Structural and Enzymatic Characterization of the Phosphotriesterase OPHC2 from *Pseudomonas pseudoalcaligenes*

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Abstract

**Background:** Organophosphates (OPs) are neurotoxic compounds for which current methods of elimination are unsatisfactory; thus bio-remediation is considered as a promising alternative. Here we provide the structural and enzymatic characterization of the recently identified enzyme isolated from *Pseudomonas pseudoalcaligenes* dubbed OPHC2. OPHC2 belongs to the metallo-β-lactamase superfamily and exhibits an unusual thermal resistance and some OP degrading abilities.

**Principal findings:** The X-ray structure of OPHC2 has been solved at 2.1 Å resolution. The enzyme is roughly globular exhibiting a α/β/α topology typical of the metallo-β-lactamase superfamily. Several structural determinants, such as an extended dimerization surface and an intramolecular disulfide bridge, common features in thermostable enzymes, are consistent with its high Tm (97.8°C). Additionally, we provide the enzymatic characterization of OPHC2 against a wide range of OPs, esters and lactones.

**Significance:** OPHC2 possesses a broad substrate activity spectrum, since it hydrolyzes various phosphotriesters, esters, and a lactone. Because of its organophosphorus hydrolase activity, and given its intrinsic thermostability, OPHC2 is an interesting candidate for the development of an OPs bio-decontaminant. Its X-ray structure shed light on its active site, and provides key information for the understanding of the substrate binding mode and catalysis.

Introduction

Organophosphates (OPs; **Fig. 1A**) are well known neurotoxic compounds which irreversibly inhibit the acetylcholinesterase, a key enzyme in the nerve signal transmission [1]. OPs are widely used as agricultural insecticides [2] and their most toxic representatives have been developed as chemical warfare agents (e.g. tabun, sarin, soman or VX) [3]. These compounds are still massively used as pesticides resulting in considerable pollutants [4,5]. Current methods for removing them are slow, cost prohibitive [6], and generate secondary pollution. Novel methods of remediation such as enzyme-mediated decontamination, are therefore highly desirable and under intensive research [7,8].

OPs pesticides have been massively used since the 1950’s, leading to the fast emergence of microorganisms that are capable of degrading OPs, and that can probably utilize them as carbon and phosphorus source [9]. Several Organophosphorus Hydrolases (OPHs) have been identified so far, belonging to different protein families: the Organophosphorus Acid Anhydrolases (OPAAs; EC 3.4.13.9) related to the prolidases [10,11], the paraoxonases (PONs; EC 3.1.8.1) [12], the PhosphoTriesterases (PTEs; EC 3.1.8.1) and the Phosphotriesterase-Like Lactonases (PLLs; EC 3.1.1.81) from the amidohydrolase superfamily, and finally the OPHs from the metallo-β-lactamase superfamily [13]. PTEs, isolated from *Brevundimonas diminuta* [14] and *Agrobacterium radiobacter* [15], are the most characterized OPHs so far and exhibit near diffusion-limit rate for the insecticide paraaxon as substrate [14]. PTEs are believed to have emerged from native lactonases with promiscuous phosphotriesterase activity such as the PLLs [16–18].

A recently identified OPH, named OPHC2 (GenBank ID: AJ605330), has been isolated from *Pseudomonas pseudoalcaligenes* [19,20] but also from *Stenotrophomonas sp.* SMSP-1 [21]. This enzyme shares about 45% sequence identity with the Methyl-Parathion Hydrolases (MPHs; EC 3.1.8.1). MPHs enzymes are isolated from several organisms, such as *Pseudomonas putida*, *Pleisiomonas sp.* M6, *Ochrobactrum sp.* M231 or *Pseudomonas sp.* WBC3, and hydrolyze methyl-parathion with high efficiency.
(i.e. $k_{cat}/K_M = 10^6 \text{ M}^{-1} \text{s}^{-1}$) [22]. The structure of MPH from *Pseudomonas* sp. WBC3 was solved and revealed an $\alpha\beta/\beta\alpha$ sandwich fold typical of the metallo-$\beta$-lactamase superfamily, forming a homodimer [22]. Containing a Zn(II) bi-metallic catalytic site bridged by a water molecule, the MPHs catalytic mechanism is presumed to be similar to that of other OPHs [22]. The bridging catalytic water molecule is activated by the bi-metallic active site and serves as the nucleophile that attacks the phosphorus center of the bound substrate via a $\text{SN}_2$ mechanism [22].

