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THE INFLUENCE OF COORDINATION COMPOUNDS WITH MALATOGERMANATE/STANNATE ANIONS AND 1,10-PHENANTHROLINE CATIONS OF 3D METALS ON α-L-RHAMNOSIDASE ACTIVITY OF *PENICILLIUM TARDUM*, *PENICILLIUM RESTRICTUM* AND *EUPENICILLIUM ERUBESCENS*

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The search for effectors capable of influencing the catalytic activity of enzymes is an important area of modern enzymology. The aim of the study was to investigate the ability of 6 coordination compounds with malatogermanate/stannate anions and 1,10-phenanthroline cations of 3d metals to modify α-L-rhamnosidase activity of Penicillium tardum, Penicillium restrictum and Eupenicillium erubescens strains. α-L-Rhamnosidase activity was determined by the Davis method using naringin as a substrate. It was demonstrated that $[Ni(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ in 0.1% concentration had the most pronounced activating effect on α-L-rhamnosidase activity of all strains studied. Noncompetitive inhibition of α-L-rhamnosidase in E. erubescens by $[Cu(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 10H_2O$ was shown. The obtained results expand the idea of glycosidases possible activators and inhibitors and indicate the perspective of their use in modern biotechnological processes.

K e y w o r d s: α-L-rhamnosidase, double coordination compounds, germanium(IV), stannum(IV), d-metals, Penicillium tardum, Penicillium restrictum, Eupenicillium erubescens.

The intensive development of biotechnology in the last decade is largely determined by the growing needs for products of microbial synthesis, which include microbial enzymes. Hydrolytic enzymes are of the greatest importance in modern industrial technological processes. The unique specificity of action, high catalytic activity, and the increasing availability of individual enzymes determine their wide use both for analytical purposes and in medicine, food industry, environmental control, and also in scientific research [1, 2]. But despite the great variety of natural enzymes, their properties are often not optimal for technological processes. Two approaches can be used to solve this problem: the first is the search for natural enzymes with appropriate characteristics, and the second − the modification of already known and well-characterized proteins. Since very few enzymes are produced in Ukraine, and their requirements are met by the expense of foreign drugs, research in both directions is relevant.

Earlier, as a result of the screening of more than 700 strains of microorganisms, 3 strains of micromycetes *Eupenicillium erubescens*, *Penicillium tardum* and *P. restrictum* were selected as producers of α-L-rhamnosidase (α-L-rhamnoside-rhamnohydrolase – EC 3.21.40) which hydrolytically splits off terminal unreduced α-1,2, α-1,4, α-1,6-linked L-rhamnose residues in α-L-rhamnosides. Cleavage of the O-glycosidic link is carried out with the inversion of the configuration of the anomeric carbon atom (C1 in the cyclic form of the monosaccharide). The isolation and purification of enzymes were described by us in previous works [3-5].

Earlier [6-9], we showed that a number of effectors can be used to increase the activity of α-L-rhamnosidases. Thus, coordination supramolecular salts with metal chelate anions based on the essential p-elements germanium (Ge(IV)) and stannum (Sn(IV)) with hydroxycarboxylic acids (tartaric, citric, xylaric) and their complementary complex

cations of 3d metals are capable of both activation and inhibition of α-L-rhamnosidases of a number of mycromycetes strains.

Coordination compounds with malatogermanate/stannate anions and 1,10-phenanthroline cations with Fe(II), Co(II), Ni(II), Cu(II) were found to be active effectors of α-L-rhamnosidase of the yeast *Cryptococcus albidus* [10]. Therefore, the aim of the work was to investigate the activity of these complexes as modifiers of α-L-rhamnosidases of three strains of micromycetes – *Penicillium tardum*, *Penicillium restrictum*, *Eupenicillium erubescens*.

Materials and Methods

The objects of the research were α-L-rhamnosidases of *Penicillium tardum*, *Penicillium restrictum*, *Eupenicillium erubescens*, the isolation and purification of which was described by us earlier [3-5].

α-L-Rhamnosidase activity was determined by the Davis method [11], using naringin as a substrate. One unit of α-L-rhamnosidase activity was defined as the amount of enzyme that re1eases 1 μmol of naringin per min in the solution.

