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Modifying the Natural Zeolite Tuff from the Mukhor-Tala Deposit with Selenium and Iodine Organic Complexes

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Abstract

Chelate complexes have been obtained for selenium and iodine ions with peptides from protein elastin. Parameters were investigated for the sorption of selenium- and iodine-containing elastin chelate complexes on naturally occurring zeolite tuff species depending on the concentration of chelate complex and the contact time between the chelate complex and the tuff.

Key words: selenium, iodine, protein hydrolysate, chelate complexes, zeolite tuff

INTRODUCTION

Microelements maintain the equilibrium and coherence between the main regulatory systems of organismal cells, since they participate in the complex biochemical metabolism, being involved in a variety of enzymes, co-enzymes and hormones. A special place among the microelements is occupies by selenium and iodine those exert a positive influence upon the functional activity of the thyroid gland as a regulator of many processes in living organisms. Iodine and selenium are functionally connected between each other, since the latter is a part of enzyme iodothyronine deiodinase that provides the transformation of thyroxine into triiodothyronine. The deficiency of these microelements in an organism can induce iodine deficiency, first of all, endemic goiter. Simultaneous iodine and selenium deficiency results in a stronger hypothyroidism, than the deficiency of iodine only, thus to provide animals and humans with these microelements is of great importance.

In order to supply the microelements one uses their inorganic species. However, the salts of mineral substances can be only incompletely absorbed in the gastrointestinal tract of animals. Increasing the bioavailability of microelements represents one of the most urgent problems in animal husbandry. For a complete and safe assimilation of the microelements one uses chelate compounds between biogenic elements and organic ligands [1].

Of particular interest are compounds between metals and amino acids, peptides. It is known that the formation of such compounds results in changing the chemical and biological properties of original substances, thereby metal ions in combination with the amino acids become less toxic and can catalyze a variety of biochemical processes. In addition, a high efficiency of organic microelemental species, the

rigorous assimilability in vivo allow 3- to 4-fold reducing the dosage of fodder supplements. As the result of this approach, there is significantly reducing the concentration of microelements in animal husbandry by-products, which could significantly reduce the environmental pollution. In connection with high environmental standards in the countries with developed animal husbandry (the USA, Germany, France) research work concerning the introduction of chelates into the feed allowance is actively conducted therein [2].

The methods of delivering bioactive substances in an organism are manifold. As a carrier, one could use naturally occurring zeolites. The aluminosilicate chemical composition involving alkali and alkaline earth metal cations as ion exchange agents, together with a nanoscale porous skeletal structure determining molecular sieve properties impart them simultaneously adsorption, ion-exchange and catalytic properties. Because of this, the zeolites exhibit a biological activity that helps to eliminate an imbalance in food concerning macro- and microelemental content and promotes thus a significant improvement in the most of organismal vital functions [3].

Earlier, we studied the adsorption of some inorganic species of selenium ions on natural zeolites [4]. The aim of this work consisted in obtaining the chelate complexes of selenium and iodine with elastin protein hydrolysate and studying the sorption of organic compounds obtained by naturally occurring zeolite tuff species, depending on different factors.

EXPERIMENTAL

The hydrolysate of protein elastin was obtained by means of biotransformation the native protein elastin [5]. This substance represents a yellow liquid with pH 7.8 containing predominantly a mixture of low molecular mass peptides. The concentration of peptides in the hydrolysate is equal to 20 mg/mL.

To obtain a complex containing iodine and selenium in bound state, the hydrolysate solutions were mixed with the mentioned microelements at a volume ratio equal to 1 : 1. The selenium and iodine binding level with respect to elastin protein hydrolysate was determined basing on the concentration of microelements in the samples, as well as with the use of IR spectroscopic method. The inorganic salt species of selenium and iodine were separated from the bound species by means of dialysis in constantly flowing water through a semipermeable membrane. The analysis of selenium was performed using fluorometric method with the help of a Fluorat-02 fluorometer according to Russian methodological standard MUK 04-33–2004; the iodine concentration was determined by means of a kinetic thiocyanate-nitrite method [6].

