

GERMANIUM COORDINATION COMPOUNDS FOR INCREASING OF α -L-RHAMNOSIDASE ACTIVITY

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The aim of the work was to determine the influence of a number of coordination compounds of germanium, in particular cation-anionic type, on the activity of α -L-rhamnosidases of three producers: *Cryptococcus albidus*, *Eupenicillium erubescens* and *Penicillium tardum* IMB F-100074. Activity of α -L-rhamnosidases was determined according to Davis method, using naringin as substrate. The specific α -L-rhamnosidase activity of preparations was 12 units/mg of protein for *C. albidus*, 120 units/mg for *E. erubescens* and 27 units/mg for *P. tardum*. Protein concentration in preparations was 0.01 mg/ml. Germanium compounds were synthesized in alcohol-water solution according to standardized methods. In all compounds, the same bis (citrate) germanate anion is realized; however, the composition of the cation changes: the protonated form of phenanthroline and bipyridine or the complex cations of d-metals. It was established that none of the complex compounds exhibited an inhibitory effect on the activity of the enzymes under study. Maximum activating effect was observed with compound tris(bipyridine)nickel(II) bis(citrate)germanate monohydrate in concentration of 0.1% on α -L-rhamnosidases of *C. albidus* (10-fold), *E. erubescens* (2.5-fold) and *P. tardum* (5-fold). Notably, enzymatic activity increased by 45, 47 and 60% respectively in presences of compound bi-pyridine bis(citrate)germanate dihydrate in same concentration. Hence, it was shown that these two compounds can be used as effectors of studied enzymes.

Key words: germanium coordination compounds, *Cryptococcus albidus* 1001, *Eupenicillium erubescens* 248, *Penicillium tardum* IMB F-100074.

One of the important problems of modern biology, from a theoretical and practical point of view, is the study of the activity of enzymes, because society, as before, now continues to be widely used in various industries and medicine.

Lately, enzymological research focuses on microbial enzyme preparations, because there are substantial benefits in using those for biotechnological processes compared to material of plant or animal origin. That is the reason why researches are now trying to find highly productive microbial strains to solve the technological problems with generating

preparations of enzymes with various action spectra. One of such enzymes with industrially important activity is α -L-rhamnosidase (α -L-rhamnoside rhamnohydrolase, EC number 3.2.1.40). It is specific to terminal α -1,2-, α -1,4- and α -1,6-non-reducing residues of L-rhamnose, which is present in the natural glycoconjugates and synthetic glycosides. In recent years, this enzyme has become of high interest for producing preparations of plant-based flavonoid glycosides intended to treat heart diseases, also with immunotropic and antiviral effects. For example, it was shown

[1] that the biologically active substance of Proteflazid preparation is effective inhibiting HIV replication. The substance targets HIV's reverse transcriptase. α -L-rhamnosidases are also used in food industry: hydrolysis of terpene glycosides (rutinosides) and bioflavonoid naringin. The enzyme contributes to the release of aromatic compounds that enhance the aroma of grape juice and wine. Application of α -L-rhamnosidases in chemical industry is linked to reduced cost of rhamnose production [2–4]. An important aspect of using α -L-rhamnosidases in biotechnological processes is finding ways to increase their activity. Various methods are used to achieve this goal. One of those is chemical modification of enzymes, which makes it possible to receive enzymes with high activity. Chemical modification can be carried out with the help of polymers, metal ions and their complexes. Complexes of germanium, cobalt, nickel with organic ligands are promising biologically active substances [5–7]. At the same time, considerable potential is inherent in the complex compounds of germanium, which is found in almost all silicate rocks, in oil, coal, leaves, roots of a number of plants, seaweeds, in mineral waters, in various microorganisms, in the blood and in certain organs of man. Currently, germanium is considered one of the essential elements, it is present in many healing plants, such as ginseng, aloe, garlic. Previously, the properties of germanium were poorly investigated, it was obtained in limited quantities, mainly for research purposes. However, that changed with the discovery of a compound of germanium-132, which showed a wide range of biological effects including antitumor activity [8]. Since then, interest in biologically active compounds of germanium has sharply increased. Many organic and complex compounds of germanium with different bioligands were synthesized in subsequent studies, in which they exhibited neurotropic, analgesic, antihypertensive, fungicidal, bactericidal, antiviral, anti-malarial, anti-radiation, anti-tumor, interferon-inducing, adaptogenic, cardioprotective, hepatoprotective, anti-toxic, anti-anemic and other activities [9–11].

