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Research paper

Biomolecular engineering of biocatalysts hydrolyzing neurotoxic organophosphates

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ABSTRACT

Novel methods of molecular modeling help solving urgent problems in drug design, directed evolution of biocatalysts and biosensors, and a lot of other research fields. Implementation of such methods to organophosphorus hydrolase being perfect research object that hydrolyzes dangerous neurotoxic organophosphates could intensify development of antidote and protective preparations to treat poisoning. Structures of enzyme-polyelectrolyte complexes (EPCs) based on hexahistidine-tagged organophosphorus hydrolase (His_6-OPH) with different biopolymers (various modifications of polyglutamic and polyaspartic acid, as well as hydroxyethyl starch and succinylated gelatin) were simulated at different pH using molecular docking. A number of EPCs with expected "positive" effect on maintaining the maximum level of His_6-OPH activity, and some "negative" options were produced, and their catalytic performance was studied. The theoretical results were experimentally confirmed for four of the six "positive" options. EPCs obtained possessed up to 20–40% higher catalytic efficiency in hydrolysis reactions of Paraoxon and Parathion-methyl as compared with that of the native His_6-OPH. The results obtained may be a good proof of concept for implementation of molecular docking to calculate model complexes of proteins with (bio)polymers of 6.4–105.5 kg/mol. Also, the approach used here could be interesting as alternative or addition to the directed modifications of enzymes to alter their catalytic characteristics.

1. Introduction

Molecular modeling is a powerful tool for solving various problems in chemistry, biology, medicine, etc. Ongoing researches in the field of drug discovery are not conceivable without using computer simulations [1], which development have played a major role in applicability of such methods.

A growing number of studies in the field of biomedicine and biocatalysis somehow utilizes a variety of nanocomposites which can be produced on the basis of (bio)polymers in the form of protein-polyelectrolyte complexes [2]. However, despite obtaining an experimental data with AFM, TEM, DLS, and other techniques, researchers know very little of the actual (or potential) structure of the nanocomposites developed. Meanwhile, the use of an approach that allows a prediction of the structure of such preparations can lead to an intensive (but not extensive, as it is the case now) or even breakthrough development in this area as happened previously with drug discovery. From our point of view, molecular modeling may become such a trigger, and few attempts to apply it to simulate protein-DNA [3], protein-RNA [4], protein-protein [5], and protein-polyelectrolyte [6] complexes are already known.

Recent developments [7] of modern antidote and protective preparations based on hexahistidine-tagged organophosphorus hydrolase (His_6-OPH) [8] to detoxify organophosphorus compounds *in vivo* have shown a promising use for this enzyme in the form of enzyme-polyelectrolyte complexes (EPCs) with PEGylated modifications of polyglutamic acid.

The purpose of this work was to approve the possibility of applying the one of molecular modeling methods (molecular docking) for biomolecular engineering and calculation of EPCs based on His_6-OPH. PEGylated modifications of polyglutamic acid (PEG-PLE), which previously proved to be the most acceptable in the composition of EPCs with His_6-OPH, were used in this study as polyanions. For comparison reason, PEGylated polyaspartic acid (PEG-PLD), as well as non-PEGylated PLE and PLD were tried also. A successful implementation of molecular docking to study the structures of EPCs obtained previously could help to understand

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the results achieved earlier, and allow targeted improvements of the catalytic performance of newly developed similar complexes. Besides, it would make possible to generalize whether this approach is appropriate for solving similar problems.

2. Materials and methods

2.1. Materials

The following polymers were purchased in Alamanda Polymers, USA: PLE_{50}, PLD_{50}, $PEG_{113}PLE_{10}$, $PEG_{113}PLE_{50}$, $PEG_{113}PLE_{100}$, $PEG_{113}PLD_{50}$, $PEG_{22}PLE_{50}$, $PLE_{50}PEG_{113}PLE_{50}$. Hydroxyethyl starch and Gelofusine® (products of B. Braun Melsungen AG, Germany) in the form of 10 wt% and 4 wt% solution, respectively, were acquired in the local pharmacy and were used as received. Phosphorous acid, diethyl 4-nitrophenyl ester (Paraoxon) and phosphorothioic acid, dimethyl 4-nitrophenyl ester (Parathion-methyl) were obtained from Sigma-Aldrich, USA. All other chemicals were of analytical grade and purchased from Chimmed, Russia.

2.2. Cell cultivation and His_6-OPH production

Recombinant *Escherichia coli* strain SG13009[pREP4] (Qiagen, Germany) transformed by plasmid encoding His_6-OPH [9] was used for enzyme production. Cells were cultivated and the enzyme was isolated and purified as it was published previously [10].

The purified His_6-OPH preparation was characterized as described previously [8] by enzymatic activity, concentration of protein determined by Bradford assay, and by protein content analyzed by SDS-PAGE in 12% polyacrylamide gel using Mini-Protean II cell (BioRad, USA) followed by Coomassie Blue (R-250) staining. According to SDS-PAGE data, the purity of His_6-OPH preparation obtained was 98%.