For the experiments on studying the sorption of the chelate complex between protein hydrolysate and the microelements, we used a sorbent used zeolite tuff from the Mukhor-Tala deposit (Buryatia) (technical specifications TU 2163-003-12763074–97) with the particle diameter within the range of 1–3 mm. The tuff species exhibited the following chemical composition (mass %): SiO_2 66.00, TiO_2 0.99, Al_2O_3 9.52, Fe_2O_3 0.48, MnO 0.02, CaO 3.05, Na_2O 1.78, K_2O 1.98, P_2O_5 0.04. The zeolite content in the samples was equal to 80 mass %, as blend minerals there were quartz, cristobalite, mica and volcanic glass present. According to the "Manual on the Use of Natural Zeolites" (1992), the particle size of zeolite for birds amounts to 1–3 mm. Studying the sorption of the chelate complex by tuff was carried out at different values of the ratio between liquid and solid phases. The concentration of adsorbed compounds was determined from the difference in the concentration of selenium and iodine in the solution.

We carried out the immobilization of selenium ions in the protein elastin hydrolysate at pH 9.0 and at a temperature of 18–20 °C by means of adding an aqueous solution of sodium selenite with the concentration of 0.001 mol/L (Fig. 1). The medium of hydrolysate is alkaline, which provides the reactivity of the selenite ion [7].

The incubation was carried out every hour during 16 h, after each incubation the bound species of selenium and water-soluble inorganic salts were separated by means of dialysis during 24 h. The maximum concentration of bound selenium was observed after 4 h of incubation, the concentration of selenium was 1 μg/mL. After 7 h, the concentration of the bound selenium reached a constant value (0.65 μg/mL).

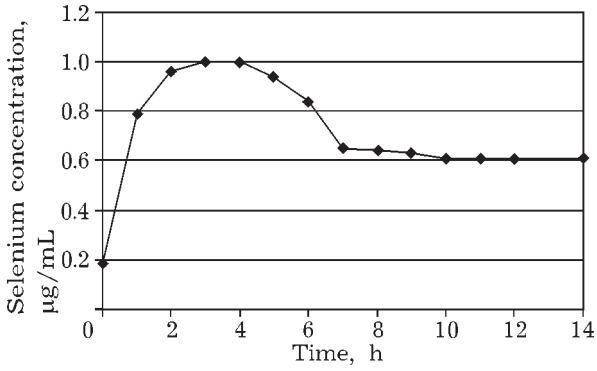


Fig. 1. Concentration of selenium bound with elastin hydrolysate, depending on the duration of incubation.

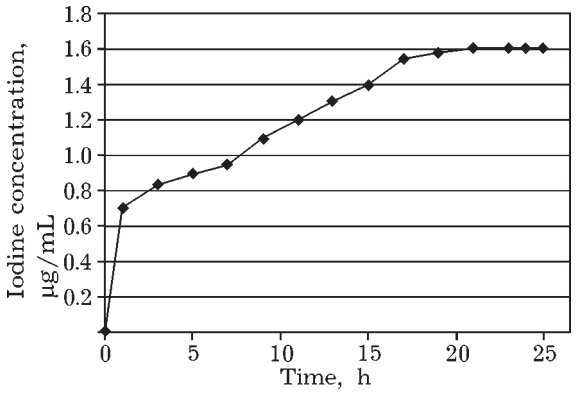
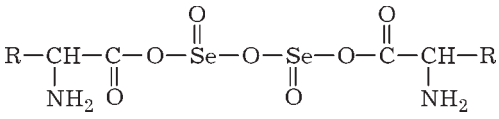


Fig. 3. Concentration of iodine bound with elastin hydrolysate, depending on the duration of incubation.

The interaction between microelements and enzymatic protein hydrolysates is not simply a mixing; this event represents a complex physical-chemical process that is accompanied by changing the structure of initial hydrolysates.

It is known that selenium is present in the solutions of protein as a dimer $-O-SeO-O-SeO-O-$ which binds together two free carboxyl groups in a similar manner as it is exhibited by metal compounds with organic substances. Appearing the absorption band at 1361.3 cm^{-1} in the IR spectrum of a complex formed by selenium and hydrolysate of protein elastin, in our opinion, could be caused by an insertion of selenite ion in the structure of the peptides in the form of dimeric bridge $Se_2O_3^{2-}$ which binds together free carboxyl groups COO^- inherent in elastin hydrolysate [8] (Fig. 2). The binding between the selenium dimer and elastin peptides could occur according to the following scheme:



In parallel with the immobilization of selenium on the elastin hydrolysate we studied binding between protein hydrolysate and iodide ions (Fig. 3). It was found that after 24 h the maximum extent of binding the iodide ions reaches 99.8 %; increasing the time up to 26 h did not result in changing the concentration of the compound absorbed. This indicates a gradual character of binding the peptides with iodide ions, whereby the microelement interacts with unfolded and available sites of the elastin