By certain properties germanium is similar to hemoglobin and can also affect various biochemical processes, in particular, stimulate tissue saturation with oxygen, help with detoxification, accelerate the rate of wound healing, improve the blood composition, and strengthen the immune system. There are also data on the stimulation of growth in plants and experimental animals [12, 13]. In this case, the investigated organic

compounds of germanium are less toxic than their silicon and carbon-containing analogues, and even less than the kitchen salt [14]. The body of scientific literature grows annually about synthesis of germanium compounds with various properties. For complex metal compounds, including germanium coordination compounds, the interaction with enzymes whether industrially important or not is studied insufficiently.

Previously [15] we've showed that cobalt (II, III) coordination compound with residues of dithiocarbamic acid $RR'NC(S)SH$ can stimulate activity of α -L-rhamnosidase of *Cryptococcus albidus*. Another compound, of germanium (IV) with nicotinamide (Nad) $[GeCl_2(Nad)_4]Cl_2$ (patented [16] as a compound with antihypoxic activity) inhibited by 70% activity of α -L-rhamnosidase of *C. albidus* but did not affect that enzyme of *Eupenicillium erubescens* [17].

Therefore, the purpose of this work was to determine the influence of a number of other on the structure of coordination compounds of germanium, in particular cation-anionic type, on the activity of α -L-rhamnosidases of three producers: *Cryptococcus albidus*, *Eupenicillium erubescens* and *Penicillium tardum*.

Material and Methods

Study objects were *Cryptococcus albidus* 1001, *Eupenicillium erubescens* 248 and *Penicillium tardum* IMB F-100074, producers of α -L-rhamnosidases. *C. albidus*, *E. erubescens* and *P. tardum* IMB F-100074 were grown in deep culture for 4 days at 28 °C, agitated at 220 rpm. Strains were cultured on media which were previously optimized as follows (g/l): *C. albidus*: rhamnose — 1, peptone — 5, yeast extract — 3, malt extract — 3, pH — 6 [18]; *E. erubescens*: $NaNO_3$ — 2; KH_2PO_4 — 1; KCl — 0.5; $MgSO_4 \cdot 7H_2O$; $FeSO_4 \cdot 7H_2O$ — 0.015; rhamnose — 2.5, pH — 5.5 [20]; *P. tardum*: rhamnose — 8, yeast autolysate — 2; KH_2PO_4 — 1; KCl — 0.5; $MgSO_4 \cdot 7H_2O$ — 0.5; $FeSO_4 \cdot 7H_2O$ — 0.015, pH — 6 [19].

α -L-rhamnosidases were obtained by precipitation of culture liquid supernatant with ammonium sulfate (90% saturation) and further purified in columns with neutral and polarized TSK-gels Toyopearl HW-55, 60 ("Toyosoda", Japan) and DEAE-650-s ("Merck", Germany) as described previously [20–22]. Homogeneity of α -L-rhamnosidase preparations was confirmed with SDS-PAGE [19, 20].

