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Studies of the Adsorption of Glutamic Acid on Brushite

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Abstract

Regularities of the adsorption of *L*-glutamic acid on brushite were studied. Brushite is a mineral component of pathogenic new growth formed at the initial stages of calculus formation. It was established that the interaction of calcium hydrophosphate dihydrate and the amino acid is described within Langmuir's theory ($r^2 = 0.99$). Monomolecular adsorption layer is formed as a result of coordination of protonated terminal carboxyl groups of glutamic acid with positively charged groups $CaOH^+$ and/or $CaH_2PO_4^+$ on the surface of brushite crystal.

Key words: brushite (calcium hydrophosphate dihydrate), glutamic acid, adsorption, active centres and groups, biogenic and pathogenic crystallization, organomineral aggregates

INTRODUCTION

Interaction between the organic and mineral components has a decisive importance in such essential processes of biogenic crystallization as the formation of the bone matrix in mammals, formation and growth of pathogenic neoplasms. The formation of bone tissues *in vivo* always proceeds due to mineral reinforcement of the structural matrix that has been formed previously – a network of collagen fibres [1]. Under the conditions of undesirable mineralization, the molecules of organic substances can serve as the centres of crystallization, which is evidenced by the studies of the morphology and texture-structure characteristics of nephroliths, salivary and dental calculi *etc*. [2]. The centres of nuclei in these aggregates are most often organic substances; layered structure can be followed by means of examination at the milli- and microlevels: there are alternating zones of organic and mineral composition with different thickness. The mechanism of the interaction between collagen and growing crystals of bone apatite or between organic and mineral components of biological media in course of physiogenic and pathogenic mineralization remains insufficiently studied yet.

There is a set of assumptions according to which mineralization processes rely on the adsorption interaction of amino acids, both free ones and those bound into protein molecules, with the inorganic components of biological liquids and the growing phases. In this connection, it is urgent to carry out the studies aimed at the ascertainment of regularities of the adsorption of amino acids, the main construction blocks of proteins, on the inorganic component of the majority of pathogenic neoplasms – calcium orthophosphates. Often hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is the object of investigation [3–6 *etc*.] as the substance close in composition to bone mineral [1, 2, 7, 8], and as the major component of phosphate calculi [1, 2, 9, 10 *etc*.]. However, it was stressed in [11–13] that at the early stages of the formation of pathogenic aggregates of various kinds of localization (less than 3 months) calcium hydrophosphate dihydrate $CaHPO_4 \cdot 2H_2O$ is often crystallized; it is an analogue of natural mineral brushite. The author of [13] defines this calcium phosphate as the primary crystal structure in dental calculi. So, brushite deposition at the initial stages of pathogenic mineralization and the presence of the organic component in the growing aggregates determine the urgency of studies of the interaction of components differing from each other in their nature.

The goal of the work was to study the adsorption of glutamic acid on calcium hydrophosphate dihydrate (brushite).

EXPERIMENTAL

Three series of experiments were carried out during the work: 1) the time of the establishment of adsorption equilibrium was determined; 2) the process of amino acid adsorption on brushite was investigated; 3) the effect of pH on adsorption value was established.

Synthetic brushite powder was used to carry out adsorption measurements. The powder was analyzed by means of XPA (D8 Advance, Bruker), IR Fourier transform spectroscopy (FT-801, SIMEKS, tablets with KBr), AES-ICP (710-ES, Varian). Diffraction patterns and IR spectra of the particles of the substance correspond to the pure phase of calcium hydrophosphate dihydrate; the ratio $Ca/P = 1.00\pm0.01$. Specific surface area of the crystals, established using BET procedure (Sorbtometr, Russia) was $(4\pm1)\ m^2/g$.

The solutions of glutamic acid were prepared directly before adsorption experiment by means of separate weighted portions of the crystal substance $C_5H_9NO_4 \cdot H_2O$ (ch. d. a. grade). *L*-isomer which is present in biological systems was used in experiments.

Adsorption on the surface of adsorbent from solution was determined from the difference of the concentrations of dissolved amino acid before and after contact. To determine the concentration of glutamic acid in the liquid phase, we used the analysis based on the transformation of amino acids into soluble copper salts followed by their photometric determination [14].

