ray apparatus DRON-3 [17, 18]. Phase identification was performed using ASTM international indexes and tables [18]. The sensitivity of the XRD method for these measurements is 3 %.

The infrared spectroscopy method was used for more information on the composition of the samples obtained. IR spectrum was obtained on the FSM 2201 spectrophotometer. Mathematical processing of the data was carried out using statistical software Statistica 10 and Static2 from the StatSoft statistical package. The sensitivity of the method of IR-spectroscopy for the measurement data was < 5 %.

The method of area measurement was used to analyze the specific surface of synthetic calcium phosphates. Analysis of the specific surface of the samples according to the BET method (SBET-N2) was carried out using techniques of single-point standard gas adsorption equilibrium at an absolute pressure in the adsorption unit Sorbtometr, IK SB RAS (OSC SB RAS, Omsk). The limit of permissible relative error of measurement of the specific surface in the mode of multiple measurements is not more than 5 %.

**The adsorption experiment.** 0.5 g of hydroxylapatite sample was placed in a flask and the solution of amino acids was poured. The concentration of amino acids was varied as follows: 2, 4, 6, 8, 10, 15, 20, 25, 30 mmol/l and pH range 5.00–8.00 ± 0.05 (except 6.50) in increments of 0.50. Shaking is carried out for 30 minutes, then it was left for 48 hours. After the specified time the content of the flasks was filtered and there was determined the content of amino acids in the filtrate by the method of transfer of amino acids in a solu-
ble copper salt and subsequent photometric determination, the pH after adsorption was measured, the mass of precipitation was determined.

Determination of the concentration of amino acids in the experiment was assessed by the photometric determination of amino acids. The essence of the method for determination of the concentration of amino acids used in the analysis is based on the conversion of amino acids into soluble copper salts and their subsequent photometric determination. KFK-2 photoelectric colorimeter was used for measurements. Determination of optical density of standard solutions is carried out in the wavelength interval that includes the value of 670 nm. A calibration curve was built for measurements. Determining the unknown concentration of amino acids was performed using a calibration chart.

Results and discussion

1. The results of the synthesis of crystals of hydroxyapatite. The methods of XRD and IR spectroscopy were established that the precipitation obtained after 48 hours of crystallization, represented by the phase of hydroxyapatite (Fig. 1), belonging to the hexagonal crystal structure (2 θ corresponds to 31.8, 32.9, 39.9).). The crystallite size was calculated of equal to D = 13 nm [19], which is consistent with the data [2].

The IR spectrum of the samples contained the whole spectrum of the bands characteristic for hydroxyapatite (Fig. 2) and have a complex structure. The presence of absorption at 1113, 1019, 958 cm⁻¹ and 604, 573, 469 cm⁻¹ correspond to vibrations of a group PO₄³⁻ as well as trough sat 3576, 3400 cm⁻¹ that are characteristic for OH- stretching vibrations of molecules of structurally bound water. One can also notice that the spectrum contains absorption bands of CO₃²⁻ with the peaks at 1460, 1420 and 865 cm⁻¹. In addition, a broad band in the region of 2700–3700 cm⁻¹ and peak at 3540 cm⁻¹ can be attributed to the stretching vibrations of H-O-H and OH, respectively.

![Figure 1. The diffraction pattern of the synthesized sample of hydroxyapatite at pH = 5.50±0.05 (48 hours)](image1.png)

![Figure 2. The IR spectrum of the sample of hydroxyapatite synthesized at pH = 5.50±0.05 (48 hours)](image2.png)

The specific surface of hydroxyapatite was calculated by the method of BET, and its value was 72.0 m²/g. Analysis of the supernatant liquid and calculation of the Ca/P ratio showed that the Ca/P in the synthesized hydroxyapatite was equal to 1.67 that corresponded to the ideal HA.