6 cation-anion compounds were used as enzyme activity modifiers:

$[Fe(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ (**1**),
$[Co(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ (**2**),
$[Ni(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ (**3**),
$[Cu(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 10H_2O$ (**4**),
$[Zn(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]$ (**5**),
$[Co(phen)_3]_2[Ge(HMal)(Mal)_2]\cdot 14H_2O$ (**6**)

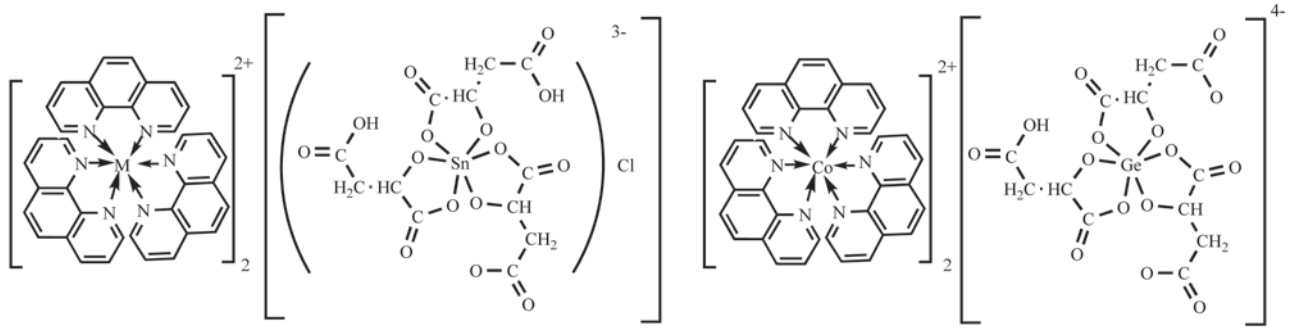
with 1,10-phenanthroline cations of 3d metals and malatostannate/germanate anions complementary to them (Fig. 1). The molecular, crystal structures of **1-4**, **6** were deposited in the Cambridge crystallographic database under the numbers: 2166164 (**1**), 2166165 (**2**), 2166166 (**3**), 2166167 (**4**), 2166168 (**6**), the properties are characterized by a set of physicochemical methods [12].

When studying the influence of various compounds on the activity of enzymes, concentrations of 0.1 and 0.01%, exposure times of 1 h and 24 h were used. The studied compounds were dissolved in 0.1% DMSO. Enzyme solutions without modifiers were used as a control.

All experiments were performed in 5-7 replicates. The analysis of the obtained results was carried out by their statistical processing by the methods of variation and correlation statistics using the Student's *t*-test. In the work, average values and standard errors ($M \pm m$) were calculated. Values with $P < 0.05$ were considered reliable. The results presented graphically were processed using Microsoft Excel 2007.

Results

α-L-Rhamnosidases with a specific activity of 33, 27 and 120 Units/mg were isolated and purified from the supernatant of the culture liquid of three strains of micromycetes *P. tardum*, *P. restrictum* and *E. erubescens* respectively. The obtained enzymes were homogeneous according to gel filtration on Sepharose 6B and did not show other glycosidase activities that were observed in the culture liquid of the producers [3, 6, 5]. 6 cation-anion compounds with 1,10-phenanthroline cations of 3d metals and malatostannate/germanate anions complementary to them were used as enzyme activity modifiers. Complexes **1-5** are isostructural and contain an additional chloride anion. In the same complex anion $[Sn(HMal)_2(Mal)]^{3-}$ (**1-5**), one of the carboxyl groups



$[M(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]$

M(II)=Fe(**1**), Co(**2**), Ni(**3**), Cu(**4**), Zn(**5**)

$[Co(phen)_3]_2[Ge(HMal)(Mal)_2]$(**6**)

*Fig. 1. Schemes of the compounds' **1-6** structures*

of the terminal malate ligand is deprotonated, and in a similar malatogermanate – $[Ge(HMal)(Mal)_2]^{4-}$ (**6**) – two. The charge of two cations $[M(phen)_3]^{2+}$ (M(II)=Fe, Co, Ni, Cu) is compensated by one $[Sn(HMal)_2(Mal)]^{3-}$ and Cl^- in **1-5**, and two $[Co(phen)_3]^{2+}$ in **6** – one $[Ge(HMal)(Mal)_2]^{4-}$. Sn/Ge atoms in anions bind to three pairs of deprotonated carboxyl and hydroxyl groups of malate ligands. The central atoms are six-coordinated, their polyhedra are twisted octahedra. The coordination polyhedron of the metal in $[M(phen)_3]^{2+}$ (M(II)=Fe(**1**), Co(**2**,**6**), Ni(**3**), Cu(**4**), Zn(**5**)) is an octahedron, the structure of which does not depend from a metal or anion that compensates for its charge.

