Design of immobilized biocatalysts for degradation of neurotoxic organophosphorous compounds

I. Lyagin[1,2], R. Ivanov[3], V. Lozinsky[3], E. Efremenko[1,2*]
[1] The N.M. Emanuel Institute for Biochemical Physics, Russian Academy of Sciences, Moscow, Russia
[2] The M.V. Lomonosov Moscow State University, Moscow, Russia
[3] The A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia
E-mail: efremenko@enzyme.chem.msu.ru

Introduction

The organophosphorous compounds (OPC) being derivatives of phosphoric and alkylphosphonic acids are widely used as pesticides in the agriculture and private life (Costa, 2006). The most toxic chemical warfare agents such as Soman, Sarin and Vx, that should be destroyed under "Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction" in the nearest future, are also among OPC of same group (Efremenko, 2001). The low velocities of environmental detoxification of OPC provoke their accumulation presumably in river and ground water.

Organophosphorus hydrolase (OPH, EC 3.1.8.1) is capable of hydrolyzing wide variety of OPC (Efremenko, 2001), thereupon its application in biotechnological decontamination of the compounds is considered to be a base of advanced bioremediation processes.

The immobilization of enzyme is usually carried out to obtain a stable form of OPH for its multiple use in OPC hydrolysis (Efremenko, 2005b; Efremenko, 2007b). The use of various amino acid sequences genetically introduced to OPH structure as ligands for enzyme immobilization recently became very popular as a tool for biocatalyst obtaining (Efremenko, 2005b; Efremenko, 2007a). In general, the use of specific tags of fusion proteins for their immobilization allows to combine the isolation, purification and immobilization of the target protein on carrier in one step (Efremenko, 2006a; Terpe, 2003).

The application of organophosphorus hydrolase (OPH) catalyzing destruction of neurotoxic organophosphorous compounds (OPC) appeared to be very efficient when enzyme is used in an immobilized form. The insertion of polyhistidine sequence containing six or twelve histidine residues at N- or C-terminus of protein molecule was shown to enable combination of effective purification and immobilization of OPH onto metal-chelating carriers. Such immobilization method was established to increase the stability of obtained biocatalysts as compared to soluble forms of OPH derivatives (Efremenko, 2006b).

It is known that effective action of immobilized biocatalyst in the flow systems depends on a catalytic activity of the enzyme and certain properties of carrier (its porosity, reusability, sorption capacity in relation to substrates and products, resistance to mechanical deformation, etc.). These aspects were taken into account, when a new immobilized biocatalyst for the treatment of water polluted by OPC was developed (Efremenko, 2006b) using polypeptide with polyHis-tag and OPH-activity (polyHis-OPH) (Efremenko, 2005b). Cryogel of synthetic polymer such as polyacrylamide (cryoPAAG) (Lozinsky, 2003) obtained as polymerization product with surface modified by metal-chelating ligands and charged by divalent metal ions was applied as a supermacroporous carrier.

Main catalytic and chemical-physical characteristics of new immobilized biocatalysts based on the polyHis-OPH and cryoPAAG in the reaction of OPC hydrolysis in the flow systems were investigated in this work.

Materials and methods

The following chemicals used in the work were purchased from Sigma (St. Louis, MO, USA): O,O-diethyl O-(4-nitrophenyl) phosphate (Paraoxon); imidazole; isopropyl-β-D-thiogalactoside (IPTG);

N,N-dimethylacrylamide (DMAA); *N,N'*-methylene-bis-acrylamide (MBAA); allylglycidile ether (AGE); *N,N,N',N'*-tetramethylethylenediamine (TEMED); cobalt chloride hexahydrate; potassium persulfate. Tryptone and yeast extract were bought from Difco (Detroit, MI, USA). All other chemicals were of analytical grade and purchased from Reachim (Moscow, Russia).
To produce the IDA-cryoPAAG, the water mixter of DMAA and MBAA was degased with Ar. Then, the AGE was added and mixter was cooled to 1-2°C. To iniciate the reaction of polymerization, the TEMED and potassium persulfate were added. The reaction composition was packed into 5-mL syringes, which were frozen at -12°C. After reaction completion the cryogels were modified by iminodiacetic acid and charged by Cu^{2+}-ions The immobilization of polyHis-tagged OPH was conducted according to previously developed method (Efremenko, 2006b).
The investigation of the catalytic characteristics of biocatalyst based on immobilized polyHis-tagged OPH was carried out as it was previously reported (Efremenko, 2005b; Efremenko, 2006b). The accumulation of 4-nitrophenolate anion (p-NP) as product of enzymatic hydrolysis was monitored (25°C, 405 nm) using 8300 Uvicord II spectroscopy system (Pharmacia, Sweden). Aqueous solution of Paraoxon (15 mM) was used as stock solution to investigate the substrate specificity of the biocatalysts. In all cases the catalytic reaction was initiated by pumping of a substrate solution in the 0.1 M Na-carbonate buffer (pH 10.5) through the column filled by immobilized biocatalyst. One unit of enzymatic activity was defined as the quantity of the enzyme necessary to hydrolyze 1 μmol of substrate per 1 min at 25°C.

