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Development of functional materials with specific activities for degradation of toxins

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Abstract. It was shown that it is possible to use different samples of hexahistidine-containing organophosphate hydrolase (His_6-OPH)-based enzyme preparations, including those obtained in the frame of functionalized materials, for the successful detoxification of organophosphorus compound such as Paraoxon and different mycotoxins (Zearalenone, Patulin, Sterigmatocystin) in their present in contaminated agricultural commodities. For the first time it was demonstrated that different toxins (xenobiotics and toxic compounds naturally produced by microscopic fungus) can be hydrolysed by the same enzyme and various biocatalysts prepared on the base of it use.

1. Introduction

Agricultural products can be contaminated with various xenobiotics, in particular, organophosphate pesticides, which are actively used at different stages of production and preservation of the crop, as well as natural toxicants, such as mycotoxins (McT), which produce various microscopic fungous cells that affect agricultural products [1]. These natural and synthetic toxins (organophosphorus compounds, OPC) cause significant harm to human and animal health, consuming agricultural products that contain them or having regular contact with various raw materials containing these substances to be stored or processed. The main problem is not the once consumed doses of these toxicants, causing acute poisoning, but regular consumption of these substances in small quantities, which leads to the formation of a cumulative effect and the development of chronic neurodegenerative diseases, nephropathy, hepatopathy, oncology.

To neutralize these toxicants, various means are being developed and applied in practice, but the most interesting and promising are combined functional materials that allow not only to ad(b)sorb toxic compounds from the mass (or surface) of agricultural raw materials or products, but also to protect them and catalyze their degradation. Since then, these materials should contain, first of all, various sorbents, including those presented in the form of fibrous materials, possessing enzymatic activity aimed at the destruction of McT or organophosphorus pesticides widely used in agriculture. Moreover, such materials are functionalized, as a result of imparting to them certain special catalytic characteristics.

It should be noted that the microbiologically confirmed absence of agricultural raw materials or the production of microscopic fungi does not mean that the object under study does not contain McT. So filamentous fungi can die during the processing of grain or other agricultural raw materials, and McT can retain their presence, since they are more resistant to chemical and heat treatments (melting point

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of zearalenone is 165°C, ochratoxins - 169–221°C, aflatoxins - 244–299°C, trichothecenes - 150–190°C). However, for people servicing storage and production facilities in which they come in contact with contaminated agricultural raw materials containing McT, protection from exposure to these substances is necessary, which can be implemented at the transdermal and inhalation levels, as well as *in vivo*.

There are no safe concentrations of MCT, since even a small amount of them has a negative effect on the animal's body while in cereal feeds, while they can accumulate in animal products (meat, milk, eggs), creating a trophic threat to human health. At the same time, even very low concentrations of McT can affect the state of health, since they "cooperate" with bacterial infections and the effects of xenobiotics, in particular, organophosphate pesticides, and thereby increase the overall negative effect. In addition, toxins reduce the body's resistance (suppress immunity), which increases its susceptibility to various diseases. Therefore, early and effective control of McT as well as defense against McT as well as against OPC is essential for animal health and the profitability of agricultural production.

However, there is another important problem associated with the accumulation of McT in agricultural products, which is that often in the feed there is the presence of several types of McT, which is due to the fact that each microscopic fungus can produce several toxins simultaneously. In this regard, in order to create effective means of neutralizing McT, it is necessary to select "maximally universal" enzymes that allow them to be used for the destruction of the widest possible range of McT.

As for the enzymatic and therefore environmentally friendly decomposition of organophosphorus pesticides in the same agricultural raw materials or products, here the selection of enzymes for such processing also involves the use of biocatalysts with the broadest substrate specificity [2]. The most interesting are the development of functional materials that allow decomposition of both McT and organophosphate pesticides.

The purpose of this work was to show that such biocatalytic functional materials can be obtained with respect to degradation of McT and OPC happening within contact or exposure of various agricultural raw materials with such biocatalytically active samples of materials. It was nesessary to show how it can lead to removal of McT and OPC from raw materials. It was decided to add additional functionality to these materials due to the presence of specially introduced stabilized forms of hexahistidine-containing organophosphate hydrolase (His_6-OPH), catalyzing not only the hydrolysis of various OPC, but also the McT [3-5]. The most effective way to stabilize this enzyme to neutralize these toxins by obtaining of non-covalent polyelectrolyte complexes of the His_6-OPH with polyaspartic acid was chosen previously [6], and was used in this work, taking into account the previously obtained positive results.