OPHC2 has been previously shown to exhibit methylparathion hydrolyzing activity [19]. Although the enzyme originates from a mesophilic soil bacterium [23], its optimum temperature for catalysis is reported to be 66°C. In this article, we report the biochemical, enzymatic and structural analysis of OPHC2 from *P. pseudoalcaligenes*. In combination, these results allows us to propose possible explanations for the thermostability of OPHC2 and its substrate preference.

Figure 1. Chemical structure of tested substrates. Chemical structures of (A.) phosphotriesters, (B.) CMP-coumarin, (C.) esters, (D.) Acyl-Homoserine Lactones, (E.) $\gamma$-lactones, (F.) $\delta$-lactones and (G.) dihydrocoumarin are presented. For phosphotriesters, R corresponds to different nature of substituents; LG corresponds to the leaving group which can be F, S-R, O-R or CN. The terminal substituent could be S atom if the molecule is a thionophosphotriester or an O atom if the molecule is an oxonophosphotriester. For esters, R corresponds to different nature of substituent. For AHLs and $\gamma/\delta$-lactones, R corresponds to different size of acyl chain.

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Materials and Methods

Sequence Alignment

The alignments were performed using the T-coffee server (expresso) [24,25], and subsequently manually improved. The phylogenetic tree was performed using PhyML [26] and default parameters. The sequence alignment was drawn with BioEdit 7.1.3. Protein sequence identities were calculated using ClustalW server [27].

Protein Production and Purification

The protein production and purification was performed as previously explained [29]. Briefly, the protein production was performed in E. coli strain BL21(DE3)-pGro7/GroEL (TaKaRa). Purification was performed using a previously described procedure [18,28] that takes advantage of the high stability of the target protein by performing a heating step of 30 minutes at 70°C followed by a differential ammonium sulfate precipitation to eliminate remaining thermostable proteins. The sample is subsequently loaded on a size exclusion column [20]. Proteins were quantified using a nanospectrophotometer (nanodrop, ThermoFisher scientific, France) with the protein molar extinction coefficient (ε280 nm = 38 390 M⁻¹ cm⁻¹) calculated by the PROT-PARAM server [29].

Determination of the Oligomerization State

Oligomerization state determination was performed using a size exclusion column S75 10/300 GL (GE-Healthcare) calibrated with the Gel Filtration Low Molecular Weight calibration kit (GE-Healthcare) in activity buffer (50 mM HEPES pH 8, 150 mM NaCl, 0.2 mM CoCl₂). 145 µg of OPHC2 enzyme was separated using a flow rate of 0.5 ml/min on an AKTA avant chromatography apparatus (GE-Healthcare) running with the UNICORN 6.1 software. Dynamic light scattering (DLS) experiments were performed at room temperature using zetasizer nano series apparatus (Malvern, UK) and the Zetasizer software. 30 µl of purified OPHC2 (5 mg.ml⁻¹) was used in the activity buffer to measure the hydrodynamic radius of particles at 633 nm allowing estimation of a theoretical enzyme molecular weight.

Kinetic Characterization

Catalytic parameters were evaluated at 25°C, and recorded with a microplate reader (Synergy HT, BioTek, USA) and the Gen5.1 software in a 6.2 mm path length cell for 200 μl reaction in 96-well plate as previously explained [18]. Catalytic parameters were obtained by fitting the data to the Michaelis-Menten (MM) equation [30] using GraphPad Prism 5 software. When Vₘₐₓ could not be reached in the experiments, the catalytic efficiency was obtained by fitting the linear part of MM plot to a linear regression using GraphPad Prism 5 software.

Standard assays were performed in activity buffer by measuring the time course hydrolysis of pNP derivatives (ε₄₀₅ nm = 17 000 M⁻¹ cm⁻¹) of OPs (3-oxo-C₆-AHL, 2 mM; C₆-AHL (i), 1 mM; 3-oxo-C₈-AHL (i), 2 mM; 3-oxo-C₁₀-AHL (i), 2 mM) [Fig. S1IX–XIV] and oxo-lactones (Fig. 1EF) [i.e. γ-caprolactone, 5 mM; γ-heptanolide (i), 5 mM; Nonanoic-γ-lactone (i), 5 mM; Nonanoic-δ-lactone (i), 5 mM; Undecanoic-γ-lactone (i), 5 mM] (Fig. S1XV–XIX). Cresol purple (pKₐ 8.3 at 25°C) is a pH indicator (577 nm) used to follow the lactone ring hydrolysis that cause an acidification of the medium.