Activity of α -L-rhamnosidases was determined according to Davis [23], using

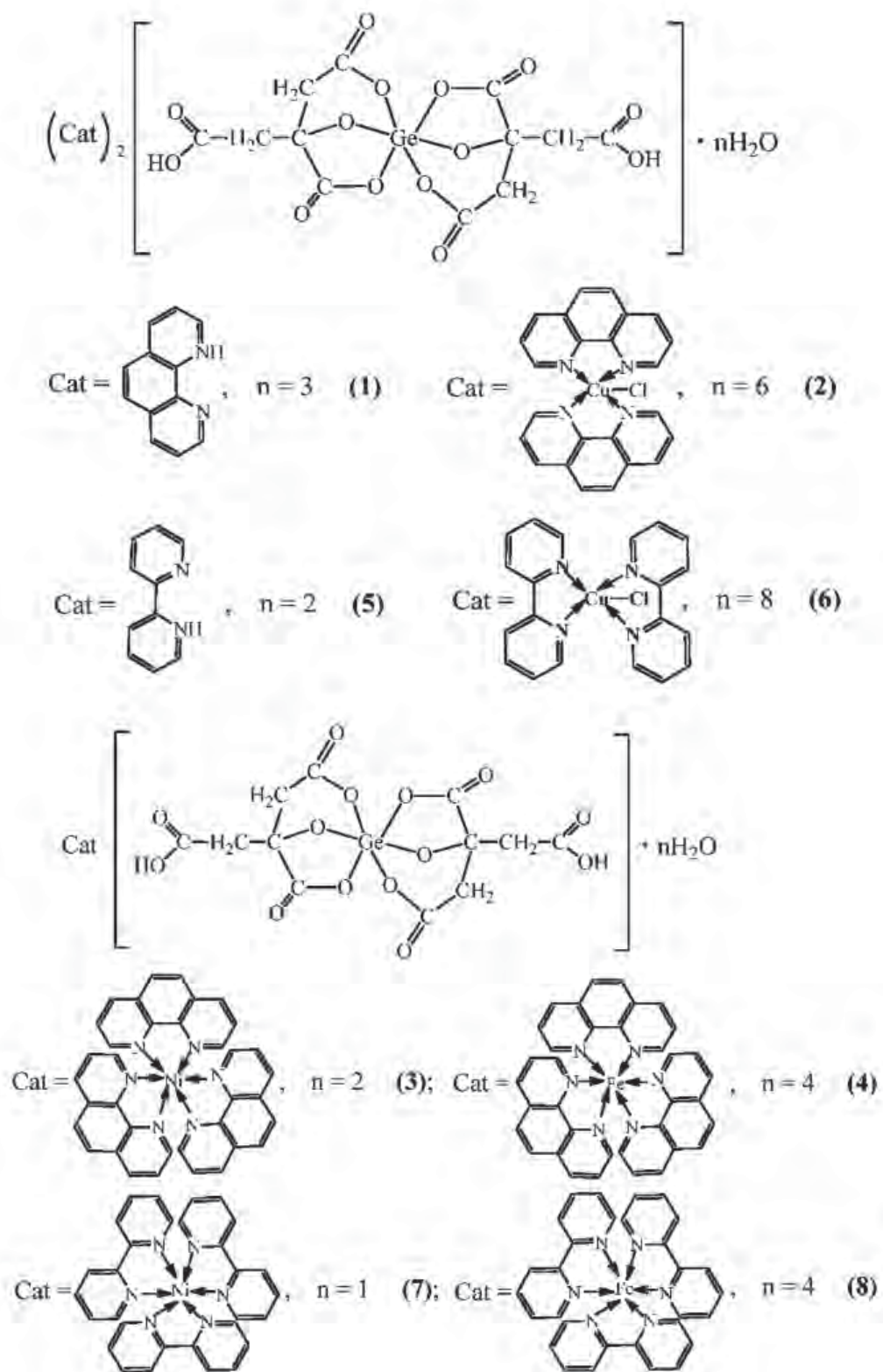


Fig. 1. Composition and structure of germanium coordination compounds 1–8

naringin as substrate. The specific α -L-rhamnosidase activity of preparations was 12 units/mg of protein for *C. albidus*, 120 units/mg of protein for *E. erubescens* and 27 units/mg of protein for *P. tardum*. Protein concentration in preparations was 0.01 mg/ml.

Germanium compounds were synthesized in alcohol-water solution according to standardized methods [24, 25] and used to modify enzymatic activity. The composition and structure of compounds are given in Fig. 1. Complexes 1–8 are cation-anion type. In all compounds, the same anion is realized (bis(citrate)germanate). Cations vary as follows: protonated phenanthroline (1) or bi-pyridine (5), or complexes of d-metals (2–4, 6–8).

To study their effect on activity of α -L-rhamnosidases, GCC were used in concentrations of 0.1 and 0.01%, time of exposure 30 min and 24 hr. GCC were dissolved in 0.1 M phosphate-citrate buffer, pH 5.2 adding 0.1% DMSO. Incubation was performed at room temperature.

The figures show average arithmetic values for five repetitions of the experiment, deviation from the average does not exceed 5%.

Results and Discussion

In the last few decades there has been a rapid development of research inorganic drug chemistry. Therapeutic uses of metallopharmaceuticals include such diverse areas as tumor treatments, antibacterial therapy and anti-inflammatory drugs [26]. Most of the metal complexes with antitumor activity act mainly through direct covalent binding to biological targets such as DNA or proteins. Thus, the mechanism of action of one of the previously studied drugs — cisplatin (cis diamminodichloroplatin (II)), similar to the action of other alkylating drugs and is disfunctions of DNA caused by chemical damage of its bases by establishing coordination bonds between the platinum atom and two bases of DNA (mainly guanine), resulting in the DNA of intra- and intermediate segregation. At the cellular level, cisplatin causes abnormal replication and transcription, leading to cellular delay and apoptosis. Cisplatin has pronounced cytotoxic, bactericidal and mutagenic properties. This complex of platinum is now widely used in medicine as an antitumor. Unlike such “classical” metal products, the focus of research has recently been shifted towards metal products that carry out their biological action by either activating or inhibiting