2. Materials and Methods

2.1. Contaminated objects for investigation

Agricultural raw materials (haylage, silage, feed grain, cake, pulp, meal) and feed (grain mixture, feed, extrudates), with the exception of flour (meat and bone, feather, fish) were prepared for the study as follows: dry substrates and substrates with low humidity thoroughly mixed and ground to 1 mm, silage, pulp and meal were ground with a knife and thoroughly mixed, flour was used without preliminary preparation. A sample of each of the feedstock was mixed with 1 mM Paraoxon solution to a final moisture content of 75 ± 5%. The obtained samples of raw materials were brought into contact with samples of functionalized materials containing enzyme or enzyme complexes, which were then exposed for 24 hours at room temperature.

2.2. Obtaining of biocatalytically functionalized materials

Recombinant cells of *Escherichia coli* SG13009[pREP4] were cultivated, and the enzyme was isolated and purified by methods published previously [7]. The purified preparation of His_6-OPH was characterized by enzymatic activity as described earlier [8]. The concentration of protein was determined by Bradford assay, and protein purity was analyzed by SDS-PAGE in 12% polyacrylamide

gel using Mini-PROTEAN II cell (Bio-Rad, Hercules, CA, USA) followed by Coomassie Blue (R-250) staining. According to SDS-PAGE (data not shown), the purity of His_6-OPH dimer preparation obtained (MW ≈ 73 $kg \cdot mol^{-1}$) was ca. 98%. The specific organophosphorus hydrolase activity of the purified enzyme was 4,300 $U \cdot mg^{-1}$. One unit of enzymatic activity (U) was defined as the quantity of the enzyme necessary to hydrolyze 1 μmol of Paraoxon per min at 25°C.

Enzyme-polyelectrolyte complexes (EPC) were produced similarly to the known procedure [9, 10]. In brief, to produce an EPC of the enzyme with polyasparaginic acid (His_6-OPH/PLD_{50}), an aliquot of 20 $g \cdot L^{-1}$ PLD_{50} water solution was added to the purified His_6-OPH (1.6 ± 0.1 $g \cdot L^{-1}$) in 50 mM phosphate buffer (pH 7.5) containing 150 mM NaCl. The aliquot volume was calculated so that "enzyme:polymer" molar ratio was 1:5. Next, the mixture was held for 30 min at 8°C.

The organophosphorus hydrolase activity was determined as described earlier [8] with 7.8 mM aqueous Paraoxon stock solution at 405 nm using the Agilent 8453 UV-visible spectroscopy system (Agilent Technology, Waldbronn, Germany) equipped with a thermostatted analytical cell. The reaction was carried out in a 0.1 M carbonate buffer (pH 10.5).

Diatomite (Kvant, Russia), DVUM-carbon-containing fibrous material (Russia) and bacterial cellulose obtained according to a previously developed and published procedure [11] were chosen for biocatalytic fictionalization using the enzyme and its EPC.

3. Results and discussion

To demonstrate the feasibility of detoxification of OPC in agricultural raw materials and feed, specially prepared samples of various materials containing His_6-OPH or its EPC as stabilized forms of the enzyme were used.

To determine the effectiveness of detoxification of OPC, which was carried out for 1 day at room temperature under the action of biocatalytically functionalized materials and different active forms of the enzyme, extraction of OPC (as which Paraoxon was applied) from the obtained samples was carried out and the residual amounts of the contaminant were determined (Table 1).

Table 1. The effectiveness of the destruction of OPC in contact with powdered feed and agricultural raw materials under the action of the created samples of biocatalytically functionalized materials

Raw material	Residual concentration of Paraoxon, %				
	His_6-OPH	His_6-OPH/PLD_{50}	His_6-OPH with diatomite	His_6-OPH/PLD_{50} with bacterial cellulose	His_6-OPH/PLD_{50} with carbon containing fibrous material
Haylage	29.5 ± 1.1	12.2 ± 0.5	16.7 ± 0.2	19.7 ± 0.4	11.7 ± 0.6
Silage	22.2 ± 0.8	13.7 ± 0.5	17.2 ± 0.3	18.6 ± 0.6	10.3 ± 0.2
Coarse grains	24.6 ± 0.9	14.6 ± 0.4	16.4 ± 0.2	17.4 ±0.3	10.2 ±0.5
Bagasse	19.8 ± 0.8	12.8 ± 0.3	19.8 ± 0.3	16.8± 0.5	9.6 ± 0.5
Beet pulp	29.4 ± 1.1	13.2 ± 0.3	19.7 ± 0.3	15.6 ± 0.5	9.8 ± 0.6
Grist	22.5 ± 0.8	12.5 ± 0.3	15.5 ± 0.2	18.3 ± 0.3	9.3 ± 0.4
Grain mixture	24.2± 0.9	12.7 ± 0.3	14.6± 0.2	18.8 ± 0.4	8.8 ± 0.4
Mixed feed	29.1 ± 1.1	10.1 ± 0.3	19.1 ± 0.3	19.0 ± 0.6	9.0 ± 0.5
Extrudate	27.3 ± 0.9	12.0 ± 0.3	17.0 ± 0.3	18.2 ± 0.3	12.0 ± 0.4
Meet and bone meal	25.0 ± 0.8	17.8 ± 0.6	15.8 ± 0.2	21.3 ± 0.7	8.8 ± 0.3
Feather meal	26.8 ± 0.9	16.8 ± 0.5	18.6 ± 0.3	20.2 ± 0.4	9.3 ± 0.5
Fish meal	20.8 ± 0.7	10.6 ± 0.3	19.1 ± 0.3	19.3 ± 0.3	9.8 ± 0.4

