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Advanced Biocatalysts Based on Hexahistidine-Containing Organophosphorus Hydrolase for Chemical and Biological Defense

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The advanced biocatalysts based on hexahistidine-tagged organophosphorus hydrolase (His_6-OPH) were recently developed for the detoxification of various organophosphorus compounds and degradation of N-acyl homoserine lactones. Due to enzyme immobilization, some of obtained biocatalysts are quite stable, easy to use and very effective/active (e.g. tens of millions of substrate solution volumes appeared to be treated with column cartridges containing immobilized His_6-OPH). Recently, the possible bioengineering of different stabilized nanocomplexes of His_6-OPH due to its non-covalent binding with different compounds (polymers, antioxidants, antimicrobials, etc.) was demonstrated. Firstly, it was realized by computer modeling via molecular docking. Polymers of amino acids (polyglutamic and polyasparctic acids) were established to be the most effective stabilizers of the enzyme that enabled effective preservation of the enzyme activity. Up to 100 %-retention of initial catalytic characteristics of the enzyme was reached in obtained enzymatic complexes. Such nanobiocatalysts were stabilized against inactivating effects of solvents, temperatures and were able to circulate in vivo for at least 25 hours. It appeared that different antioxidants can be applied as partners of the enzyme in the nanocomplexing. Thus, a new set of original enzymatic antidotes were developed possessing dual action: both hydrolytic activity against organophosphorus neurotoxins and improved antioxidant activity. Additionally, it was shown that different organophosphorus compounds and N-acyl homoserine lactones could be molecularly docked directly to the active centers of His_6-OPH dimer, thus allowing to theoretically clarify some new prospective substrates for the enzymatic hydrolysis. It appeared that new type of nanocomplexes of the enzyme with antibiotics also can be prepared. In this case the combination of antibiotics with enzyme quenching the quorum of the pathogenic gram-negative bacteria was performed. The enzyme being stabilized by the various antibiotics (especially those containing β-lactame ring) played the role of a carrier for the antimicrobial compounds significantly improving their efficiency of the action. Such biocatalysts and/or method of their design have a great potential and can be very useful for both chemical and biological defense.

Keywords: *antibiotics; antioxidants; hydrolysis; N-acyl homoserine lactones; nanocomplexes; organophosphorous hydrolase; organophosphorus compounds.*

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In the modern world, the possible effective decomposition of neurotoxic organophosphorus compounds (OPCs) is of great importance. OPCs include chemical warfare agents (CWAs) such as Sarin, Soman, Vx, which were destroyed in the Russian Federation (but are still in the process elsewhere), as well as agricultural pesticides (Coumaphos, Methylparathion, Malathion, Chlorpyrifos, Diazinon, etc.). The need for storing, transporting and disposing of OPCs, and in the case of pesticides, their use in hundreds of thousands tons annually, requires to develop and apply modern safe and environmentally friendly technologies.

To decompose various OPCs, several biocatalytic technologies based on organophosphorus hydrolase (OPH) and its genetically modified analogues [1] have been developed. In terms of activity, some of these enzyme derivatives, e.g. hexahistidine containing OPH (His_6-OPH), appeared to be catalytically better in orders of magnitude and can be expediently used as a basis for development of multiple biocatalysts.

The technology for enzyme application has been developed for decomposition of CWAs in the form of pure substances [2], as well as in the reaction masses after chemical destruction of CWAs by

different formulations [3]. This technology is quite simple (just mixing and exposing) and effective. When it was combined with microbial destruction of enzymatic decomposition products, in particular, methylphosphonic acid [4], wastewater acceptable for dumping into the city sewage system could be obtained. This design still has no analogues in the world.

Immobilization of His_6-OPH on various carriers allowed to produce a wide range of preparations for OPCs detoxification. So, when His_6-OPH was immobilized on a polyacrylamide cryogel modified with iminodiacetic acid and charged with divalent metal ions, biocatalytically active column cartridges were obtained to totally degrade various OPCs in flow systems [5]. This technology is also simple and reliable. The enzyme binds strongly to the carrier and can be regenerated in a controllable manner though it is quite effective. For example, 1 ml of biocatalyst can detoxify up to 10.8 m^3 of wastewater contaminated with 10 μM Paraoxon for a half-life period.