enzymes [14]. It is difficult to say, before, how this or that metal will act on enzyme activity. It depends on many factors, among which: the nature of the enzyme (metal- or metal-independent), the properties of the metal, its concentration. Germanium is unique in its complexing properties and the ability to use its compounds in various industries and medicine. But, despite the fact that every year it grows the number of publications devoted to the application of germanium compounds with different properties, remains to be clarified the question of their influence on the activity of enzymes. Such studies are mainly devoted to selective inhibition of specific enzymes that are associated with the development of tumor processes. It was established [27] that complex metal compounds are able to interact with active regions of the enzyme, increasing its selectivity and the ability to coordinate with the residues of proteins, which may result in an increase in their inhibitory activity. Information concerning the application of complex compounds of germanium to increase the activity of enzymes of glycolytic action, we have not found in the available literature. The most promising and, at the same time, the least studied, in our opinion, is the coordination compounds of germanium with biologically active metals and organic molecules. Such compounds are characterized by a synergistic effect [12]. Of those substances, researchers are especially drawn to supramolecular coordination compounds which incorporate both complex cations, and complex anions with biometals and various ligands (heteroaromatic bis-chelate amines of 2,2'-bi-pyridine (bipy), 1,10-phenanthroline (phen) and biologically active citric acid (H_4Cit)). In our study, we chose compounds with the same bis(citrate) germanate anion and organic or complex cations (Fig. 1).

We found that GCC at different exposure and concentration affected the activity of studied α -L-rhamnosidases differently. For example, 30-minute incubation with compounds (1), (2), (4) and (6) in concentration of 0.1% increased the activity of *C. albidus* α -L-rhamnosidase by 10–20% compared to control (Fig. 2, A). Under the same conditions, enzymatic activity increased by 45% with compound (5), twice with compound (3) and 10-fold with compound (7). GCC in concentration of 0.01% had a different effect on the enzymes. Enzymatic activity stayed at the control level except for compound (7), incubation with which increased activity of *C. albidus* α -L-rhamnosidases by 150%.

A slightly different picture was noted after 24 hours exposure (Fig. 2, B). Significant effects were observed only for compounds (3), (5) and (7) at a concentration of 0.1% (increase by 200, 45 and 1000%, respectively). When the concentration decreased to 0.01%, enzymatic activity was observed at the control level in the presence of all substances except for the compound (7). In the latter case, a significant activating effect on enzyme activity was maintained (by 280%).

Study results of GCC influence on the activity of *E. erubescens* α -L-rhamnosidase after 30 min incubation are showed on Fig. 3, A. Maximum activation was 150%, as in the case of *C. albidus* α -L-rhamnosidase and occurred under the action of compound (7) at a concentration of 0.01%. It is noted that higher concentrations (0.1%) of this substance contribute to increase of activation at different times of exposure. Additionally, activation by 10 and 40% was seen for compounds (5) and (6), respectively, for *E. erubescens* α -L-rhamnosidase. With longer exposure, an insignificant (20 and 47%) increase in activity was observed, both in the case of 0.1% and 0.01% of the concentration of active substances (Fig. 3, B). As a whole, it can be noted that the activation occurred quickly in all cases, during the first 30 minutes of incubation, and almost did not change for 24 hours. An increase in enzyme activity (40%) as a result of increased incubation time was noted only in the presence of compound (1).

The effect of GCC in concentration of 0.01% and exposure time of 30 minutes was studied also for *P. tardum* α -L-rhamnosidase (Fig. 4, A). It was established that all compounds except for (6) contributed to an increase in enzymatic activity by 5–45%. With an increase in the

concentration of substances (1), (2), (6), (7), (8) to 0.1%, the enzymatic activity inanced by 10–275%. A similar pattern was observed if exposure were prolonged to 24 hours (Fig. 4, B). At a concentration of 0.01%, all complexes contributed to an increase in the activity of *P. tardum* α -L-rhamnosidase by 3–53%. Compounds (3) to (8) had a greater effect when the concentration increased to 0.1%.

The stimulating effect of GCC on activity of α -L-rhamnosidases was previously unknown. Earlier, we established the inhibitory effect of germanium (IV) complex with nicotinamide (Nad) on activity of α -L-rhamnosidases of *C. albidus* and *E. erubescens* [17]. The obtained results are of considerable interest both from a practical and a theoretical point of view, and need further study. Future studies may be devoted to the creation of specially developed coordinating compounds of biometals and bioligands with improved characteristics.

To summarize, the maximum activating effect was observed using 0.1% compound (7) [tris(bi-pyridine) nickel (II) bis(citrate)germanate monohydrate] for α -L-rhamnosidase of *C. albidus* (10-fold), *E. erubescens* (2.5-fold) and *P. tardum* (5-fold). In contrast, compound (5) [bi-pyridine bis(citrate)germanate dihydrate] at the same concentration increased enzymatic activity only by 45, 47 and 60%, respectively. In all cases (except for incubating α -L-rhamnosidase of *P. tardum* with compound (7)), 30 min exposure was more effective than 24 hours. The obtained results indicate that compound (7) can be used in further studies as an effector of α -L-rhamnosidases of investigated strains.

The obtained data demonstrate the influence of cation composition in complexes 1–8 on their enzyme activating action in relation to the investigated rhamnosidases.