2. The results of adsorption experiment. As a result of the adsorption experiment, isotherms of adsorption of amino acids to hydroxyapatite were obtained (Fig. 3). Referring to the curves, it can be concluded that the adsorption reaches saturation. This allows for Cₓm = 0.030 mol×L⁻¹ to compare the values of adsorption while varying the pH of the solution to finding the values of maximum adsorption (Table 1).
The maximum adsorption of amino acids at varying pH, \( q, \text{ mol} \times \text{kg}^{-1} \)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>pH 5.00</th>
<th>pH 5.50</th>
<th>pH 6.00</th>
<th>pH 7.00</th>
<th>pH 7.50</th>
<th>pH 8.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>0.400</td>
<td>0.350</td>
<td>0.400</td>
<td>0.400</td>
<td>0.450</td>
<td>0.400</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.150</td>
<td>0.150</td>
<td>0.200</td>
<td>0.150</td>
<td>0.150</td>
<td>0.150</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.150</td>
<td>0.150</td>
<td>0.200</td>
<td>0.200</td>
<td>0.250</td>
<td>0.300</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.300</td>
<td>0.250</td>
<td>0.250</td>
<td>0.200</td>
<td>0.200</td>
<td>0.200</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.200</td>
<td>0.200</td>
<td>0.200</td>
<td>0.150</td>
<td>0.150</td>
<td>0.150</td>
</tr>
</tbody>
</table>

The pH values, at which the maximum adsorption of amino acids on the surface of hydroxylapatite occurs, are shown in the table 1. These amino acids are in charged ionic forms at given pH values of the solution. To determine the equation describing the adsorption, the experimental data were processed from the position of the Langmuir theory (1) and the Freundlich theory (2) (Table 2):

\[
q = \frac{q_m K_f C}{1 + K_q C},
\]

where \( q_m \) — is the limiting amount of adsorption, \( \text{mol} \times \text{kg}^{-1} \); \( K_q \) — is the adsorption equilibrium constant; \( C \) — is equilibrium concentration of adsorbate, \( \text{mol} \times \text{L}^{-1} \).

\[
q = K_F C^n^{1/2},
\]

where \( K_F \) — is coefficient of proportionality; \( n \) — is exponent, \( n<1 \).
Equations described by the models of Freundlich and Langmuir, for adsorption of amino acids on hydroxylapatite by varying the pH of the solution

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>$q_{\text{max}}$, mol×kg$^{-1}$</th>
<th>pH$_{\text{max}}$</th>
<th>Langmuir</th>
<th>Freundlich</th>
<th>Langmuir</th>
<th>Freundlich</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>0.450</td>
<td>7.50</td>
<td>$q = 0.749 \times \frac{46.49 \times C}{1 + 46.49 \times C}$</td>
<td>$q = 9.158 \times C^{0.722}$</td>
<td>0.960</td>
<td>0.958</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.200</td>
<td>6.00</td>
<td>$q = 0.239 \times \frac{11.40 \times C}{1 + 11.40 \times C}$</td>
<td>$q = 0.938 \times C^{0.401}$</td>
<td>0.958</td>
<td>0.899</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.300</td>
<td>8.00</td>
<td>$q = 0.368 \times \frac{33.47 \times C}{1 + 33.47 \times C}$</td>
<td>$q = 2.965 \times C^{0.534}$</td>
<td>0.969</td>
<td>0.831</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.300</td>
<td>5.00</td>
<td>$q = 0.368 \times \frac{33.15 \times C}{1 + 33.15 \times C}$</td>
<td>$q = 2.966 \times C^{0.534}$</td>
<td>0.969</td>
<td>0.831</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.200</td>
<td>5.00–6.00</td>
<td>$q = 0.216 \times \frac{13.08 \times C}{1 + 13.08 \times C}$</td>
<td>$q = 0.782 \times C^{0.361}$</td>
<td>0.957</td>
<td>0.846</td>
</tr>
</tbody>
</table>

The obtained values of $q_{\text{in}}$ are adequate, as all practical values of $q$ are less than $q_{\text{in}}$. As can be seen from Table 2, adsorption of amino acids has a good correlation in the linear coordinates of the model of Langmuir.