His_6-OPH was immobilized on environmentally friendly carriers being agricultural scraps to clean up soil contaminated by OPCs [6]. Thus, on the one hand, the important problem of soil decontamination is simply solved, and on the other hand, these carriers act as soil structurizers, as well as additional organic fertilizers. The maximum efficiency was observed with wheat straw resulting in complete degradation of 630 mg/kg of Paraoxon for 7 days by 300 U/kg of biocatalyst.

OPH was immobilized within a fabric-based chitosan gel in another interesting work to detoxify various surfaces contaminated by OPCs [7]. Due to covalent coupling to carrier by glutaraldehyde, such biocatalysts have been stable for at least 6 months.

Several biocatalytic technologies have been developed to be used as protective materials. E.g., a multilayer protective material can contain the following layers: 1) polyamide cotton fabric with a polyfluoroolefin or polyurethane membrane having oleophilic properties; 2) the middle layer for sorption and self-degassing with His_6-OPH immobilized within a polyacrylate gel; 3) bottom (hygienic) layer of woven or non-woven cellulose material [8]. Such material effectively prevents penetration of toxic substances like VX for a long time and is very promising.

Recently, completely different biocatalytic systems have been developed for *in vivo* protection against poisoning by OPCs [9]. The use of self-assembly of nanosized complexes of enzyme with a block copolymer is expected for this purpose, and the procedure of their production is extremely simple. Such nanobiocatalysts are stabilized against inactivating effects of solvents, temperatures, etc. and are able to circulate *in vivo* for at least 25 hours. These preparations have high activity towards various OPCs *in vitro* and *in vivo*, and can be improved even further [10, 11].

Several representative examples of enzyme nanocomplexes [12–14] and their catalytic characteristics are listed in Table 1.

Such nanocomplexes can be formed with a number of charged polymers [9–14] and have high enough catalytic activity. Moreover, interaction of His_6-OPH with antioxidants [15, 16], antimicrobial agents [14, 17–19] and other chemicals can be computationally simulated (Figure 1) and predicted to choose the best one for activity preservation.

Recently it has been shown, that His_6-OPH can be used to eliminate biological threats also. Namely, N-acyl homoserine lactones (AHLs) are known as inducers of Quorum Sensing, and synthesized by most gram-negative pathogenic bacteria. Quorum Sensing improves resistance of microorganisms to the action of various antimicrobials. His_6-OPH was shown to possess the lactonase activity towards various AHLs

Table 1 – *Catalytic characteristics of free His_6-OPH and its nanosized complexes with various "partners" (determination was done in 0.1 M carbonate buffer (pH 10.5) towards Paraoxon)*

Block copolymer	K_m (μM)	V_{max}/e_o (s^{-1})	$V_{max}/(e_o \times K_m)$ (10^6 $M^{-1}s^{-1}$)	Ref.
–	10.0 ± 0.5	5100 ± 100	510 ± 30	[12]
$PEG_{113}PLE_{50}$	16.7 ± 0.9	5005 ± 60	300 ± 20	[13]
PLD_{50}	12.1 ± 1.0	5240 ± 140	434 ± 46	[12]
PLD_{50}/Ampicillin	14.6 ± 1.2	4450 ± 120	305 ± 33	[14]
$PEG_{22}PLE_{50}$	9.8 ± 0.3	4550 ± 50	466 ± 22	[12]
$PLE_{50}PEG_{113}PLE_{50}$	10.8 ± 0.8	4840 ± 110	450 ± 42	[12]

Note. *PEG – polyethylene glycol, PLE – polyglutamic acid, PLD – polyaspartic acid*
K_m – Michaelis constant, V_{max}/e_o – catalytic constant