The conducted studies showed that compounds **1-6** at different exposure times and concentrations had different effects on the activity of the studied α-L-rhamnosidases. Their most diverse effect was observed on the activity of α-L-rhamnosidase of *P. tardum* (Fig. 2, *A*, *B*).

The greatest activation of α-L-rhamnosidase of *P. tardum* was observed when compound **3** was used at a concentration of 0.1%, both at exposure for 1 h (by 84%) (Fig. 2, *A*) and at exposure for 24 h (by 89%) (Fig. 2, *B).* A completely different picture was observed when compound **3** was used in a concentration of 0.01%. The activating effect (by 48%) (Fig. 2, *A*) was more pronounced after exposure for 1 h than after 24 h (Fig. 2, *B*). When exposed for 1 h, compounds **1** and **5** in both concentrations practically did not affect the activity of the studied α-L-rhamnosidase (the activity was almost at the same level as the control) (Fig. 2, *A*, B). Compounds **2** and **6** in both concentrations inhibited the activity of *P. tardum* α-L-rhamnosidase by 16-64%. The most significant decrease in activity (by 80%) was observed with the action of compound 4 at a concentration of 0.01% during exposure for 1 h. It turned out to be interesting that this compound at a higher concentration (0.1%) increased the activity of the studied α-L-rhamnosidase by 20%.

At exposure for 24 h, all the studied compounds at a concentration of 0.01% had almost no effect on the activity of *P. tardum* α-L-rhamnosidase, while at a concentration of 0.1% they increased it from 30 to 89% (Fig. 2, *B*). In comparison with the enzyme of *P. tardum*, compounds **1-6** had a less pronounced effect on *P. restrictum* α-L-rhamnosidase (Fig. 3, *A*, *B*).

It was noted that with an exposure time of 1 h and different concentrations of substances **2**, **4** and **5** there was a slight increase in activity (from 5 to 27%) (Fig. 3, *A*), which when the exposure time increased to 24 h (Fig. 3, *B*) almost leveled off to control values. Compound **1** inhibited α-L-rhamnosidase of *P. restrictum* by 14-16% at both tested concentrations (Fig. 3, *A*), an exposure time of 1 h. It was shown that compound **3** increased the activity of *P. restrictum* α-L-rhamnosidase by 9% at an exposure time of 1 h at a concentration of 0.01%, although a higher concentration of the latter compound inhibited the activity by 14% (Fig. 3, *A*). Compound **6** in both concentrations at the given exposure time had no effect on the activity of the studied α-L-rhamnosidase (Fig. 3, *A*). The activity of *P. restrictum* α-L-rhamnosidase under the action