It was found that even taking into account the obtained initial results of the experiments without any optimization of the conditions for the detoxification of OPC, as well as the applied enzyme

activity, we can say that the enzyme, as well as its EPC with polyaspartic acid, has a high catalytic activity, which allows to count on the creation of new functionalized materials with special properties that form protection from exposure to toxicants.

Further, various biocatalytically active samples prepared on the basis of His_6-OPH, allowing to maintain the stability of the enzyme action, were used to remove McT from different types of raw materials and types of animal feeds (Table 2).

It should be noted that there is a known method for bioprotection of zearalenone (43.4 mg L^{-1}) in Tris-HCl buffer (pH 7.5) under the action of highly purified polyhistidine-containing recombinant lactonase, taken at a concentration of 1.2 mg L^{-1}, which allows to reach 100% efficiency of the mycotoxin decomposition within 38 hours [12]. The use of recombinant lactonase for the application of this method in practice demands the production of the enzyme in the required quantity and purification degree to the maximum level of catalytic activity, but the procedure is not optimized yet. However, a significant disadvantage of this method is the low rate of hydrolysis of Zearalenone (1.142 mg L^{-1} h^{-1}) and the possibility of using this enzyme method only for the hydrolysis of one mycotoxin, Zearalenone.

Table 2. Destruction of McT under the action of various forms of His_6-OPH

Raw material or fodder	Mycotoxin (concentration)	Catalytically active sample tested in the work	Period of 100%-decomposition, h
Wheat	Zearalenone (300 mg kg^{-1})	His_6-OPH	3
Meet and bone meal	Sterigmatocystin (100 mg kg^{-1})	His_6-OPH/PLD_{50} with diatomite	2
Grain mixture	Patulin (2 mg kg^{-1})	His_6-OPH/PLD_{50} with bacterial cellulose	1

In comparison with this method, the results achieved here are unique because:

- the possibility of carrying out the enzymatic biological neutralization of McT present in media with a complex chemical composition, and not only in the form of pure substances, was shown,
- a significant increase in the rate of effective decomposition of McT is shown while reducing the time required for their 100% decomposition (only in comparison with a pure substance - Zearalenone - an increase in the decomposition rate by an order of magnitude).

It should be noted that oxidative enzymes tested for the decomposition of McT, in particular, oxidoreductases (laccase, cytochrome P-450, F420-dependent oxidoreductase, Mn-containing peroxidase) [13, 14] provided a rather high rate of neutralization McT. For example, a maximum (90%) degradation of McT was achieved in 48 hours at a concentration of 312 mg L^{-1}, and the rate of decomposition of McT was 5.85 mg L^{-1} h^{-1}[15]. However, these methods of enzymatic oxidative decomposition of McT have a number of significant drawbacks: instability of the used enzymes, requirements for the special conditions of their storage and use in methods of McT detoxifying (use of additional expensive redox-mediators, introduction of hydrogen peroxide to the environment, the need to ensure elevated oxygen concentrations in media containing McT, etc.), and others. However, their main disadvantage is that as a result of the use of these oxidative enzymes, products are not less, but sometimes more toxic than the original McT [16, 17].

The results achieved herein enable a highly efficient biocatalytic detoxification of not one, but several different McT having a lactone ring in their molecular structures, using the same enzyme His_6-OPH, due to which the solution of the problem of decomposition and detoxification of McT produced by different filamentous fungi can be significantly simplified in practice.