Thermostability Analysis

Circular Dichroism (CD) spectra were recorded as previously described [18] using Jasco J-810 spectropolarimeter equipped with Pelletier type temperature control system (Jasco PTC-4235) in 1 mm thick quartz cell and using the Spectra Manager software. To determine the melting temperature of the protein, the denaturation was recorded at 222 nm by increasing the temperature from 20 to 95°C (at 2°C/min) in 10 mM sodium phosphate buffer at pH 8 containing increasing concentrations (1–3 M) of guanidinium chloride. The theoretical Tₘ without guanidinium chloride was extrapolated at the y-intercept by a linear fit using the GraphPad Prism 5 software.

Cryocrystallization

Cryocrystallization was performed as previously published [20]. Briefly, crystals were obtained by the siting drop vapour diffusion method set up in a 96-well plate. Crystals grow reproducibly after three months at 293 K in drops (2:1 and 1:1 protein/reservoir ratio) of condition 1 (10% PEG 8000, 100 mM Tris-HCl pH 7 and 200 mM MgCl₂) and condition 2 (10% PEG 3000, 100 mM Sodium cacodylate pH 6.5 and 200 mM MgCl₂).

Data Collection and Structure Refinement

Crystals were transferred into a cryo-protectant solution composed of the reservoir solution and 20% (v/v) glycerol prior to flash-freezing in liquid nitrogen. X-ray diffraction dataset was collected at 100 K using synchrotron radiation at the Proxima-1 beam line (SOLEIL, Gif-sur-Yvette, France) and a PILATUS-6M detector (DECTRIS, Switzerland). X-ray diffraction data were integrated and scaled with the XDS package [31] [Table 1]. The phases were obtained by molecular replacement with PHASER [32] as previously described, using the MPH structure as a starting model (PDB ID 1P9E) [20]. The model was subsequently built with Coot [33] and refined using REFMAC5 [34] and PHENIX [35]. A total of five monomers (two dimers and one monomer of a symmetry related dimer) were found per asymmetric unit. One of these dimers is highly agitated in the crystal, resulting in a poor electron density. The model and structure factors were deposited under the Protein Data Bank (PDB) code 4LE6.

Structural Modeling

The region 168 to 210, lacking from OPHC2 structure, was modelled using CHIMERA [36] and Modeller 9.11 [37] with the structure of MPH as template.

Anomalous X-ray Scattering Experiments

The chemical nature of the bi-metallic center was studied using anomalous X-ray fluorescence. Two datasets were collected consisting of 3600×0.1° at 2.6 and 3.1 Å resolution at respective wavelengths lower (1.2835 Å) and higher (1.2822 Å) than the Zn-K absorption edge. Moreover, the X-ray fluorescence spectrum of OPHC2 crystal has been collected.
metallo-
are concentrated in the helix/loop parts of the enzymes.

MPH, AiiA and AiiB sequences ([38]). Vacuum electrostatic potentials and surface representation were computed using PISA available from a deviations (RMSD) were calculated on command under PyMOL. Results Structure Analysis MPH (identity = 48%; similarity = 58.6%), and lower sequence shares significant homology with other representatives such as OPHC2 belongs to the metallo-
-lactamase superfamily and OPHC2 enzyme was initially characterized for its ability to hydrolyze OPs in crude extracts [19]. We have determined the enzyme kinetic parameters for several insecticides: ethyl/methyl-paraoxon (Fig. S1H–I), ethyl/methyl-paralathion (Fig. 1SIII–IV) and malathion (Fig. 1SV). (Table 2). Methyl-paraoxon (kcat/KM = 1.49±0.34×10^2 M^-1s^-1) and methylparalathion (kcat/KM = 2.68±0.73×10^1 M^-1s^-1) are the best substrates. As previously observed in PLLs [28], the KM for methyl-paralathion is better than for methyl-paraoxon. Ethyl-paraoxon (kcat/KM = 13.3±2.9 M^-1s^-1), and malathion (specific activity = 329±82 μmol.mol^-1.s^-1) are poor substrates for OPHC2, whereas CMP-coumarin (Fig. 1B) is hydrolyzed with significant rates (kcat/KM = 2.96±0.48×10^1 M^-1s^-1). Overall, OPHC2 exhibits rather low organophosphate hydrolyase activity (kcat/KM=10^3 M^-1s^-1) as compared to other enzymes such as PTEs, mainly because of a low catalytic rate (kcat~10^-1 s^-1).