It was further used to calculate the change of Gibbs energy according to the formula (3) (Table 3):

$$
\Delta G = -RT \ln K,
$$

where $R$ — is the universal gas constant, $R = 8.314 \text{kJ/(Kmol×K)}$; $K$ — is the constant of adsorption equilibrium.

The value of the Gibbs free energy for adsorption of amino acids, $-\Delta G$, kJ×mol$^{-1}$

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>pH</th>
<th>5.00</th>
<th>5.50</th>
<th>6.00</th>
<th>7.00</th>
<th>7.50</th>
<th>8.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>8.94</td>
<td>8.87</td>
<td>9.08</td>
<td>9.08</td>
<td>9.51</td>
<td>8.80</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>6.47</td>
<td>7.42</td>
<td>6.03</td>
<td>6.47</td>
<td>7.42</td>
<td>7.42</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8.53</td>
<td>8.53</td>
<td>8.50</td>
<td>8.50</td>
<td>8.56</td>
<td>8.70</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>8.67</td>
<td>8.53</td>
<td>8.53</td>
<td>8.46</td>
<td>8.46</td>
<td>8.46</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>6.37</td>
<td>6.37</td>
<td>6.37</td>
<td>7.42</td>
<td>7.42</td>
<td>7.42</td>
<td></td>
</tr>
</tbody>
</table>

The values of the Gibbs energy were calculated and there was revealed that the interaction of amino acids with the surface of the hydroxyapatitewas characterized by physical adsorption due to forces of electrostatic interaction of positively and negatively charged centers, as well as due to the formation of hydrogen bonds.

Analysis of the surface charge of the solid phase of hydroxyapatite at pH $= 5.00, 6.50, 8.00 \pm 0.05$ has shown that its surface is positively charged under the given pH values of the solution. It can be seen that after adsorption ($C_{aa} = 4 \text{mmol/l}$), glycine addition does not change the sign of the surface charge, because it is possible to incorporate glycine into the hydroxyapatite structure. With the addition of alanine, there is also no surface recharge, since physical adsorption binds its positive and negative groups at the ends, and as a result, the uncharged side group does not affect the charge of the surface. When adsorption of arginine is also binding its end groups, but at these pH values it is in the form of positively charged zwitterions, and as a result, outside the surface is it’s charged positively side group, which does not change the charge of the surface. However, with the addition of aspartic and glutamic acids, it can be seen that the surface is recharging, they are at the given pH values of the solution in the form of negatively charged zwitterions. Because of this, adsorption on the surface has a negatively charged side group that forms a layer on the surface, which as a result changes the charge of the surface of the hydroxyapatite (Table 4).
Table 4

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>pH</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.00</td>
<td>6.50</td>
<td>8.00</td>
</tr>
<tr>
<td>Without amino acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Arginine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The result of IR spectroscopy confirmed adsorption. On the IR spectrum of the sample after the adsorption experiment (Fig. 4), the vibration band at 1650 cm\(^{-1}\) should be considered as the total, reflecting the vibrations of C = O, N–H, O–H bonds in amino acid and OH-ions in hydroxylapatite. Also, the appearance of specific absorption bands associated with the vibrations of N–H and C–N bonds, which appear in the 3500–3300, 1650–1500, 1360–1000 cm\(^{-1}\) regions, is registered, which confirms the possibility of adsorption of amino acids on hydroxylapatite.

![Figure 4. The IR spectrum of the sample after the adsorption experiment (48 hours)](image)

Previously we have found that glycine, aspartic and glutamic acids adsorption is described by Freundlich model. For alanine and arginine adsorption is described by the model of Langmuir [20, 21].

Conclusions

1. It was carried out the synthesis of hydroxylapatite, the structure of which was confirmed by XRD and IR spectroscopy. The values of the sign of the surface charge of hydroxylapatite were found to be positive. τ

2. The processes of adsorption of amino acids on the hydroxylapatite were investigated. There was identified that the values of pH of maximum adsorption were as follows: glycine at pH = 7.50 ± 0.05, the alanine at pH = 6.00 ± 0.05, aspartic acid at pH = 8.00 ± 0.05, glutamic acid at pH = 5.00 ± 0.05, arginine at pH = 5.00–6.00 ± 0.05.