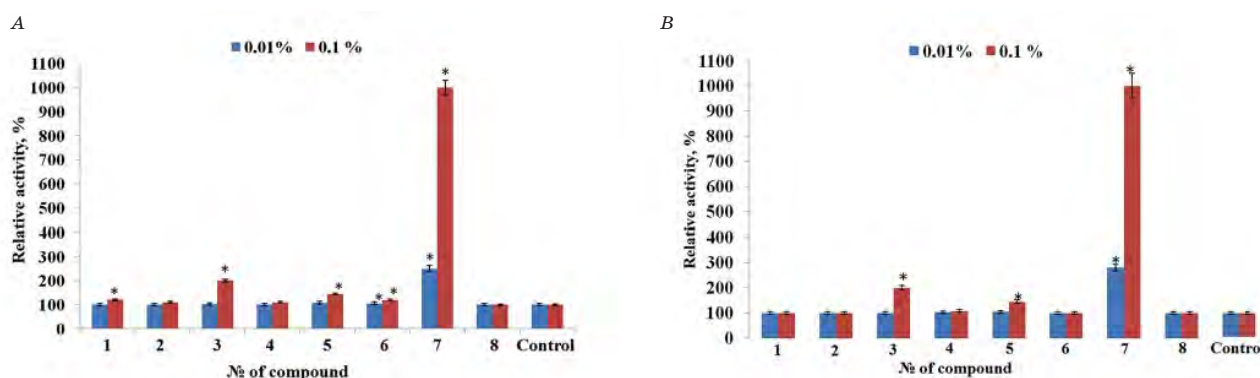


Fig. 2. Influence of germanium coordination compounds on activity of α -L-rhamnosidase of *Cryptococcus albidus*

Here and after: A — 30 min exposure; B — 24 hr exposure;

* — $P \leq 0.05$ with compare to control

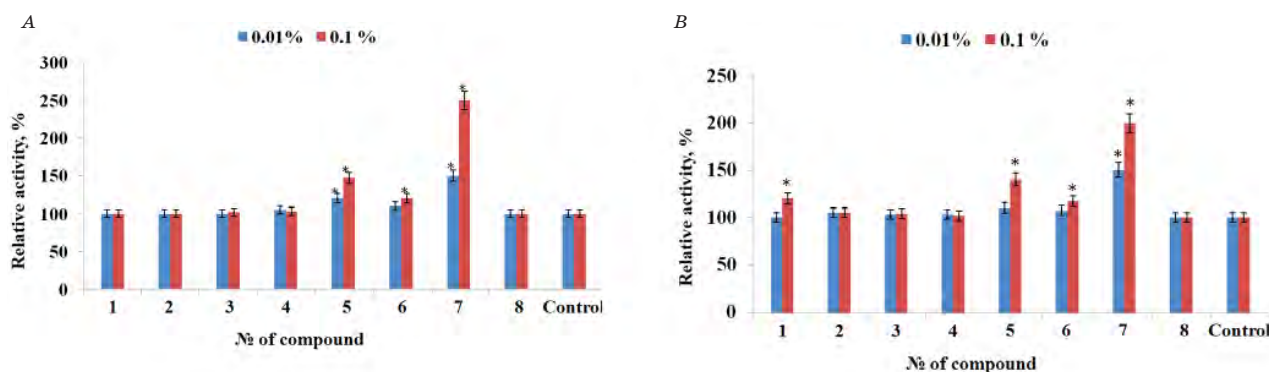


Fig. 3. Effect of germanium coordination compounds on activity of *E. erubescens* α -L-rhamnosidase

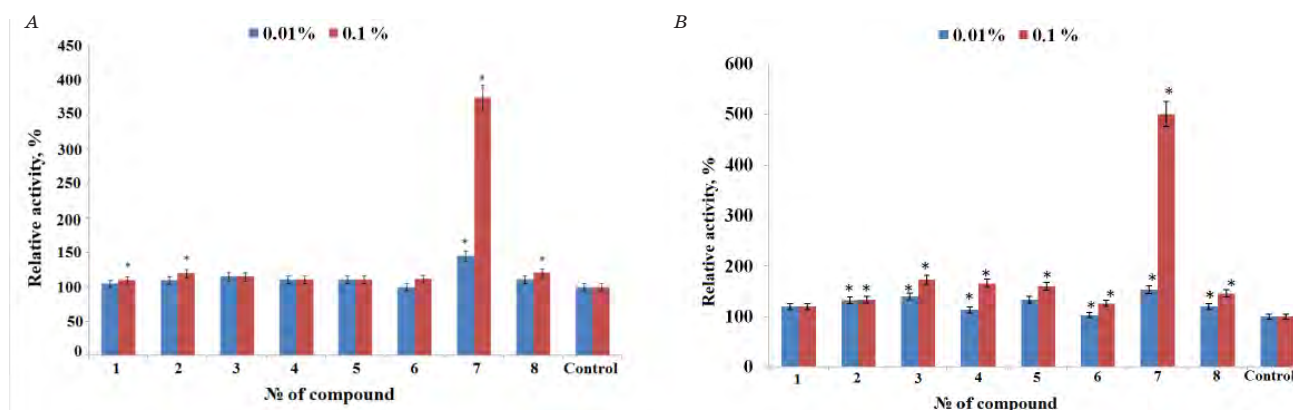


Fig. 4. Effect of germanium coordination compounds on activity of *P. tardum* α -L-rhamnosidase