Concomitantly to the phosphotriesterase activity, esterase or lactonase activities are systematically observed in other enzyme superfamilies such as paraoxonases or PLLs [12,13]. We have thus characterized OPHC2’s activity against various esters (Fig. 1C) and lactones (Fig. 1D–F) (Table 2). OPHC2 hydrolyzes phenylacetate, pNP-acetate and pNP-decanoate with low catalytic efficiencies (kcat/KM<10^2 M^-1s^-1) because of low catalytic rates (kcat~10^-2 s^-1). The best ester substrate for OPHC2 is pNP-decanoate (kcat/KM=2.33±0.83×10^2 M^-1s^-1). Concerning lactones, no hydrolysis of AHLs (Fig. 1D) or oxo-lactones (Fig. 1E) could be detected, even at high enzyme concentration (i.e. 250 μg/mL). However, interestingly, the best substrate for OPBNCH2 of all tested molecules is the lactone dihydrocoumarin, with a catalytic efficiency of 5.93±1.55×10^3 M^-1s^-1.

Structural Characterization X-ray structure of OPHC2. The structure of OPBNCH2 was solved at 2.1 Å resolution (Table 1) and reveals that OPBNCH2 forms a homodimer in the crystal. The dimer interface consists in a large, mainly hydrophobic, contact area between the two dimers (2453.2 Å^2) (Fig. 3A), which is bigger than that of MPH (2243.9 Å^2). The dimer is reinforced by the interaction of the N-terminal extremities of the chain that contact the second monomer (Fig. 3A). Both monomers interact intensively, forming 29 hydrogen bonds and 15 salt bridges, involving 61 residues (256 atoms). In comparison, MPH monomers perform 45 hydrogen bonds and 8 salt bridges. OPBNCH2 exhibits a very large, hydrophobic contact area (2453.2 Å^2) (Fig. 3A) with several surface salt bridges (Fig. S3) that stabilize, for example, the protein extremities (Fig. S3B).

The monomer of OPBNCH2 is roughly globular with overall dimensions of approximately 44 Å×50 Å×37 Å. As for MPH, its
Figure 2. Phylogenetic analysis of OPHC2 enzymes. A. Simplified phylogenetic tree of several OPHs (OPHC2s and MPHs) and lactonases (AiiAs and AiiBs) exhibiting a $\alpha$/$\beta$ or $\beta$/$\alpha$ topology. Sequences were selected from NCBI blast (sequence identity >40%) using input query of OPHC2 from *P. pseudoalcaligenes*, MPH from *Stenotrophomonas* sp. Dsp-4, AiiA from *B. thuringiensis* and AiiB from *A. fabrum* str. C58. Alignment was performed using the T-coffee server and the tree was built using PhyML. The tree has been arbitrarily rooted for clarity. Bootstraps values are indicated. B. Sequence alignment of OPHC2 from *P. pseudoalcaligenes* (OPHC2-1) and *Stenotrophomonas* sp. SMSP-1 (OPHC2-2), MPH from *Stenotrophomonas* sp. Dsp-4, AiiA
structure could be described as an αβ/βα sandwich topology, typical of the metallo-β-lactamase superfamily [42]. Two mixed twisted β-sheets, each composed of six strands, are flanked by seven α-helices exposed to the solvent (Fig. 3C). The catalytic center is located between the two β-sheets and surrounded by the connecting αβ-loops. In OPHC2 structure, residues 168 to 210 are absent from the electron density maps and therefore have not been modelled. A tentative model of these missing residues based on MPH structure yielded a model that is unfortunately not compatible with OPHC2’s crystal packing (Fig. S4).

The superposition of OPHC2 with MPH and AiiA yields RMSD values for α-carbon atoms of 0.74 Å (over 213 residues) and 2.41 Å (over 105 residues), respectively. While OPHC2 and MPH structures are very similar, major structural differences are visible between OPHC2 and AiiA. These differences mainly concern the loops size and conformations (Fig. 3D).