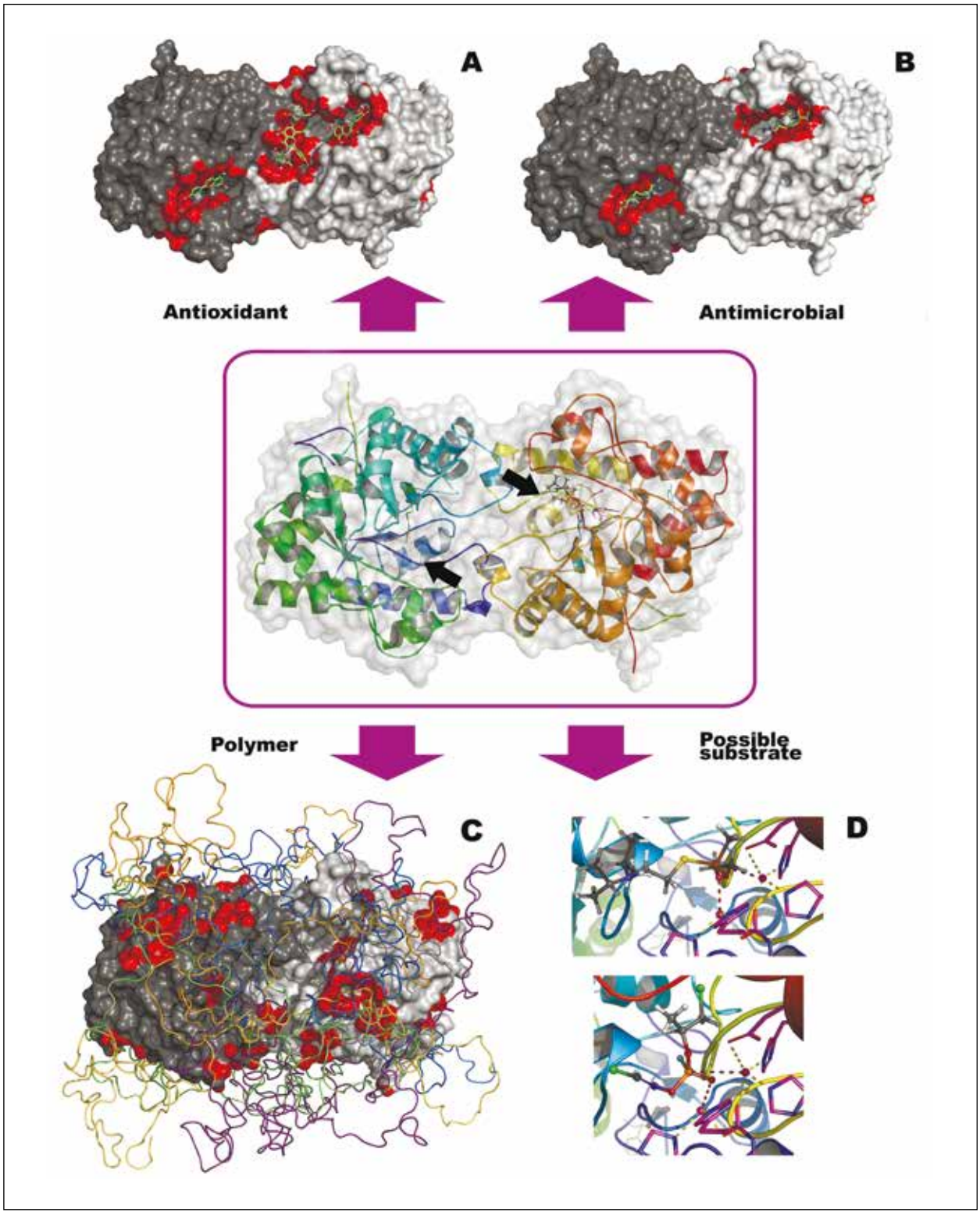

Figure 1 – *Front view of His6-OPH homodimer (in center). General approach has been used to simulate enzyme interaction with antioxidants (A) [16], antimicrobials (B) [14, 19], polymers (C) [12] and possible substrates (D) [22] by molecular docking (two subunits of enzyme are colored by grey and dark grey. The enzyme atoms located within 4 Å of any atom of docked ligand and the corresponding molecular surface, are colored red. The entrances to the active sites of His_6-OPH dimer are marked with black arrows. Binding of two OPCs within active center of His_6-OPH is zoomed (D), and catalytically important amino acid residues are shown with magenta sticks. The most crucial interactions between Co^{2+} ions (purple spheres) of enzyme and substrates' phosphorous (orange sphere) and oxygen (red sphere) atoms are highlighted)*

and could be used for Quorum Quenching [20]. Nanocomplexes of the enzyme can be obtained with various antibiotics so as a number of enzyme forms (complexes with other compounds) can be applied [14, 17–19].