To determine the time of adsorption experiment, accurately measured and identical in all series volume of the solution of glutamic acid with the concentration of 0.010 mol/L with accurately calculated NaCl additive (ch. d. a. grade) used to achieve the ionic force of the system equal to 0.1 mol/L was added to the weighted portions of brushite with the mass of 0.5000 (±0.0002) g. The acidity of the solutions was brought to pH 6.50±0.05 with the help of NaOH solution (20 %) and/or HCl (1 : 1). After that, adsorption vessels were tightly closed, shaken for 20 min and left for the adsorbate and adsorbent to interact. Examination was carried out after contact for different time intervals τ (0–72 h). After the required time, the solid phase was separated from the solution by filtering.

To study adsorption process, the concentration of glutamic acid was varied within the range 0.002–0.020 mol/L (this concentration range is characteristic of human mouth fluid [15]). In the experiments aimed at the determination of the effect of pH on adsorption value, the acidity of the medium was made to be equal to 4.5, 5.0, 5.5, 6.0, 7.0 or 8.0 (±0.05). Contact time was chosen to be 48 h. In other features the experiments of this series were similar to those described above.

Solid phases that were separated from solutions after adsorption and dried in the air were analysed by means of IR Fourier transform spectroscopy (FT-801, tablets with KBr).

All experiments and measurements were carried out three times and treated statistically to reveal the regularities of adsorption process.

RESULTS AND DISCUSSION

Equilibrium in a chemical process may be achieved within different time intervals depending both on the nature of interacting particles and on the kinetic and thermodynamic factors acting together. Similarly, in adsorption processes: their conditions, properties and structure of adsorbents and adsorbates affect the duration of period within which the system will approach the equilibrium state.

It was demonstrated in [6] that glutamic acid exhibits surface-active properties towards calcium orthophosphate of different stoichiometric compositions. On this ground, under the conditions of the contact of aqueous solution of glutamic acid with the solid phase

TABLE 1

Results of experiments on the adsorption of glutamic acid on brushite

Concentration of amino acid, mol/L			Γ_{av}, mol/kg	Degree of adsorbent surface filling Θ
initial	equilibrium			
	in solution after adsorption	in the surface layer of adsorbent		
0.002	0.0009 ± 0.0002	0.0011 ± 0.0002	0.055 ± 0.010	0.27 ± 0.01
0.004	0.0022 ± 0.0005	0.0018 ± 0.0005	0.091 ± 0.012	0.43 ± 0.01
0.006	0.0034 ± 0.0005	0.0026 ± 0.0005	0.13 ± 0.02	0.58 ± 0.04
0.008	0.0051 ± 0.0004	0.0029 ± 0.0004	0.15 ± 0.02	0.68 ± 0.03
0.010	0.0069 ± 0.0007	0.0031 ± 0.0007	0.16 ± 0.03	0.75 ± 0.01
0.015	0.012 ± 0.001	0.0034 ± 0.0006	0.17 ± 0.02	0.82 ± 0.02
0.020	0.016 ± 0.001	0.0037 ± 0.008	0.18 ± 0.02	0.86 ± 0.01

$CaHPO_4 \cdot 2H_2O$, organic molecules probably get concentrated on the surface of brushite crystals. In this situation, in agreement with the accepted theories [16] describing adsorption phenomena, adsorbate concentration will remain constant in the bulk liquid phase and in the surface layer of the adsorbent, so the system under consideration will achieve equilibrium.

To determine the time of equilibrium establishment and the time necessary for adsorption experiment, at the first stage of investigation we carried out a series of experiments with different time of contact between the 0.010 g/L solution of glutamic acid and brushite. According to the data obtained the concentration of the amino acid in the surface layer of the crystals of calcium hydrophosphate dihydrate increases with an increase in the time of adsorbent to adsorbate contact from 0 to 40 h. Under the conditions of longer interaction between the components in the system, no changes of adsorption value Γ are observed. On this ground, as well as from the viewpoint of practice, we chose adsorption time to be equal to 48 h in further experiments. Experimental data