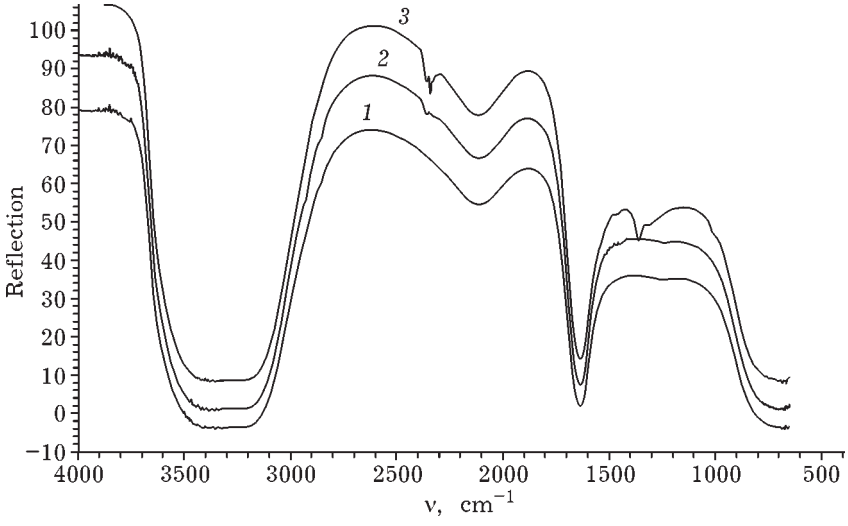
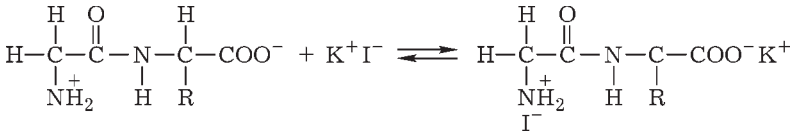


Fig. 2. IR spectra of elastin protein hydrolysates: *1* – initial; *2*, *3* – modified with iodine (*2*) and selenium (*3*).



Scheme 1.

hydrolysate. Basing on the experimental data we have chosen processing rules for treating the elastin hydrolysate by an aqueous solution of potassium iodide at a concentration of 0.001 mol/L, which can provide completely binding the amount of iodine introduced: the duration of exposure with the solution of potassium iodide being equal to 24 h at a temperature of 0–4 °C. Under these conditions, 1 mL of elastin protein hydrolysate could bind as much as 1.6 μg of iodine.

With introducing the potassium iodide solution into the solution of elastin hydrolysate, an ionic bond could occur between the protonated NH_3^+ groups of elastin hydrolysate and I^- ions [9]. Taking into account the content of glycine in the hydrolysate (35.5 %) as well as the selective pepsin-induced cleavage of the peptide bond formed with glycine, there is a high probability of I^- interaction just with the glycine NH_3^+ group according to Scheme 1.

In the IR spectrum of the complex between iodine and hydrolyzed protein elastin there were no changes observed (see Fig. 2), which indicates the absence of covalent bonds with the presence of ionic interactions only.

The main objective in developing selenium and iodine-containing fodder supplements basing on naturally occurring zeolite tuff species consists in studying the sorption capacity of the latter with respect to the selenium and iodine-containing chelate complexes depending on different factors (such as concentration and exposure time). In order to obtain a complex containing both selenium and iodine in bound state, we mixed between each other the solutions of protein elastin hydrolysate modified with selenium and iodine (1 : 1), with the concentration of selenium and iodine equal to 1 and 1.6 μg/mL, respectively. In this case the acidity of the hydrolysate containing selenium decreased with from pH 9 to pH 8 due to neutralizing by the iodine-containing chelate, which allowed saving the obtained bonds between iodine and peptides. Simultaneously, the selenium concentration decreased down to 0.5 μg/mL, whereas the iodine concentration exhibited a decrease down to 0.8 μg/mL.