Compounds 5 and 7 containing 2,2'-bi-pyridine were most active, the latter was most effective. Probably, adding Ni^{2+} cation to the compound containing 2,2'-bi-pyridine increases the action of complex 7 compared to 5.

Further researches on revealing of interconnections between features of structure of coordination compounds and their biological activity will allow to create the basis for the targeted synthesis of compounds with the predicted character of action on living objects. Change of activity is possible by varying the specific metal, as well as its coordination medium (such as the ligand, the degree of oxidation, the coordination number

and geometry). However, the conclusions about the differences in the activity of individual central atoms should be based only on complexes having (almost) identical ligands, coordination numbers and coordination geometry, differing only in the central atom. Even then, an exact comparison will make it more difficult the influence of other factors (for example, almost complete lack of data on solubility in water and stability of complexes in solution).

In general, it can be argued that heterometallic complexes of germanium (IV) with various ligands are promising objects for creating enzyme effectors on their basis.

REFERENCES

1. Trokhymchuk T., Zavelevich M., Liulchuk M., Starosyla D., Rybalko S., Rudenko A. In vitro study of anti-HIV activity of Ptoteflazid herbal composition. *Amer. J. Fundam. Appl. Experim. Res.* 2017, 4 (7), 87–91.
2. Mueller M., Zartl B., Schleritzko A., Stenzl M., Viernstein H., Unger F. M. Rhamnosidase activity of selected probiotics and their ability to hydrolyse flavonoid rhamnoglucosides. *Bioprocess Biosyst. Eng.* 2018, 41 (2), 221–228. <https://doi.org/10.1007/s00449-017-1860-5>
3. Yadav V., Yadav P. K., Yadav S., Yadav K. D. S. Alpha-L-rhamnosidase: a review. *Proc. Biochem.* 2010, 45 (8), 1226–1235. <https://doi.org/10.1016/j.procbio.2010.05.025>.
4. Puri M. Updates on naringinase: structural and biotechnological aspects. *Appl. Microb. and Biotechnol.* 2012, 93 (1), 49–60.
5. Shutka A. O., Kravetz D. S., Seifullina I. I., Martsinko E. E., Pesaroglo A. G. Toxicometric