3. Adsorption of amino acids was described by the Langmuir model.

4. The values of the Gibbs energy were calculated. There was revealed that the interaction of amino acids with the surface of the hydroxylapatite was characterized by physical adsorption.

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References


О.А. Голованова, К.К. Головченко
Гидроксилапатит бетони аминкышқылдар сорбциясының физика-химиялық заңдылықтары

Кальций фосфаттары физиогендік және патогендік минералды ауылдық қызметтің бір болғы болады. Гидроксилапатит субқретін, тіс эмалы және дентинің негізгі минералды компонент бірлестік табиғатының белгілі. Органикалық және минералды компоненттердің озара әрекетті биогенди кристалдану үдерістерінде суктораксиілердің сүйек матрицасының қалыңтаяшуы, соңай-ақ патогенді тұлғақтардың нүлгеағышы және олардың қамтамасыз етуде ұлардын мәнінен сияқты. Мұндай минералдану процестерінің негізді биофизикадың әйбергірекеті мен қалыңтаяшуы фазалары бар ауыз мүкөлділірген байланысты бірме аминкышқылдардың және ауыз мүкөлділірген адсорбциялық процесс нәрселерінің бірінше білімі болмайды. Алайда олардың озара әрекетесулерінің тегі тольк зерттеледі. Макуалау гидроксилапатит бетони аминкышқылдар әрқайшы зерттелді. Қалыпты әрекетті әрекеттінеже әрекетті әрекеттінеже, РФА және НК спектроскопиясының нәрселеріне сүйене отырып, тұрғына гидроксилапатит фазасы арықталған. ВЗТ еңсізен гидроксилапатиттің бетінің аударын 72,0 мг/л болғанға айналды. Аминкышқылдардың адсорбциясы олардың концентрациясы мен әрекеттілер pH-нің кен ауызымда.
Физико-химические закономерности сорбции аминокислот на поверхности гидроксиapatита

Фосфаты кальция входят в состав физиогенных и патогенных минеральных образований. Известно, что гидроксиapatит является основой минеральной составляющей костной ткани, зубной эмали и дентина. Взаимодействие органической и минеральной составляющих имеет большое значение в таких процессах биогенной кристаллизации, как формирование костного мatriкса млечопитающих, а также зарождение и рост патогенных образований. Существует ряд предположений, согласно которым в основе процессов минерализации лежит адсорбционное взаимодействие свободных аминокислот и связанных белковых молекул с неорганическими компонентами биожидкостей и формирующихся фаз. Однако механизмы их взаимодействия до конца не изучены. В работе изучена адсорбция аминокислот на поверхности гидроксиapatита. Осуществлен синтез фосфатов кальция из раствора. По результатам РФА и ИК-спектроскопии установлено, что осадки представлены фазой гидроксиapatита. С помощью метода БЭТ рассчитан удельную поверхность гидроксиapatита, равную 72,0 м²/г. Исследована адсорбция аминокислот в широком интервале вариации их концентраций и рН раствора. Доказано, что адсорбция аминокислот на поверхности гидроксиapatита достигает насыщения. Рассмотрено влияние рН раствора на значение максимальной адсорбции аминокислот на поверхности гидроксиapatита. Доказано, что адсорбция аминокислот на поверхности гидроксиapatита описывается моделью Ленгмюра. Результаты ИК-спектроскопии приведены для подтверждения факта адсорбции. Проведено определение знала заряда поверхности твердой фазы гидроксиapatита. Получено, что значение знала заряда поверхности гидроксиapatита положительное. Осуществлен расчет значений энергии Гиббса и выявлено, что взаимодействие аминокислот с поверхностью гидроксиapatита характеризуется физической адсорбцией.

Ключевые слова: гидроксиapatит, адсорбция, аминокислота, растворение, поверхностный заряд, раствор, модель Ленгмюра, модель Фрейндлиха.

References