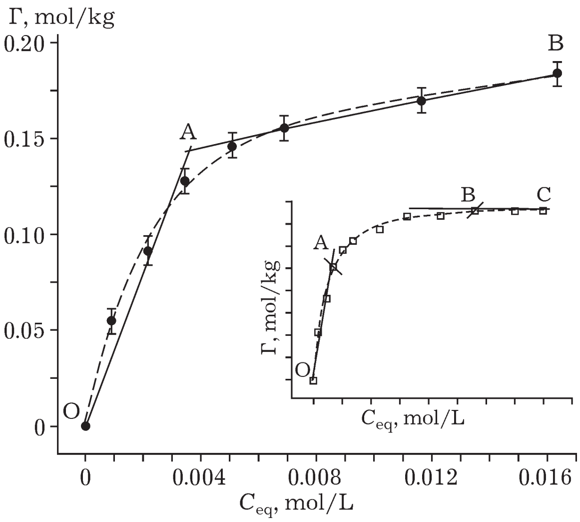

Fig. 1. Adsorption isotherm of glutamic acid on brushite. Theoretical curve is shown in the insert.

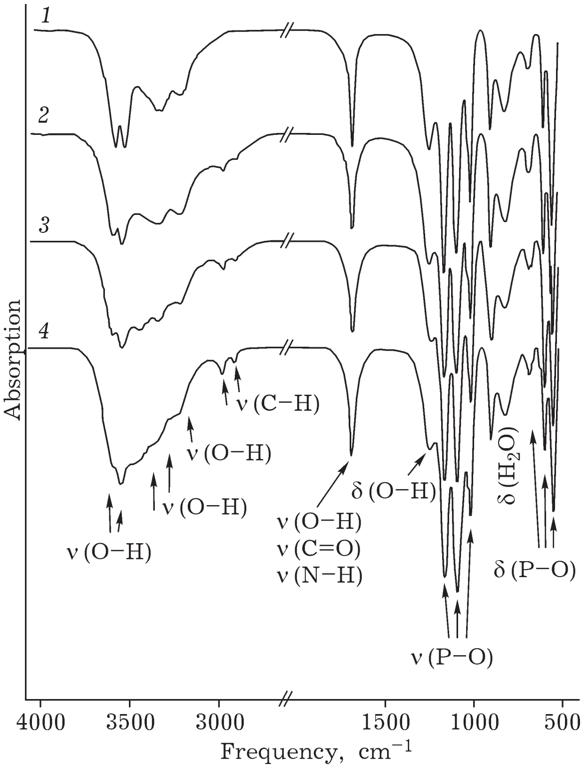

Fig. 2. IR spectra of brushite: *1* – before adsorption; *2*–*4* – after 48 h of contact with 0.010 mol/L solution of glutamic acid at pH 5.5 (*2*), 4.5 (*3*) and 8.0 (*4*).

TABLE 2

Freindlich and Langmuir equations for the adsorption of glutamic acid on brushite

Equations	Freindlich model					Langmuir model				
	$1/n$	n	$\ln K$	K	r^2	$1/\Gamma_\infty$, kg/mol	Γ_∞, mol/kg	$1/b\Gamma_\infty$, kg/mol	b	r^2
$y = -0.14 + 0.41x$	0.41	2.44	−0.14	1.15	0.91	–	–	–	–	–
$y = 0.012 + 4.74x$	–	–	–	–	–	4.74	0.21	0.012	397	0.99

Note. n and K are coefficients depending on temperature and adsorbent and adsorbtive kind (Freindlich model); b is the constant of adsorption interaction (Langmuir model); r^2 is correlation coefficient.

obtained under variation of the concentration of glutamic acid are presented in Table 1.

Two regions may be distinguished in the graphical plot of adsorption value on the equilibrium concentration of glutamic acid in solution (Fig. 1), plotted according to the data of Table 1. These two regions differ from each other by the curve behaviour. Within the initial region of the isotherm (OA region), adsorption is directly proportional to the equilibrium concentration of the amino acid in solution ($r^2 = 0.95$). However, the trend of the dependence is close to linear within a very narrow range of adsorbate concentrations – not more than 0.004 mol/L. This is likely caused by a decrease in the fraction of the free adsorbent surface Θ during adsorption (see Table 1), which leads to deceleration of adsorption increase and to the transition of the law of Γ value dependence to the exponential one at the AB region ($r^2 = 0.99$). In comparison with the theoretical adsorption isotherm, the experimental curve is lacking for BC region corresponding to saturation conditions, when adsorption value is independent of concentration. This may be due to the limitation of glutamic acid concentration under experimental conditions.