**Active site description.** The active site of OPHC2 consists of a cavity with two metal cations: one buried (β metal) and one more solvent exposed (α metal). The α metal is coordinated by His294, His144, Asp143 and the Asp247, the latter coordinates also the β metal together with His226, His139, His141 and a water molecule (Fig. 4A). Both metals are bridged by the putative catalytic water molecule. The chemical nature of metal cations was investigated using anomalous X-ray data collection at the Zn-K edge (1.2822 Å) and under (1.2835 Å) (Table S2). The presence of two peaks for each metals in Bijvoet difference Fourier maps at the Zn-K edge (18.5 and 14.7 σ in height) and their drop in the maps calculated from data collected under the Zn-edge (3.9 and 11.6 σ) clearly indicates the presence of zinc cations in the active site, but not only (Table S2). Indeed, the residual fluorescence observed under the Zn absorption edge may be due to the presence of iron or cobalt, as observed in the X-ray fluorescence spectrum (Fig. S5). Therefore, the active site of OPHC2 contains, at both α and β positions, a mixture of zinc and possibly cobalt and/or iron.

The substrate binding pocket is mainly composed of hydrophobic residues. It can be subdivided, based on the MPH structure [45], into three subsites: the leaving group pocket (Phe111, Met188 and Trp172), a first specificity subsite (Leu250, Leu61, Phe263 and Phe265) and a second specificity subsite (Val55 and Leu67) (Fig. 4B and Fig. S6). Residues Met188 and Trp172 that belong to the leaving group subsite in MPH, belong to the disordered protein fragment that has not been modelled. Residues Cys110 and Cys146 that form a disulphide bridge comprise second shell active site residues (Fig. 4B). It covalently bridges helix z1 and loop β1, and may thus rigidly the active site, especially Phe111, a residue possibly involved in the leaving group subsite.

**Discussion**

OPHC2 is a Dimeric, Thermostable Enzyme

OPHC2 enzyme, as observed for MPH [22], crystallizes as a homodimer, and biochemical evidences suggest that this dimer exists in solution. This homodimer exhibits (i) a very important surface interaction, (ii) a high number of intermolecular hydrogen bonds and salt bridges. The higher thermal stability of OPHC2, as compared to its closest known homologue MPH, may stem from a high dimer interface area and an intramolecular disulphide bridge. Moreover, the structure reveals several ion bridges at the protein surface, a feature commonly observed in thermostable enzymes [46,47] and usually linked to thermal stability. Altogether, these structural determinants may contribute to the enzyme thermal stability (T m of 97.8 ± 3.2°C). Because of this very high stability, OPHC2 can be purified by a fast and easy procedure: a heating step followed by ammonium sulfate precipitation, and a polishing step by gel filtration. Moreover, being a thermostable enzyme [47], OPHC2 is expected to exhibit high stability toward various chemicals like organic solvent, and resist to long-term storage. These properties and its measured organophosphatase-degrading

| Substrates | kcat (s⁻¹) | km (μM) | kcat/km (M⁻¹s⁻¹) |
|------------|------------|---------|------------------|
| **Phosphoesters** | | | |
| Ethyl-paraoxon (I) | 4.05(±0.01) x 10⁻³ | 94±19 | 1.33(±0.02) x 10⁴ |
| Methyl-paraoxon (II) | 3.87(±0.29) x 10⁻⁵ | 261±56 | 1.48(±0.34) x 10⁶ |
| Ethyl-parathon (III) | ND | ND | ND |
| Methyl-parathon (IV) | 5.71(±0.33) x 10⁻⁵ | 21±6 | 2.68(±0.73) x 10⁶ |
| Malathion (V) | ND | ND | VLH |
| CMP-coumarin (VI) | 3.38(±0.19) x 10⁻⁵ | 114±17 | 2.96(±0.48) x 10⁵ |
| **Esters** | | | |
| Phenyl-acetate (VII) | 9.03(±1.26) x 10⁻² | 1620±563 | 5.56(±0.28) x 10⁸ |
| αNP-Acetate (VIII) | ND | ND | 2.17(±0.08) x 10⁵ |
| βNP-Decanoate (IX) | 3.22(±0.25) x 10⁻² | 138±48 | 2.33(±0.83) x 10⁷ |
| **Lactones** | | | |
| AHls (X-XV) | ND | ND | ND |
| o xo-lactones (XVI-XX) | ND | ND | ND |
| Di-hydrocoumarin (XXI) | 2.39±0.20 | 403±100 | 5.93(±1.55) x 10⁵ |

Roman numbers correspond to the related chemical structure of the substrate presented in Figure S1. Data obtained with cobalt as cofactor. ND corresponds to not determined values because of no or too low catalytic rate. VLH correspond to Very Low Hydrolysis observed without the possibility to record a value.
ability make OPHC2 an interesting candidate for developing an efficient OP biodecontaminant by protein engineering.