- studies of the new antihypoxante OK-5 on the basis of the coordination compound of germanium with bioligands. *Toxicol. Med. Prod.* 2010, V. 2–3, P. 50–53.
6. Zelenin K. N. Physiologically active complexes of hydrazones. *Soros Educat. J.* 1996, V. 12, P. 41–46. (In Russian).
 7. Zinchenko O. Yu., Shmatkova N. V., Seifullina I. I., Galkin B. N., Fillipova T. O. Antimicrobial activity of isonicotinic acid derivatives and tin (IV) complexes based on them. *Microbiology and biotechnology.* 2012, V. 2, P. 69–78. [https://doi.org/10.18524/2307-4663.2012.2\(18\).93037](https://doi.org/10.18524/2307-4663.2012.2(18).93037). (In Russian).
 8. Asia K. Organic germanium. A medical godsend. *Tokyo: Kogakusha Ltd.* 1977, 154 p.
 9. Lukevic E. Y., Gar T. K., Ignatovich L. M., Mironov V. F. Biological activity of germanium compounds. *Riga: Zinati.* 1990, 191 p. (In Russian).
 10. Seifullina I. I., Martsinko E. E., Afanasenko E. V. Design and synthesis of new homo- and heterometal coordination compounds of germanium(IV) for preparation of low toxic drugs with a wide therapeutic action. *Odessa National University Herald. Chemistry.* 2015, 20 (4), 6–17. [https://doi.org/10.18524/2304-0947.2015.4\(56\).56690](https://doi.org/10.18524/2304-0947.2015.4(56).56690)
 11. Luk'yanchuk V. D., Seifullina I. I., Litvinenko D. F., Martsinko E. E. Pharmacological properties of organic and coordination compounds of germanium — modern presentation. *Pharmacol. Drug Toxicol.* 2016, 1 (47), 3–13. (In Russian).
 12. Martsinko E. E., Seifullina I. I. Design and synthesis of molecular complexes and germanium complexones (IV) with a wide spectrum of pharmacological action. *Odessa: ONU I. I. Mechnikov.* 2018, 144 p. (In Ukrainian).
 13. Martsinko E. E., Seifullina I. I., Chebanenko E. A., Pesaroglo A. G., Pozharic'kij O. P. Plant growth promoter iron(II) bis(citrate) germanate(IV). *Ukraine. Patent 131091*, January 10, 2019. (In Ukrainian).
 14. Fedoruk R. S., Khrabko M. I., Tsap M. M., Martsinko E. E. Growth, development and reproductive function of female rats and their offspring viability at the condition of the watering of different doses of citrate germanium. *The Animal Biology.* 2016, 18 (3), 97–106. <https://doi.org/10.15407/animbiol18.03.097>. (In Ukrainian).
 15. Varbanets L. D., Matselyukh O. V., Nidyalkova N. A., Avdiyuk E. V., Gudzenko E. V., Seifullina I. I., Masanovets G. N., Khitrish N. V. The coordination compounds of cobalt (II, III) with dithiocarbamic acid derivatives — modifiers of hydrolytic enzymes activity. *Biotechnol. acta.* 2013, 6 (1), 73–80. (In Ukrainian).
 16. Luk'yanchuk V. D., Vitohina N. V., Seifullina I. I., Martsinko E. E., Tkachenko V. M., Kravec' D. S. The molecular complex of germanium tetrachloride with nicotinamide, which exhibits antihypoxic activity with thermoprotective properties. *Ukraine. Patent 59089*, May 10, 2011. (In Ukrainian).
 17. Varbanets L. D., Matselyukh O. V., Nidyalkova N. A., Gudzenko O. V., Avdiyuk E. V., Shmatkova N. V., Seifullina I. I. Influence of coordinative compounds of germanium (IV) and stanum (IV) on activity of some microbial enzymes with glycolytical and proteolytical action. *Microbiol. Zh.* 2014, 74 (6), 11–18. (In Russian).
 18. Gudzenko O. V., Borzova N. V., Varbanets L. D. Optimization of cultivation conditions of alpha-L-rhamnosidases producers — representatives of different taxonomic groups of microorganisms. *Microbiol. Zh.* 2011, 73 (3), 46–53. (In Ukrainian).
 19. Gudzenko O. V., Varbanets L. D. Optimization of cultivation conditions of penicillium tardum — the α -l-rhamnosidase producer. *Microbiol. Zh.* 2015, 77 (4), 25–31. (In Ukrainian).
 20. Varbanets L. D., Gudzenko O. V., Borzova N. V. Rhamnosidase from *Eupenicillium erubescens*: purification and characterization. *Nauka i Studia.* 2013, 41 (109), 11–23.
 21. Borzova N., Gudzenko O., Varbanets L. Purification and characterization of a naringinase from *Cryptococcus albidus*. *Appl. Biochem. Biotechnol.* 2018, 184 (3), 953–969. <https://doi.org/10.1007/s12010-017-2593-2>
 22. Gudzenko O. V., Varbanets L. D. Purification and physico-chemical properties of α -L-rhamnosidase *Penicillium tardum*. *Microbiol. Zh.* 2016, 78 (1), 13–22. (In Ukrainian).
 23. Davis D. W. Determination of flavonones in citrus juice. *Anal. Biochem.* 1947, V. 19, P. 476–478.
 24. Seifullina I., Martsinko E., Chebanenko E., Pirozhok O., Dyakonenko V., Shishkina S. Synthesis and structural characteristics of bis(citrate)germanates(IV) $(\text{Hbipy})_2[\text{Ge}(\text{HCit})_2] \cdot 2\text{H}_2\text{O}$ and $[\text{CuCl}(\text{bipy})_2]_2[\text{Ge}(\text{HCit})_2] \cdot 8\text{H}_2\text{O}$. *Chemistry Journal of Moldova. General, Industrial and Ecological Chemistry.* 2016, 11 (2), 52–57. [https://doi.org/10.19261/cjm.2016.11\(2\).11](https://doi.org/10.19261/cjm.2016.11(2).11)
 25. Seifullina I. I., Martsinko E. E., Chebanenko E. A., Pirozhok O. V., Dyakonenko V. V., Shishkina S. V. Synthesis, Thermal Stability, and Structure of Bis(citrato)germanates: $[\text{Co}(\text{Phen})_3][\text{Ge}(\text{HCit})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Fe}(\text{Phen})_3][\text{Ge}(\text{HCit})_2] \cdot 4\text{H}_2\text{O}$ (H_4Cit = Citric acid, Phen = 1,10-Phenanthroline). *Rus. J. Coord. Chem.*, 2017, 43 (8), 505–511. <https://doi.org/10.7868/S0132344X17080060>
 26. Habala L., Devnsky F., Egger A. Metal complexes as urease inhibitors. *Journal of Coordination Chemistry.* 2018. <https://doi.org/10.1080/00958972.2018.1458228>
 27. Kelly J., Kilpin P., Dyson J. Enzyme inhibition by metal complexes: concepts, strategies and applications *Chem. Sci.* 2013, V. 4, P. 1410–1419. <https://doi.org/10.1039/C3SC22349>