The adsorption of organic molecules on brushite is confirmed by the data of IR Fourier transform spectroscopy. The IR spectra of all the samples after contact with the solution of glutamic acid (Fig. 2) in the region of 2800–3000 cm^{-1} contain the bands corresponding to the vibrations of C–H bonds in methylene groups of the organic component. The vibration band at 1650 cm^{-1} can be considered as a sum depicting the vibrations of C=O, N–H and O–H bonds in amino acid molecules and OH ions in calcium hydrophosphate.

To determine a model describing the adsorption of glutamic acid on brushite, the experimental data were treated from the viewpoint of Freindlich and Langmuir theory. Equation coefficients calculated graphically are presented in Table 2.

So, Freindlich equation

$$\Gamma = 1.15C^{0.41} \quad (1)$$

and Langmuir equation

$$\Gamma = 0.21 \cdot 397C/(1 + 397C) \quad (2)$$

were obtained.

On the basis of Langmuir and Freindlich equations, adsorption values for the equilibrium concentrations of glutamic acid in solution were calculated (Table 3). The dependences of

TABLE 3

Data on the adsorption of glutamic acid on brushite: obtained experimentally and calculated according to Freindlich and Langmuir equations

C_{in}, mol/L	C_{eq}, mol/L	Γ_{exp}, mol/kg	Γ_{Fr}, mol/kg	$\lvert\Gamma_{exp} - \Gamma_{Fr}\rvert$	Γ_{Lang}, mol/kg	$\lvert\Gamma_{exp} - \Gamma_{Lang}\rvert$
0.002	0.001	0.055	0.065	$1.04 \cdot 10^{-2}$	0.056	$9.39 \cdot 10^{-4}$
0.004	0.002	0.091	0.093	$1.98 \cdot 10^{-3}$	0.097	$6.18 \cdot 10^{-3}$
0.006	0.003	0.13	0.11	$1.52 \cdot 10^{-2}$	0.12	$6.36 \cdot 10^{-3}$
0.008	0.005	0.15	0.13	$1.42 \cdot 10^{-2}$	0.14	$5.69 \cdot 10^{-3}$
0.010	0.007	0.16	0.15	$5.94 \cdot 10^{-3}$	0.15	$1.58 \cdot 10^{-3}$
0.015	0.012	0.17	0.19	$1.55 \cdot 10^{-2}$	0.17	$2.98 \cdot 10^{-3}$
0.020	0.016	0.18	0.21	$2.90 \cdot 10^{-2}$	0.18	$1.85 \cdot 10^{-3}$

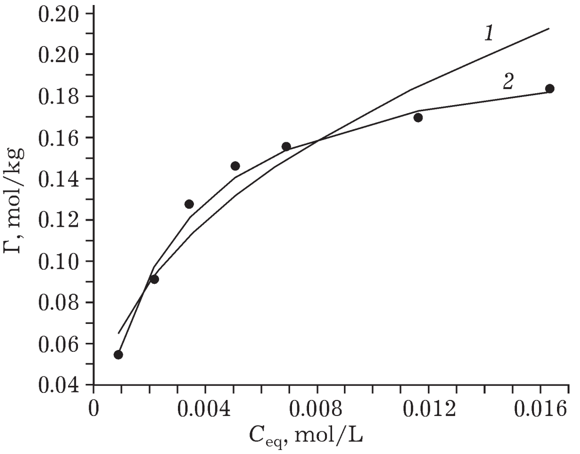

Fig. 3. Isotherms of adsorption Γ of glutamic acid on brushite plotted on the basis of the equations of Freindlich (*1*) and Langmuir (*2*).

$\Gamma = f(C)$ in the linear coordinates of Langmuir and Freindlich models, plotted using the calculated data, are shown in Fig. 3.