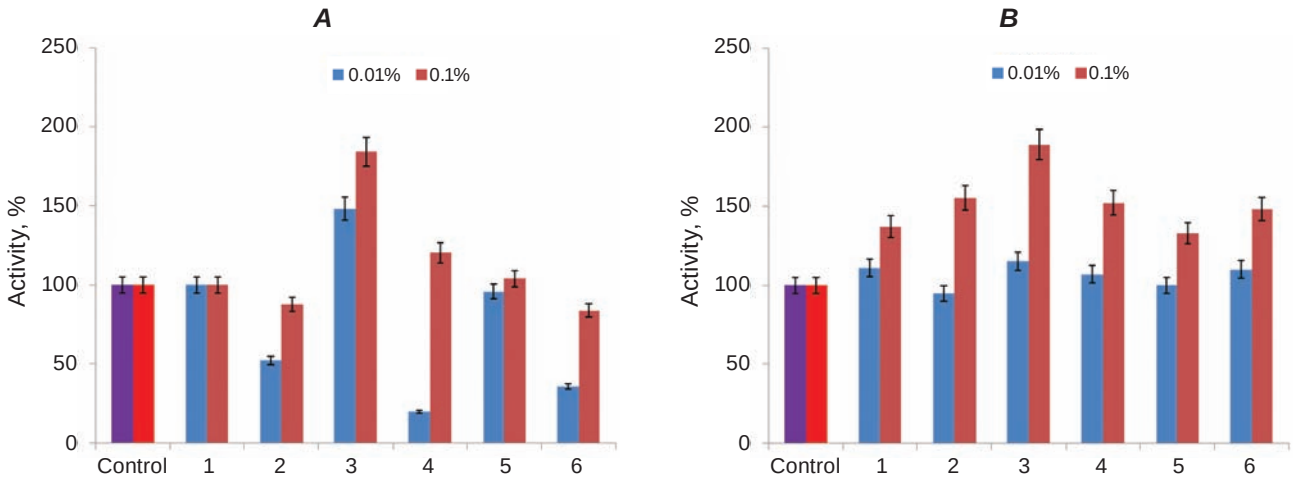


*Fig. 2. Effect of compounds **1-6** on the activity of P. tardum α-L-rhamnosidase. **A** – exposure time 1 h, **B** – exposure time 24 h*

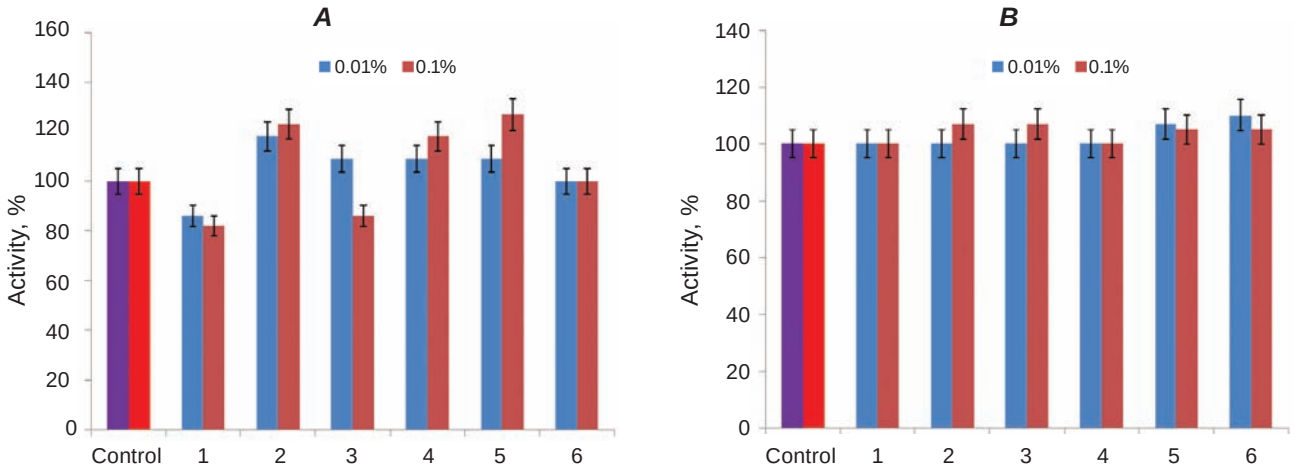


Fig. 3. Effect of compounds ***1-6*** *on the activity of P. restrictum α-L-rhamnosidase.* ***A*** *– exposure time 1 h,* ***B*** *– exposure time 24 h*

of compounds **1-6** and the exposure time of 24 h was almost no different from the control values. A slight increase in activity (by 7-10%) was observed under the action of compounds **5** and **6** at a concentration of 0.01% and an increase in activity by 5-7% under the action of compounds **1**, **2**, **3**, **5** and **6** at a concentration of 0.1%.