КООРДИНАЦІЙНІ СПОЛУКИ ГЕРМАНІЮ ДЛЯ ПІДВИЩЕННЯ АКТИВНОСТІ α -L-РАМНОЗИДАЗ

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Метою роботи було дослідити вплив ряду координаційних сполук германію, зокрема катіон-аніонного типу, на активність α -L-рамнозидаз трьох продуцентів: *Cryptococcus albidus* 1001, *Eupenicillium erubescens* 248 та *Penicillium tardum* IMB F-100074. α -L-рамнозидазну активність визначали методом Davis, використовуючи як субстрат нарингін. Специфічна α -L-рамнозидазна активність препаратів становила 12 од/мг протеїну для *C. albidus*, 120 од/мг для *E. erubescens* і 27 од/мг для *P. tardum*. Вміст протеїну в препаратах — 0,01 мг/мл. Як модифікатори активності ензимів застосовували координаційні сполуки германію, що їх було синтезовано у водно-спиртовому розчині за однотипними методиками. У всіх сполуках реалізується однаковий біс(цитрато)германатний аніон, але змінюється склад катіона: протонувана форма фенантроліну і біпіридину або комплексні катіони d-металів. Встановлено, що жодна з комплексних сполук не виявляла інгібуючої дії на активність досліджуваних ензимів. Максимальний активуючий ефект відзначено у разі використання 0,1%-ї концентрації трис(біпіридин)нікель(II) біс(цитрато)германат моногідрату для α -L-рамнозидаз *C. albidus* (у 10 разів), *E. erubescens* (у 2,5 раза) та *P. tardum* (у 5 разів). Водночас біпіридиній біс(цитрато)германат дигідрат у цій самій концентрації підвищував активність на 45, 47 та 60% відповідно. Таким чином, у результаті проведеної роботи показано, що ці дві сполуки можуть слугувати ефекторами досліджуваних ензимів.

Ключові слова: комплексні сполуки германію, α -L-рамнозидази *Cryptococcus albidus* 1001, *Eupenicillium erubescens* 248, *Penicillium tardum* IMB F-100074.

КООРДИНАЦИОННЫЕ СОЕДИНЕНИЯ ГЕРМАНИЯ ДЛЯ ПОВЫШЕНИЯ АКТИВНОСТИ α -L-РАМНОЗИДАЗ

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Целью работы было исследовать влияние ряда координационных соединений германия, в частности катион-анионного типа, на активность α -L-рамнозидаз трех продуцентов: *Cryptococcus albidus* 1001, *Eupenicillium erubescens* 248 и *Penicillium tardum* IMB F-100074. α -L-рамнозидазную активность определяли методом Davis, используя в качестве субстрата нарингин. Специфическая α -L-рамнозидазная активность препаратов составляла 12 ед/мг протеина для *C. albidus*, 120 ед/мг для *E. erubescens* и 27 ед/мг для *P. tardum*. Содержание протеина в препаратах — 0,01 мг/мл. Как модификаторы активности энзимов использовали координационные соединения германия, которые были синтезированы в водно-спиртовом растворе по однотипным методикам. Во всех соединениях реализуется одинаковый бис(цитрато)германатный анион, однако изменяется состав катиона: протонированная форма фенантролина и бибиридина или комплексные катионы d-металлов. Установлено, что ни одно из комплексных соединений не проявляло ингибирующего действия на активность исследуемых энзимов. Максимальное активизирующее действие отмечено в случае использования 0,1% -й концентрации соединения трис(бибиридин)никель(II) бис(цитрато)германат моногидрата для α -L-рамнозидаз *C. albidus* (в 10 раз), *E. erubescens* (в 2,5 раза) и *P. tardum* (в 5 раз). В то же время соединение бибиридиний бис(цитрато)германат дигидрат в этой же концентрации повышало активность на 45, 47 и 60% соответственно. Таким образом, в результате проведенных исследований показано, что эти два соединения могут служить эффекторами исследуемых энзимов.

Ключевые слова: комплексные соединения германия, α -L-рамнозидазы *Cryptococcus albidus* 1001, *Eupenicillium erubescens* 248, *Penicillium tardum* IMB F-100074.

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