The influence of the concentration in the mixture of iodine and selenium-containing chelate complexes upon the sorption capacity of zeolite tuff species was studied within the selenium concentration range of 0.05–0.5 μg/mL during a constant time (30 min.) The dilution of the solutions was performed using a pure protein hydrolysate. From Fig. 4 one can see that with increasing the concentration of the initial solution higher than 0.05 μg/mL the concentration of absorbed selenium compound exhibited

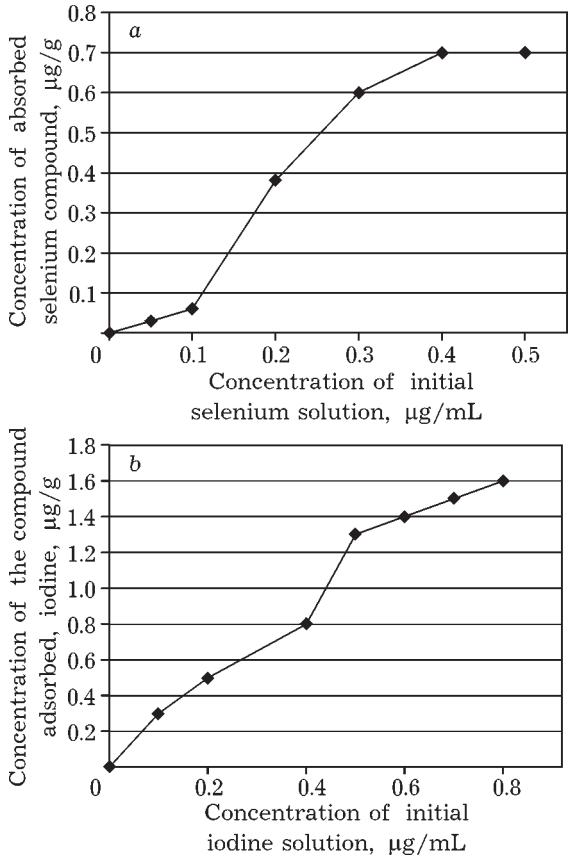


Fig. 4. Sorption isotherms for selenium (*a*) and iodine (*b*) organic species on the zeolite tuff.

an increase, whereas when the concentration of selenium in the initial solution was equal to 0.4 μg/mL, the concentration of the selenium compound absorbed reached 0.7 μg/g. Further increasing the selenium concentration in the initial solution did not result in changing the concentration of absorbed selenium compound.

The results obtained indicate changing the concentration of selenium-containing chelate complex adsorbed onto the surface of zeolite tuff species: very dilute solutions are weakly adsorbed onto zeolite tuff species, whereas with increasing the concentration of the solution the complexes adsorbed, to all appearance, become the centres of adsorption for the further complexes, which results in increasing the sorption capacity of zeolite tuff species.

The influence of the concentration in the mixture of iodine and selenium-containing chelate complexes upon the sorption capacity of tuff species was studied within the range of iodine concentration from 0.1 to 0.8 μg/mL during 30 min. With increasing the concentration of iodine in the complex higher than 0.8 μg/mL, the concentration adsorbed compound on the zeolite tuff amounted up to a constant value 1.6 μg of iodine per 1 g of zeolite (see Fig. 4, *b*).

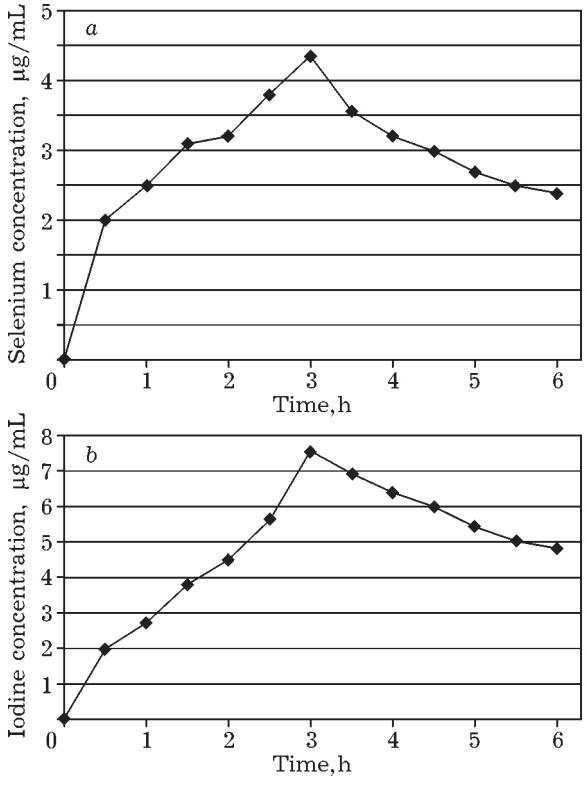


Fig. 5. Kinetic curves for the adsorption of selenium ions (*a*) and iodide ions (*b*) bound with elastin protein hydrolysate on zeolite tuff.