In comparison with the enzymes of *P. tardum* and *P. restrictum*, compounds **1-6** at a concentration of 0.01% had a more pronounced inhibitory effect in the case of *E. erubescens* α-L-rhamnosidase (Fig. 4, *A*, *B*). Thus, at the given concentration and exposure time of 1 h, compounds **1-6** inhibited the α-L-rhamnosidase activity of *E. erubescens* by 28-82% (Fig. 4, *A*). Increasing the exposure time to 24 h contributed to the complete inhibition of the activity of the studied enzyme (Fig. 4, *B*). The effect of compounds **1-5** at a concentration of 0.1% on the α-L-rhamnosidase activity of *E. erubescens* was more diverse (Fig. 4, *A*, *B*). The maximum activation (by 82-118%) of *E. erubescens* α-L-rhamnosidase occurred under the action of compounds **5**, **3** and **2** at exposure for 24 h (Fig. 4, *B*). A somewhat smaller increase in activity (by 68-23%) was noted under the action of compounds **3**, **4** and **2** during exposure for 1 h (Fig. 4, *A*). In general, it should be noted that with an increase in exposure time, the activating effect of compounds **1-6** at a concentration of 0.1% increased, while at a concentration of 0.01%, their inhibitory effect increased.

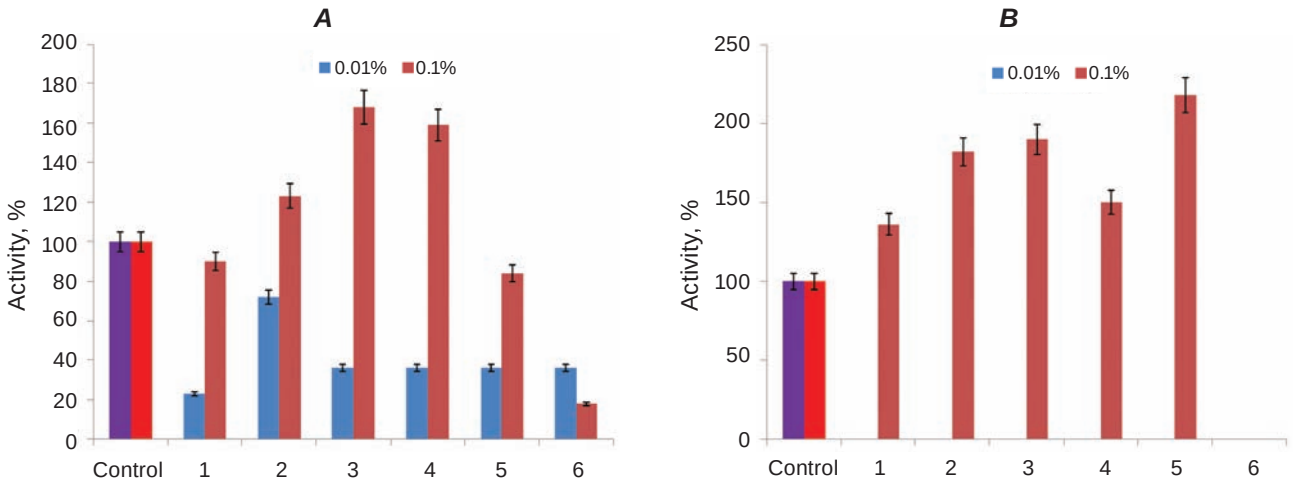


Fig. 4. Effect of compounds ***1-6*** *on the activity of E. erubescens α-L-rhamnosidase.* ***A*** *–exposure time 1 h,* ***B*** *– exposure time 24 h*

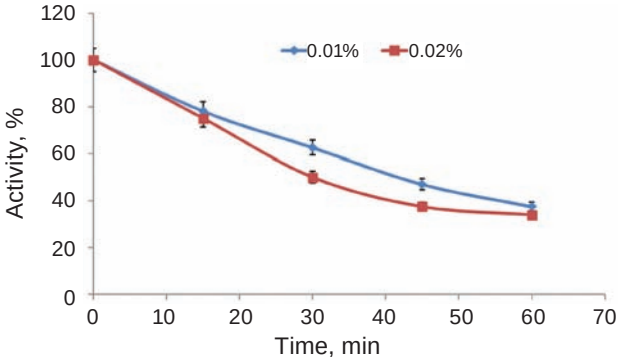


*Fig. 5. Effect of compound **4** on the activity of E. erubescens α-L-rhamnosidase in dynamic (pH 5.2; t 20°C)*

To study the inhibition of *E. erubescens* α-L-rhamnosidase by these compounds, we chose complex **4**, where the cation contains a Cu atom. The dynamics of inhibition was studied in the presence of rhamnose (reaction product) and cysteine (protector of sulfhydryl groups).