Results and discussion

The pH-profiles for all immobilized preparations are known to be very similar independently on the length of the tag or the tag attachment site (Efremenko, 2006b), while the ranges of pH-optimum of immobilized biocatalysts action were much wider as compared to soluble native OPH and polyHis-tagged OPH (Efremenko, 2006b; Efremenko, 2007b; Gudkov, 2006). The Na-carbonate buffer (pH 10.5) was used in the reaction of enzymatic hydrolysis of substrate (Paraoxon), since that buffer appeared to guarantee the optimal conditions for N-His_6-OPH, OPH-His_6-C and N-His_{12}-OPH activity in the soluble form.
To investigate the efficiency of action of immobilized biocatalyst, various biocatalysts with different specific activity were prepared (Fig.1). It was established that 90% of 1.1 mM Paraoxon solution can be hydrolyzed under operational conditions by immobilized biocatalyst for 6 min, when it possessed high enough specific activity localized on the immobilizing carrier.
Obviously, the increase in amount of protein immobilized on a carrier could result in the enhahcement of catalytic activity of biocatalyst in general. In our case, the developed cryogels allowed to immobilize the enzymes up to 0.7 mg/mL of carrier. Thus, theoretical specific activity of the immobilized biocatalyst could be 2100 U/mL, whereas we have only one fourth part of it. Therefore, using the biocatalyst with protein loaded to the limit it is possible to hydrolyze even "bad" substrates extensively used in agriculture as pesticides, for example, Malathion, Chlorpyrifos or Coumaphos in the flow-through system.
Comparison of the results obtained with immobilized and soluble forms of polyHis-tagged OPH showed that immobilization procedure caused 2.5-fold decrease in the activity of enzyme.
The dependence of efficiency of biocatalyst action on paraoxon concentration in reaction solution was revealed (Fig.2). The amount of hydrolyzed Paraoxon increased concurrently with increase in substrate concentration. The saturation concentration of Paraoxon for 1990 U biocatalyst was 2.2 mM. At the same time the reduction of substrate conversion degree from 100% (with 0.55 mM Paraoxon) to 41% (with 2.2 mM Paraoxon) was observed.

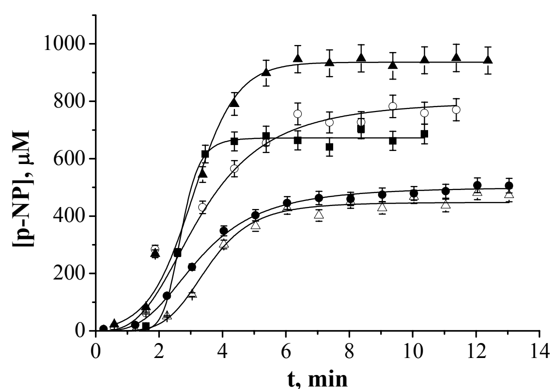

Fig.1. Degradation of 1.1 mM Paraoxon solution at flow rate 1 mL/min by immobilized biocatalysts with different initial specific activity per column: 1020 U (●), 1270 U (Δ), 1990 U (○), 2150 U (■), 2500 U (▲).

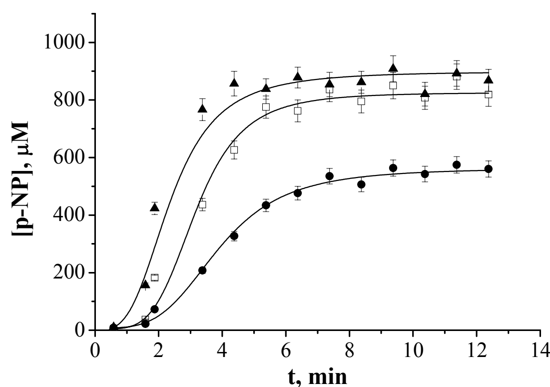

Fig.2. Degradation of 0.55 mM (●), 1.1 mM (□) and 2.2 mM (▲) Paraoxon at flow rate 1 mL/min by immobilized biocatalyst with 1990 U specific activity loaded onto carrier.

Conclusions

Thus, new immobilized biocatalysts based on polyHis-tagged OPH analogues and metal-chelating carrier were obtained. The influence of initial catalytic activity of biocatalyst and substrate concentration on the efficiency of action of immobilized enzyme was revealed in the flow-through system. The possibility of effective hydrolysis of neurotoxic organophosphorous compounds in the flow-through system was shown.

Acknowledgements

This research was financially supported by the *Federal Agency on Science and Innovation of Russian Federation* (Contract No. 02.515.11.5002) and *Russian Foundation of Basic Research* (Grant 07-04-12025_ofi).

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