Enzyme-polyelectrolyte complexes of His_6-OPH were produced as described elsewhere [7,11]. Briefly, solutions of His_6-OPH (0.2 mg mL^{-1}) in 50 mM phosphate buffer (pH 7.5) containing 150 mM NaCl, or 0.1 M carbonate buffer (pH 10.5) was mixed with polymer solution at molar ratio “enzyme: polymer” equal to 2:1 and 1:5. The mixture was stirred for 30 min at +8°C and sterile filtered using Millex GP with 200 nm pores filter (Millipore, USA). Effective hydrodynamic diameter of EPCs was determined at 25 °C by DLS using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK) of necessity.

2.3. Determination of enzyme activity

Enzyme activity was determined as described previously [8] with 10 mM aqueous Paraoxon solution or 10 mM Parathion-methyl solution in methanol at 405 nm using Agilent 8453 UV–visible spectroscopy system (Agilent Technology, Germany) equipped with a thermostatted cell. Enzyme concentration (e_o) was 0.1–1 nM in the reaction mixture of 0.1 M carbonate buffer (pH 10.5). One unit of enzyme activity was defined as the quantity of the enzyme necessary to hydrolyse 1 μmol of Paraoxon per min at 25 °C.

The enzymatic reaction rates were calculated using the initial linear parts of kinetic curves ($V_o = tg\ \alpha$). To determine the catalytic parameters of enzyme (K_m and V_{max}), the initial rates at various initial substrate concentrations were analyzed by hyperbolic approximation using least square method in Origin Pro 8.1 SR3.

All results are presented as averages from at least three independent experiments; the errors cited are standard deviations calculated using SigmaPlot 11.0 software.

2.4. Computer modeling

The following free resources were used: I-TASSER server (ver. 4.4, http://zhanglab.ccmb.med.umich.edu/I-TASSER/) [12] to correctly fold aminoacid sequences; GLYCAM-Web server (force field ver. GLYCAM06, http://glycam.org/) [13] to correctly fold polysaccharides; APBS & PDB2PQR server (ver. 1.4.2.1 and 2.1.1, respectively, http://www.poissonboltzmann.org/) [14,15] to calculate surface charge distribution of polypeptides; AutoDockTools (as part of MGLTools ver. 1.5.6) [16] to calculate partial charges of PEG and polysaccharide atoms and to convert files with various formats; PyMOL Molecular Graphics System (ver. 1.7.6, Schrödinger, LLC) to work with structures obtained and to generate illustrations.

Known structure of OPH dimer (RSCB PDB number 1QW7) was modified with predicted His_6-tag (Fig. S1) to obtain structure of His_6-OPH dimer.

As a basis for PEG, segment PEG_4 available via Cambridge Crystallographic Data Centre (number 707050) was used. By duplication and deletion it was elongated to the necessary size (PEG_{113} and PEG_{22}) and was merged with other polypeptides after charge calculation within Gasteiger-Marsili method (without considering pH influence).

To obtain gelatin structure, known aminoacid sequence of human collagen type III (RSCB PDB number 3DMW) was used. Further, it was modified by aminoacid substitution to the average gelatin of animal origin (Tables S1–S2) and was folded. During elongation and ‘succinylation’, the model of succinylated gelatin (commercially available Gelofusine®) was obtained and it contained 294 aminoacid residues, 5 wt% of succinic acid and total molar mass ca. 27.8 kg mol^{-1}.

Primary moiety of amylopectin technically allowing its computer modeling was selected to conform to literature data about its characteristics of X-ray structural analysis. The most adequate model was revealed to contain 111 glucose residues with 6 branching. Such ‘subunit’ was modified with hydroxyethyl groups and was merged by 10-glucose segments of hydroxyethylated ‘amylose’ with the same subunits. At all, model of hydroxyethyl starch (HES) contained 554 glucose residues (4 amylopectin subunits and 11 amylose subunits) with total molar mass ca. 105.5 kg mol^{-1} and degree of substitution 0.646. After that, atom charges were calculated within Gasteiger-Marsili method (without considering pH influence).

Models of PLEs and PLDs with different chain length were predicted *in silico*. After that, surface charge distribution for all polypeptides (incl. succinylated gelatin and His_6-OPH) was calculated at pH 7.5 and 10.5 with PARSE force field and default settings.

Enzyme-polyelectrolyte complexes were calculated using Supercomputer “Lomonosov” of Lomonosov Moscow State University [17], utilizing up to 1024 cores of Intel Xeon X5570 2.93 GHz and 1.5 TB of operating memory. AutoDock Vina (ver. 1.1.2) [18] was used with Intel MPI Library (ver. 5.0.1). Calculations were performed with default program options (if not stated otherwise).

More details of computer modeling are available in the Supporting Information.

3. Results and discussion

3.1. Computer modeling

Since there is no literature data (X-ray analysis, NMR spectroscopy, etc.) about structure of polyanion used in this study, computer simulation was implemented to generate their models (Figs. S2–S7). Although these molecules were subsequently subjected to numerous conformational changes (incl. unfolding), the use of the initial conditions as close as possible to the real ones and