4. Conclusion

Thus, it was shown that it is possible in principle to use different enzyme preparations based on His6-OPH, including in the composition of fibrous functionalized materials, for the detoxification of OPC

using the example of paraoxon and several McT, which are often present in agricultural raw materials. In addition, for the first time, the possibility of decomposition of individual McT, in particular, Patulin and Sterigmatocystin, for which similar enzymatic decomposition has never been described anywhere else, has been shown. These results can solve the significant environmental and economic problem associated with the disposal of McT and an increase in the level of safe contact for the storage and processing of such raw materials.

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References

[1] Jard G, Liboz T, Mathieu F, Guyonvarc'h A, and Lebrihi A 2011. Review of mycotoxin reduction in food and feed: from prevention in the field to detoxification by adsorption or transformation. *Food Addit Contam Part A*, **28(11)** 1590-1609.
[2] Efremenko E N, Lyagin I V, Aslanli A G and Makhlis T A 2017 The method of bioneutralization of mycotoxins. Ru Patent 2634914
[3] Efremenko E N, Senko O V and Maslova O V 2017 Optimization of the use of His_6-OPH-based enzymatic biocatalysts for the destruction of chlorpyrifos in soil. *Int. J. Environ. Res. Public Health.* **14** 1438.
[4] Efremenko E N, Lyagin I V, Makhlis T A, Maslova O V, Senko O.V., Stepanov N A 2017 Enzymatic detoxification of mycotoxins: known and promising solutions. *Acta Naturae* **special issue** 124-125.
[5] Maslova O V, Stepanov N A, Grigoryeva A I, Bruyako M G, Efremenko E N 2016 New effective His6-OPH-containing mineral carriers pretreated by low-temperature plasma for destruction of organophosphates in different types of soil. *IJPT* **8(4)** 27317-27333.
[6] Maslova O V, Senko O V and Efremenko E N 2018 The influence of enzymatic removal of chlorpyrifos from feed grain mixes on biochemical parameters of rat blood. *Biochem (Mocs) Suppl Ser B* **12(2)** 181–185.
[7] Efremenko E, Votchitseva Y, Plieva F, Galaev I and Mattiasson B 2006 Purification of His_6-organophosphate hydrolase using monolithic supermacroporous polyacrylamide cryogels developed for immobilized metal affinity chromatography. *Appl. Microbiol. Biotechnol*. **70**, 558–563.
[8] Votchitseva Y A, Efremenko E N, Aliev T K and Varfolomeyev S D. 2006 Properties of hexahistidine-tagged organophosphate hydrolase. *Biochemistry (Moscow)* **71** 167–172.
[9] Efremenko E N, Lyagin I V, Klyachko N L, Bronich T, Zavyalova N V, Jiang Y and Kabanov A V 2017 A simple and highly effective catalytic nanozyme scavenger for organophosphorus neurotoxins. *J. Control. Release* **247** 175–181.
[10] Lyagin I V and Efremenko E N 2018. Biomolecular engineering of biocatalysts hydrolyzing neurotoxic organophosphates. *Biochimie* **144** 115–121.
[11] Stepanov N and Efremenko E 2018 "Deceived" concentrated immobilized cells as biocatalyst for intensive bacterial cellulose production from various sources *Catalysts* **8** 1–16.
[12] Vekiru E, Fruhauf S, Hametner C, Schatzmayr G, Krska1 R, Moll W D and Schuhmacher R 2016 Isolation and characterisation of enzymatic zearalenone hydrolysis reaction products *World Mycotoxin J*. **9(3)** 353-363.
[13] Wu Y Z, Lu F P, Jiang H L, Tan C P, Yao D S, Xie C F and Liu D L 2015 The furofuran-ring selectivity, hydrogen peroxide production and low Km value are the three elements for highly effective detoxification of aflatoxin oxidase. *Food Chem. Toxicol*. **76** 125-131
[14] Alberts J F, Gelderblom W C A, Botha A and van Zyl W H 2009 Degradation of aflatoxin B1 by fungal laccase enzymes Int. J. Food Microbiol **135** 47–52.

[15] Yehia R S 2014 Aflatoxin detoxification by manganese peroxidase purified from *Pleurotus ostreatus Braz. J. Microbiol*. **45(1)** 127-133.
[16] Zeinvand-Lorestani H, Sabzevari O, Setayesh N, Amini M, Nili-Ahmadabad A and Aramarzi M 2015 Comparative study of in vitro prooxidative properties and genotoxicity induced by aflatoxin B1 and its laccase-mediated detoxification products *Chemosphere* **135** 1–6.
[17] Karlovsky P 1999 Biological detoxification of fungal toxins and its use in plant breeding, feed and food production *Nat. Toxins* **7(1)** 1-23.