OPHC2 Exhibits Esterase and Phosphotriesterase Activities

We here show that OPHC2 hydrolyzes a broad range of esters, from phosphotriesters to the lactone dihydrocoumarin. Being isolated as a phosphotriesterase [19], we show that the enzyme hydrolyzes various insecticides and a nerve agent analogue with relatively low catalytic efficiencies. OPHC2 exhibits clear preference for small substituents (e.g. OPHC2 processes methyl-parathion better than ethyl-parathion). Notably, OPHC2 is a less efficient phosphotriesterase than its closest homologue MPH (e.g. against methyl parathion), catalytic efficiencies of OPHC2 and MPH are \( \sim 10^3 \text{M}^{-1}\text{s}^{-1} \) and \( \sim 10^5 \text{M}^{-1}\text{s}^{-1} \) [22], respectively. This lower activity seems to reside in low \( k_{cat} \) values of OPHC2 for phosphotriesters. While structures of MPH and OPHC2 are overall similar (RMSD = 0.74 Å), some differences are observed in active site residues, loops lengths, and second shell residues. Laboratory evolution studies have repeatedly shown that substrate preferences are mediated, at least partly, by length and conformation of active site surrounding loops [16,48–50]. OPHC2's active site comprises a highly hydrophobic substrate binding pocket which thus seems well adapted for the accommodation of the hydrophobic molecules that comprise OPs. The sub-sites architecture of OPHC2 was established on the basis of that of MPH [7,22] (see Fig. S6). Comparison of both active site highlights amino acids differences in the leaving group pocket (e.g. Met188OPHC2 instead of Phe196MPH), in the side pockets (e.g Phe265OPHC2 instead of Leu273OPHC2 and Leu61OPHC2 instead of Arg72MPH, and the presence of an additional Phe263OPHC2 in the binding pocket), as well as the floppy region 168–210. All these differences probably account for the differences in substrate specificity and catalytic efficiencies of both proteins. These residues may thus represent interesting targets for mutational studies, with the aim of increasing the phosphotriesterase activity and widening the specificity spectrum of OPHC2.
The similarities between catalytic centers of OPHC2 and other OPs, however, suggest a similar enzymatic chemistry. The catalytic center is composed of two metals bridging an activated water molecule as observed in MPH and AiiA structures [22,51], but also other esterases such as SisPoX or SiLaC [17,18]. Consequently, the hydrolysis mechanism of OPHC2 might be common to these related enzymes.

Additionally, OPHC2 exhibits lactonase activity. Amongst the 12 tested lactones, OPHC2 processes, however, only dihydrocoumarin. Despite the absence of the conserved Tyr residue, characteristic of lactonases in the metallo-β-lactamase superfamily [52] and in the PLLs [16,17,53], the catalysis of dihydrocoumarin is significant (kcat/KM = 5.93 × 10^5 M^-1 s^-1). A low lactonase activity, interestingly, has been recorded for many phosphotriesterases, the molecular promiscuity between both activities being hypothesized to stem from a molecular overlap between substrate binding of the phosphotriesters and the transition state of the lactone hydrolysis [13].

Finally, we here show that OPHC2 exhibits relatively low catalytic efficiencies against the range of tested substrates (∼10^-5 s^-1M^-1 against the best substrates). The average catalytic efficiency of enzymes being ∼10^-5 s^-1M^-1 [54], this work suggests that OPHC2 natively processes a substrate, yet unknown, that is different in chemical nature from the tested molecules of this study. OPHC2 may therefore have a different biological function than being a lactonase, a phosphotriesterase or an esterase.

**Supporting Information**

**Figure S1** Chemical structure of phosphoesters (I-VI), esters (VII-IX) and lactones (X-XXI).

(DOCX)

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