A study of the effect of substance **4** at concentrations of 0.01 and 0.02% showed that the inhibitory effect increases with a longer duration of action. The degree of inhibition depends on the concentration of the inhibitor and decreases by more than 60% in the first 45 min of incubation (Fig. 5).

L-rhamnose added to the reaction mixture (final concentration 1 mM) prior to addition of substance **4** exhibits an insignificant protective effect on the *E. erubescens* enzyme molecule (Fig. 6). Given the low protective effect and the fact that compound **4** by its nature cannot compete with the product or substrate of this enzyme, it can be assumed that the complex compound binds to groups located outside the active site of the *E. erubescens* enzyme.

The inhibitory effect of substance **4** increases with the addition of L-cysteine before the introduction of **4**. It was found that with an exposure time of 15 min, the inhibitory effect of substance **4** increases (from 78% to 55%). After a 30 min exposure, the activity in the presence of cysteine was 27% at 0.01% of substance **4** and 27% at 0.02% of substance **4**. At an exposure of 45 minutes, the activity in the presence of cysteine was 25% at 0.01% and 0.02% concentrations of substance **4**. At 60 min exposure, the activity in the presence of cysteine was only 6% at 0.01 and 0.02% concentrations of substance **4** (Fig. 7).

Dialysis of the *E. erubescens* α-L-rhamnosidase preparation inhibited by substance 4 against 0.01 M phosphate-citrate buffer (PCB), pH 5.2 during the day did not lead to the restoration of the enzyme activity.

Discussion

α-L-Rhamnosidases remove L-rhamnose residues from various rhamnose-containing glycoconjugates, which are widely distributed in plants and bacteria (pectins, O-antigens of pathogenic bacteria, flavonoids, terpenes, rhamnolipids) [13]. For many years, α-L-rhamnosidases have been used as biocatalysts in many industrial processes: in the food industry for the production of fruit juices, for improving wine aromas by deglycosylationg terpenes, in the pharmaceutical industry for derhamnosylation of flavonoids, which are used to treat a number of diseases. At the same time, it was shown that monoglycosylated compounds have better bioavailability than their disaccharide counterparts [14, 15]. A number of authors have shown that prunin, a derhamnosylated product of naringin, has a strong effect on the inhibition of enzyme systems associated with dia-

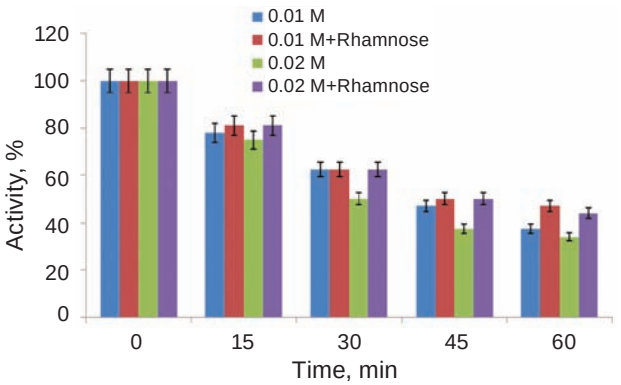


*Fig. 6. Effect of L-rhamnose on inhibition of E. erubescens α-L-rhamnosidase by substance **4***

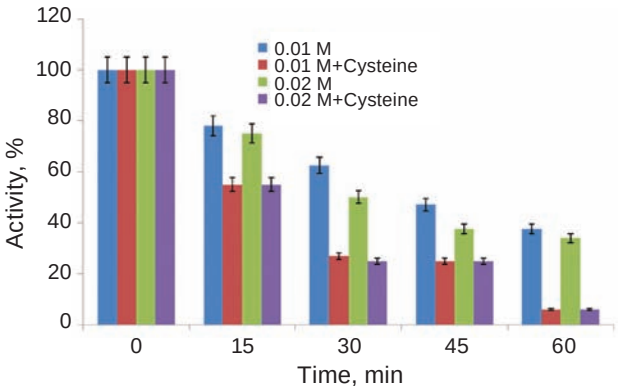


*Fig. 7. Effect of L-cysteine on the inhibition of E. erubescens α-L-rhamnosidase by substance **4***