In order to make a fodder supplement we investigated the following values of volume ratio between solid and liquid phases: 4 : 1, 3 : 1, 2 : 1, 1,5 : 1, 1 : 1, 1 : 1.5, 1 : 2. At the values of liquid to solid phase ratio equal to 4 : 1, 3 : 1, the zeolite tuff was completely immersed in the solution, thereby first of all we observed foaming the solution, whereas the supernatant layer of the liquid was high. So, at the ratio equal to 2 : 1 the height liquid layer of above the adsorbent ranged within 1–2 mm. At the ratio values equal to 1.5 : 1, 1 : 1, 1 : 1.5, 1 : 2 the liquid phase covered the solid phase to an incomplete extent. In the case when the level of liquid above the solid phase was high the drying time exhibited an increase. When the zeolite tuff was partially immersed in the solution of selenium-elastin protein hydrolysate and iodine-elastin protein hydrolysate complexes, the uniform distribution of the complexes over the zeolite was violated. Consequently, the optimum value for the ratio between the substance adsorbed and the adsorbent could be considered equal to 2 : 1.

Next, we investigated the sorption capacity of zeolite tuff depending on the time of exposure for the phase ratio chosen. The maximum capacity of the tuff with respect to selenium ions bound with protein elastin hydrolysate amounted to 4.35 μg/g, for the case of the sorption duration equal to 3 h (Fig. 5, *a*).

The maximum capacity of the zeolite tuff with respect to iodine ions bound to the hydrolysate of protein elastin amounted to 7.56 μg/g (see Fig. 5, *b*), for the case of contacting between the phases during 3 h at a temperature of 18–20 °C.

The maximum sorption level for the complexes on zeolites was observed after 3 h, which could be, to all appearance, caused by the nature and properties of zeolites from the Mukhor-Tala deposit.

Further, the samples obtained were dried at a temperature of 40 °C to gain a constant mass. In order to modify the zeolite tuff species from the Mukhor-Tala deposit by the organic complexes of selenium and iodine with elastin protein hydrolysate, we have chosen the following conditions: the ratio between sub-

stance adsorbed and adsorbent equal to 2 : 1, pH 8, the sorption procedure duration 3 h, the optimal concentration for selenium ions 0.5 μg/mL, that for iodine 0.8 μg/mL.

The modified zeolite tuff species we obtained are proposed for using as a fodder supplement. With the use of culture *Tetrachimena Pyriformis* [10] we studied the toxicity of sodium selenite and potassium iodide solutions of as well as elastin hydrolysate sample modified with iodine and selenium, deposited onto the zeolites. The concentration values for the solutions under investigation were equal to 0.01, 0.001 and 0.0001 mol/L.

It was established that 0.01 M Na_2SeO_3 solution exhibits strongly pronounced toxicity: the death of infusoria was observed after a few min, the cells were lysed. On the contrary, 0.001 M Na_2SeO_3 solution is low-toxic, because after 30 min there was the death of 30 % of animal cells observed, infusoria were not active. The solution with the concentration of Na_2SeO_3 amounting to 0.0001 mol/L is not toxic. Potassium iodide solution demonstrated no pronounced toxicity for all the values of concentration. The hydrolysate of protein elastin supported on zeolite is not toxic at the concentration values of sodium selenite and potassium iodide solutions amounting to 0.001 mol/L. Thereby the quantity of selenium on the carrier was equal to 4.35, that of iodine being of 7.56 μg/g.

Basing on the premises we could draw a conclusion that the elastin hydrolysates modified with selenium and iodine applied onto zeolite tuff species are non-toxic as to compare to inorganic species of microelements. Thus, the studies concerning the toxicity demonstrated that a fodder supplement containing selenium and iodine is safe due to bound state of microelemental species.

CONCLUSION

In order to obtain non-toxic and bioavailable iodine and selenium species there was used complexation between inorganic salts and hydrolysate followed by the adsorption on zeolite.

Optimum conditions for immobilizing the selenium ions and iodine on the peptides from elastin hydrolysate have been experimentally chosen. Basing on the IR spectroscopic analysis it was suggested that dimers $Se_2O_3^{2-}$ could be incorporated into the structure of peptides *via* binding with free carboxyl groups COO^- inherent in elastin hydrolysate. Iodide ions are bound by means of interaction with free NH_3^+ groups inherent in peptides. As a carrier of the bound species of the microelements we used cheap, available naturally occurring zeolites. It was found that at the temperature of 18–20 °C, the ratio between solid and liquid phases 2 : 1 and sorption duration equal to 3 h the selenium concentration in the zeolites tuff was equal to 4.35 μg/g, whereas the iodine concentration amounted to 7.56 μg/g. The drying temperature of the fodder supplement obtained was equal to 40 °C.

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