One can see (see Tables 2, 3, Fig. 3) that the results of experiments on the adsorption of glutamic acid on brushite are more precisely described within the framework of Langmuir model ($F_{\mathrm{exp}} = 2.3 < F_{\mathrm{tab}} = 4.3$ [17]) (Table 4). Freindlich model turned out to be inadequate.

According to Langmuir's theory, adsorption proceeds with the formation of a monomolecular layer [16]. The formation of the latter under the conditions under study is pointed to by the experimental data. With an increase in the concentration of amino acid in the system the degree of adsorbent surface filling (Θ) increases (see Table 1). However, the calculated Γ_∞ values (0.21 mol/kg) provide evidence that adsorption does not reach saturation under experimental conditions (its maximum is 0.18 mol/kg).

TABLE 4

Test of the adequacy of Langmuir and Freindlich models

Statistical parameters	Langmuir model	Freindlich model
$\Sigma(\Gamma_{\mathrm{exp}} - \Gamma_{\mathrm{calc}})^2$	$1.27 \cdot 10^{-4}$	$1.66 \cdot 10^{-3}$
S^2_{ad}	$2.11 \cdot 10^{-5}$	$2.77 \cdot 10^{-4}$
S^2_{mn}	$9.05 \cdot 10^{-6}$	*
F_{exp}	2.3	*
F_{tab}	4.3	4.3

Note. S^2_{ad} is adequacy variance; S^2_{mn} is reproducibility variance; S^2_{m} is experiment variance; F_{exp} and F_{tab} are Fischer criterion values calculated for the experimental data and the tabulated critical values, respectively [17].

* Calculation is impossible because experiment variance values S^2_{m} are nonuniform.

As the results of the third series of experiments showed, the density of surface filling of brushite crystals depends also on the acidity of the medium. For instance, with a decrease in pH an increase in adsorption value is observed, along with an increase in the fraction of adsorbent surface occupied with glutamic acid molecules (Table 5). This fact may be connected with different adsorption activity of the forms of amino acid molecules coexisting in the solution.

It is most probable that the adsorption of glutamic acid on brushite proceeds due to electrostatic interaction of charged or strongly polar groups ($-\mathrm{COO}^-$, $-\mathrm{HN}_3^+$, $-\mathrm{COOH}$) with the active surface centres of the crystal solid. A change of the amount of adsorbed amino acid under variation of the acidity of medium indicates that adsorption proceeds due to the groups the forms of which change with an increase in acidity from pH 4.5 to pH 8.0. Under experimental conditions, practically only two zwitter ions are present in solution at the same time:

$\mathrm{HOOC(CH_2)_2CH(HN_3^+)COO^-}$

$\mathrm{^-OOC(CH_2)_2CH(HN_3^+)COO^-}$

with the predominance of the latter zwitter ion in solution, and its fraction in the mixture increases while pH increases (Fig. 4). In each of these forms, the amino group is protonated and exists only as $-\mathrm{HN}_3^+$. The invariance of the state of this group gives us ground to suppose that it does not play any important part in the interaction between adsorbate and adsorbent, and the carboxylic groups of glutamic acid are responsible for the possibility of adsorption. Experimental data confirm this assumption. A substantial decrease in adsorption value at the background of an increase in the fraction of $\mathrm{^-OOC(CH_2)_2CH(HN_3^+)COO^-}$ in the mixture defines the nature of the major stage of adsorption as the interaction of protonated terminal carboxylic groups with the active centres on the surface of brushite crystals. This is probably due to the fact that glutamic acid, similarly to other a amino acids, in fact carries no charge either at the amino group or at the

TABLE 5

Results of experiments on adsorption of glutamic acid on brushite under variations of pH value

pH of solution	Γ_{av}, mol/kg	Θ	pH of solution	Γ_{av}, mol/kg	Θ
8.00	0.12±0.02	0.59±0.07	5.50	0.16±0.03	0.79±0.08
7.00	0.13±0.02	0.66±0.06	5.00	0.18±0.03	0.84±0.06
6.00	0.15±0.04	0.73±60.08	4.50	0.21±0.02	0.94±0.05

Note. Θ is the degree of adsorbent surface.

neighbouring carboxyl group. Zwitter ions are internal salts in which the carboxylic group is bound with the ammonium nitrogen atom: $H_3N^+{-}CH(R){-}COO^-$ [18]. This region of the molecule is electrically neutral. Partial charges are present on the oxygen and hydrogen atoms of HOOC group located at the opposite side of the molecule.