betes [16, 17]; diosmetin 7-O-glucoside has hepatoprotective, as well as antioxidant, antiarrhythmic and anticomplementary effects [18]; quercetin 7-O-glucopyranoside has been shown to be effective in inhibiting the viral RNA polymerase of influenza A and B viruses [19]; hesperetin 7-O-glucoside inhibits the growth of *Helicobacter pylori,* the causative agent of gastric diseases [20]. Unfortunately, most of them are not widely distributed in nature or are difficult to isolate from plant sources, and their chemical synthesis remains laborious, which, with all the ensuing consequences, leads to expensive natural compounds. That is why, one of the effective methods of large-scale production of such compounds is the use of enzymatic biotransformation of natural glycosides of flavonoids by glycosidases, including α-L-rhamnosidases of microorganisms.

Chemical modification of technologically important enzymes makes it possible to obtain drugs of long-term and repeated use, but the complexity of the structure of proteins does not allow us to offer universal approaches to their stabilization. The different availability of reactive groups determines the need for an individual approach to establishing the influence of effectors on catalytic activity. The conducted studies testify to the peculiarities of the influence of compounds on the activity of α-L-rhamnosidases of various producers. Regarding strains of *P. tardum* and *P. restrictum,* the compounds showed themselves as activators and inhibitors when exposed for 1 h, and only as activators when the time was increased to 24 h. At the same time, $[Ni(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ (**3**) had the most pronounced activating effect for all strains. Complexes **1-5** contain the same complex anions and differ only in the d-metal in the cation, so the high activity of compound **3** is due to the presence of nickel in its composition. Nickel-containing compounds with tartrate, xylaratogermanate anions also proved to be the best enzyme effectors in previous studies [7, 8, 21]. The effect of complexes **1-5** on α-L-rhamnosidase of *E. erubescens* turned out to be more diverse: at a low concentration of 0.01-0.02%, they inhibited the enzyme, in some cases – completely, and activated it when it increased to 0.1%. Compound **6** proved to be a powerful α-L-rhamnosidase inhibitor under any conditions. The change in the nature and intensity of the action of the selected compounds depending on the concentration and duration of the experiment indicates the complexity of the modification mechanisms in which they participate. As mentioned above, the efficiency of the enzyme depends, first of all, on the nature of the producer and the molecule of the complex as a whole.

Based on literature data and regularities revealed as a result of the study of the influence of coordination cation-anion malatostannates (germanates) on α-L-rhamnosidases, the mechanisms of their activating and inhibiting action are proposed. It can be assumed that the used effectors act as allosteric modulators that change the conformation of the enzyme and increase its activity due to binding to reaction centers located not far from the active center. Probably, the complexes are able to form the intermediate "enzyme (E)-activator (A)-substrate (S)", which increases the activity of α-L-rhamnosidase, or vice versa, inactivates it. Noncompetitive inhibition takes place, in which the effector prevents the formation of an active complex (E-S) due to conformational changes in the protein. Malatostannates/germanates **1-6** are reversible inhibitors for the *P. tardum* enzyme – their complexes with the enzyme disintegrate over time, and irreversible – for *E. erubescens* and increase their effect with increasing exposure up to 24 h. The irreversibility of inactivation of *E. erubescens* α-L-rhamnosidase is also evidenced by dialysis data: no recovery of activity was observed after 24 h of enzyme dialysis against 0.1 M PCB. The given mechanism of enzyme activation due to its conformational change is confirmed by previous studies of the UV spectra of similar xylaratogermanates, which additionally increase the thermal stability of the considered α-L-rhamnosidases as a result of a change in the $n\rightarrow\pi^*$ interaction of protein chains [21].

The influence of the central metal atom in the complex malatostannate **1-5**/germanate **6** anion on the effectiveness of the considered supramolecular salts was traced using the example of similar complexes $[Co(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ (**2**), $[Co(phen)_3]_2[Ge(HMal)(Mal)_2]\cdot 14H_2O$ (**6**). Their effect on α-L-rhamnosidase of *P. tardum* is almost the same, while for *E. erubescens* compound **6** is a powerful inhibitor that completely inactivates the enzyme at both concentrations at 24-hour exposure. However, the state-containing complex **2** in a concentration of 0.01% inhibits the activity of the enzyme, and in 0.1% – activates it by 150%. The bulkier malatostannate anion at a high concentration undergoes steric hindrance when binding to the enzyme, similar to the mechanism described