Moreover, an improvement of both catalytic activity and enzyme stability was revealed for the complexes of His_6-OPH with antibiotics. That resulted in improving the antibiotics' efficiency action and significant decrease of minimal inhibiting concentrations of the complexes containing both enzyme and antibiotics towards gram-negative bacteria [19].

Besides, interactions of various chemicals with His_6-OPH can be simulated by the same way to reveal possible enzyme substrates [21, 22]. It allows preliminary predicting not only possible decontamination, but its efficiency also.

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All biocatalysts on the basis of His_6-OPH are characterized by simplicity of obtaining and application and by their environmental friendliness, whereas all of them are highly active towards wide range of OPCs. Therefore they may be interesting to both civilian consumers and specialized services.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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The article has been peer reviewed by two experts in the respective field. Peer reviews are available from the Editorial Board.

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Современные биокатализаторы на основе гексагистидинсодержащей органофосфатгидролазы для химической и биологической защиты

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Разработаны биокатализаторы на основе органофосфатгидролазы, модифицированной полигистидиновой последовательностью (His_6-OPH), предназначенные для детоксикации фосфорорганических соединений (ФОС) и разложения N-ацилгомосеринлактонов. Их создание стало возможным благодаря способности фермента His_6-OPH к нековалентному связыванию с различными веществами (полимерами, антиоксидантами, антимикробными средствами и др.). Это же свойство фермента His_6-OPH позволило получить различные стабилизированные нанокомплексы. Показано, что молекулярный докинг разных ФОС и N-ацилгомосеринлактонов может быть проведен с использованием компьютерного моделирования непосредственно к активным центрам димера His_6-OPH, что позволяет теоретически установить новые субстраты для ферментативного гидролиза. Полученные по разработанной технологии биокатализаторы обладают большой стабильностью в различных условиях окружающей среды. Установлено, что полимеры аминокислот (полиглутаминовая и полиаспаргиновая кислоты) являются наиболее эффективными стабилизаторами фермента His_6-OPH, обеспечивающими максимальное сохранение активности фермента. В полученных ферментативных комплексах достигнуто сохранение первоначальных каталитических характеристик фермента до 100%. Фермент His_6-OPH был иммобилизован на полиакриламидном криогеле, модифицированном остат-

ками иминодиуксусной кислотой и заряженном ионами двухвалентных металлов, что позволило получить биокаталитически активные колоночные картриджи для полной деградации различных ФОС в проточных системах. Разработана технология применения иммобилизированного фермента His_6-ОРН для разложения ФОС в виде чистых веществ, а также в составе реакционных масс, получаемых после химического разрушения отравляющих веществ. Также иммобилизированную His_6-ОРН можно использовать для создания многослойных защитных материалов, эффективно предотвращающих проникновение через них токсичных веществ, таких как VX, в течение длительного времени. Установлено, что нанобиокатализаторы на основе фермента His_6-ОРН обладают антидотными свойствами и способны циркулировать в крови экспериментальных животных по меньшей мере в течение 25 ч. Получены нанокомплексы фермента с соединениями с антиоксидантной активностью, а также с антибиотиками. Наиболее эффективно идет формирование комплекса фермента His_6-ОРН с антибиотиками, содержащими ß-лактамное кольцо. Взаимодействие различных химических веществ с His_6-ОРН может быть компьютерно смоделировано таким образом, чтобы выявить новые возможные каталитически активные комбинации для фермента. Это позволяет предварительно прогнозировать возможность и эффективность использования ферментных биокатализаторов как антидотов или дегазирующих средств в отношении различных ФОС.

Ключевые слова: *антибиотики; антиоксиданты; гидролиз; лактоны N-ацилгомосеринлактоны; нанокомплексы; органофосфатгидролаза; фосфорорганические соединения.*

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Сведения о рецензировании

Статья прошла двойное рецензирование двумя рецензентами, специалистами в данной области. Рецензии находятся в редакции журнала.

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