It is likely that brushite surface is charged in the aqueous solution under experimental conditions. Hypothetically, as a result of protolysis of hydrophosphates and calcium ions in contact with the medium, ion associates $CaOH^+$ and $CaH_2PO_4^+$ are formed; they are characterized by close stability constants ($\beta_{[CaOH^+]} = 1.22$, $\beta_{[CaH_2PO_4^+]} = 1.45$ [19]). The dominating type of coordination is determined by pH: with an increase in the latter, the fraction of $CaOH^+$ increases with respect to $CaH_2PO_4^+$; in general, adsorbent surface is always charged positively.

In our opinion, ion associates $CaOH^+$ and $CaH_2PO_4^+$ act as the active centres in the process of glutamic acid adsorption. Their approach to the terminal uncharged carboxylic groups of the amino acid causes a strong polarization of the O–H bonds in –COOH and redistribution of the electron density between Ca and O with the formation of $[CaH_2PO_4^+][^-OOC(CH_2)_2CH(HN_3^+)COO^-]$ and/or $[CaOH^+][^-OOC(CH_2)_2CH(HN_3^+)COO^-]$. Probably, further on, due to rotation, the hydrocarbon radical of the adsorbate may take the configuration for which the additional coordination of α-COO^- and $-HN_3^+$ (in fact $-NH_2$ as a result of a loss of a proton during approaching) with the neighbouring groups $CaOH^+$ and/or $CaH_2PO_4^+$ occurs; chelate complexes on brushite surface are formed. Thus, this is the way how the zwitter ion gets fixed on brushite surface.

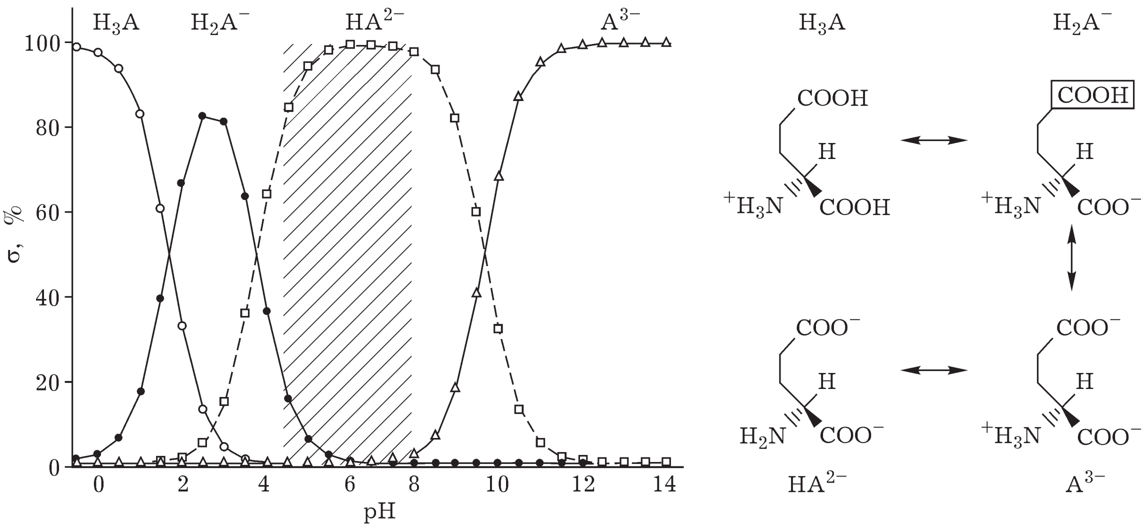

Fig. 4. Ionic diagram of glutamic acid.

CONCLUSION

It was established that *L*-glutamic acid in contact with brushite crystals in solution forms the adsorption monomolecular layer on their surface. The state of saturation is not achieved due to the limitation of amino acid concentration in the systems under study, similarly to the human mouth fluid. The dependence of adsorption value on pH of the medium points to the higher adsorption activity of the protonated form of the amino acid in comparison with deprotonated one.

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