in the literature [22]. At the same time, the compound continues to interact with the center "responsible" for the activation of α-L-rhamnosidases and forms the necessary intermediate A-E-S complex. Thus, the investigated supramolecular coordination compounds **1-6** with malatostannate/germanate anions and 3d-metal cations with 1,10-phenanthroline, as well as the previously considered xylarato-, tartratestannates/germanates, are effectors of α-L-rhamnosidases of *P. tardum, P. restrictum, E. erubescens.* The diversity of their influence under variable conditions is explained by the peculiarities of the structure of complex compounds (metals in cations and anions, the number of protonated carboxyl groups, an additional chloride anion in malatostannates), as well as the variability of the reaction centers in α-L-rhamnosidases molecules and the peculiarities of electrostatic interactions between the effector and the enzyme. Studies of tartratostannates consisting of the same octahedral cations of divalent Fe, Co, Ni, Cu, Zn with 1,10-phenanthroline $[M(phen)_3]^{2+}$ and isostructural complex anions $[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]^{4-}$ showed [21] that all of them are activators of α-L-rhamnosidases and α-galactosidases. It was established that their effectiveness is mainly influenced by complex cations and the presence of a large number of water molecules in crystal hydrates, which strengthen the structure of the intermediate complex "effector-enzyme-substrate" and change its conformation. The study of the UV spectra of *P. restrictum* α-L-rhamnosidases under the influence of xylaratogermanates made it possible to establish that the reactive binding groups in the enzyme are the aromatic residues of tryptophan, tyrosine, and phenylalanine, which participate in the formation of π-stacking interactions with phenanthroline rings of complex cations. The study of thermal stability showed that such conformational changes in the intermediate complex increase the thermal stability of the enzyme [21].

Thus, changes in the catalytic activity of α-L-rhamnosidases with the use of complex compounds of different structures can be due to the stabilization of the structure by their electrostatic interactions, π-stacking, hydrogen bonds, etc. of the protein with the components of the studied compounds – individual cations and anions, free carboxyl groups of ligands, water molecules. Therefore, the obtained results expand the concept of enzyme activators and inhibitors among compounds of a new type and are aimed at solving the general issue of enzyme regulation, which is important from the perspective of their use in modern biotechnological processes.

Thus, coordination compounds **1-6** are currently of special scientific and practical interest. It is most likely that compounds capable of exerting an effector action on the activity of α-L-rhamnosidases of *P. tardum* and *E. erubescens* will find wide practical application.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВПЛИВ КООРДИНАЦІЙНИХ СПОЛУК ІЗ МАЛАТОГЕРМАНАТ/СТАНАТНИМИ АНІОНАМИ ТА КАТІОНАМИ 1,10-ФЕНАНТРОЛІНУ 3D-МЕТАЛІВ НА α-L-РАМНОЗИДАЗНУ АКТИВНІСТЬ *PENICILLIUM TARDUM, PENICILLIUM RESTRICTUM, EUPENICILLIUM ERUBESCENS*

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Пошук ефекторів, здатних впливати на каталітичну активність ензимів, є важливим напрямком сучасної ензимології. Метою дослідження було дослідити здатність 6 координаційних сполук з аніонами малатогерманату/станату та катіонами 1,10-фенантроліну 3d-металів модифікувати α-L-рамнозидазну активність штамів *Penicillium tardum, Penicillium restrictum* та *Eupenicillium erubescens*. Активність α-L-рамнозидази визначали методом Девіса з використанням нарингіну як субстрату. Встановлено,

що 0,1% $[Ni(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\bullet 14H_2O$ найбільш виражено активував α-L-рамнозидазу усіх досліджуваних штамів. Показано неконкурентне інгібування α-L-рамнозидази *E. erubescens* $[Cu(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\bullet 10H_2O$. Отримані результати розширюють уявлення про можливі активатори та інгібітори глікозидаз та вказують на перспективність їх використання в сучасних біотехнологічних процесах.

Ключові слова: α-L-рамнозидаза, подвійні координаційні сполуки, германій(IV), станум(IV), d-метали, *Penicillium tardum, Penicillium restrictum, Eupenicillium erubescens.*

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