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Structural Basis for Natural Lactonase and Promiscuous Phosphotriesterase Activities

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Organophosphates are the largest class of known insecticides, several of which are potent nerve agents. Consequently, organophosphate-degrading enzymes are of great scientific interest as bioscavengers and biodecontaminants. Recently, a hyperthermophilic phosphotriesterase (known as *Sso*Pox), from the Archaeon *Sulfolobus solfataricus*, has been isolated and found to possess a very high lactonase activity. Here, we report the three-dimensional structures of *Sso*Pox in the apo form (2.6 Å resolution) and in complex with a quorum-sensing lactone mimic at 2.0 Å resolution. The structure also reveals an unexpected active site topology, and a unique hydrophobic channel that perfectly accommodates the lactone substrate. Structural and mutagenesis evidence allows us to propose a mechanism for lactone hydrolysis and to refine the catalytic mechanism established for phosphotriesterases. In addition, *Sso*Pox structures permit the correlation of experimental lactonase and phosphotriesterase activities and this strongly suggests lactonase activity as the cognate function of *Sso*Pox. This example demonstrates that promiscuous activities probably constitute a large and efficient reservoir for the creation of novel catalytic activities.

Keywords: lactonase; phosphotriesterase; hyperthermophilic enzymes; 3D structure; active-site mutants

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Abbreviations used: PEG, polyethylene glycol; PTE, phosphotriesterase; *Sso*Pox, *Sulfolobus solfataricus* phosphotriesterase; PLL, phosphotriesterase-like-lactonase; Opd, organophosphorus degrading; OP, organophosphates; rmsd, root-mean-square deviation; AHL, *N*-acyl-homoserine lactone; AhlA, *N*-acyl homoserine lactone acylase from *Rhodococcus erythropolis*; OpdA, phosphotriesterase from *Agrobacterium radiobacter*; C10 HTL, *N*-decanoyl-L-homocysteine thiolactone; C8 HSL, N-octanoyl-L-homoserine lactone.

Introduction

Organophosphates (OPs) are well-known potent toxic compounds that inhibit acetylcholinesterase irreversibly, a key enzyme of the central nervous system. They have been used extensively since the end of World War II. Their toxic properties have been exploited for the development of chemical warfare agents such as sarin, soman and VX, and principally for the development of agricultural insecticides.[1] Enzymes that are capable of degrading these OPs are therefore attractive as potential antidotes for both organophosphate-based pesticides and nerve agents.[2] Enzymatic detoxification of OPs has become the subject of numerous studies because current methods of removing them, such as treatment with bleach and incineration, are slow, expensive and cause environmental concerns. For this application, OP hydrolases are appealing due to their broader substrate specificity and higher catalytic rate.[2]

Phosphotriesterases (PTEs) are members of the amidohydrolase superfamily,[3] enzymes catalyzing hydrolysis of a broad range of compounds with different chemical properties (phosphoesters, esters, amides, etc.). Their coding genes, *opd*s (*o*rgano *p*hosphate *d*egradation), were isolated from *Pseudomonas diminuta*,[4] *Flavobacterium* sp.,[5] *Agrobacterium radiobacter*,[6] and genes similar to *opd* were also located in Archaea.[7] Since synthesis of their most efficient substrate, known as paraoxon, was described in the 1950s, it has been postulated that phosphotriesterases might have evolved specifically to this high level of catalytic efficiency over a relatively short period of time.[8] Several PTE structures are available in the PDB database, including those from *P. diminuta*,[9] and a very similar (90% sequence identity) OpdA homologue from *A. radiobacter*.[10] PTEs are $(\beta/\alpha)_8$-barrels and have a binuclear metal centre located at the C-terminal end of the barrel.[9,10] These enzymes show a high catalytic turnover, and their activity is modulated by the presence of divalent metal cations. It has been reported that the most active isoenzyme is Co^{2+}-substituted PTE.[11] Although complete active site metal substitution has been described,[9] recent results using anomalous fluorescence have shown both an incomplete metal substitution and a potential role for an iron cation (Fe^{2+}) in catalysis.[12] A catalytic mechanism for the hydrolysis of phosphotriesters has been proposed,[13] and it is reviewed in the present article.

A protein from the hypertermophilic archaeon *Sulfolobus solfataricus*, *Sso*Pox, has recently been cloned and characterized for its phosphotriesterase activity.[7] Although it displays only about 30% sequence identity with mesophilic PTEs, all amino acids coordinating the binuclear metal-centre are conserved. Furthermore, *Sso*Pox catalyzes the hydrolysis of paraoxon and other pesticides with a lower efficiency. Similar to the *Pseudomonas* PTE, its activity depends on the presence of metal ions, with cobalt significantly enhancing catalysis.[7] *Sso*Pox has been proved to have a high level of thermal stability, with denaturation half-lives (T_m) of 4 h and 90 min at 95 °C and 100 °C, respectively. This property allows rapid high-yield purification of the recombinant enzyme by simply heating cell lysates and thus precipitating host proteins. Owing to its exceptional stability, this PTE may be an excellent candidate in biotechnological studies seeking an efficient biodecontaminant of organophosphorus compounds.

Recently, a high level of catalytic activity and specificity with lactones as substrates has been reported for *Sso*Pox. Afriat and co-workers [14] proposed *Sso*Pox, *N*-acyl-homoserine lactone acylase from *Rhodococcus erythropolis* (AhlA) and the putative parathion hydrolase from *Mycobacterium tuberculosis*, to be members of a new group of enzymes dubbed phosphotriesterases-like-lactonases (PLLs), based on the observation of sequence features not present in mesophilic PTEs and the recognition of significant differences in enzyme specificity. In particular, the activity detected against natural homoserine lactones may relate these proteins to a precise biological function. Indeed, cell-to-cell communication mediated by small diffusible molecules is a common occurrence in several bacteria,[15] a phenomenon known as quorum sensing. For example, many Gram-negative bacteria use *N*-acyl-homoserine lactones (AHLs) as signalling molecules that regulate gene expression patterns, which in turn allow the bacteria to display "group behaviour". Displaying a high level of activity against AHLs, PLLs could have a role in these signalling pathways as well,[14] or be involved simply in the utilization of these compounds as a carbon and energy supply.

Here, we report the crystallographic structures of the hyperthermophilic *Sso*Pox in its apo form at 2.6 Å and in complex with a quorum-sensing lactone mimic at 2.0 Å. The structure identifies this enzyme as the prototype of the newly identified PLL family. The structural analysis permits us to propose a lactonase mechanism, to refine the previously proposed catalytic mechanism for PTEs and, by adding data supporting the hypothesis that the *Sso*Pox lactonase activity is in fact its native function, to exemplify the promiscuous relationship between lactonases as *Sso*Pox and optimized phosphotriesterases.

Results

X-ray structure of the hyperthermophilic phosphotriesterase

The structure of *Sso*Pox was refined at 2.6 Å resolution (Table 1). *Sso*Pox is homodimeric, and the monomer is roughly globular with overall dimensions of approximately 40 Å × 54 Å × 46 Å. *Sso*Pox displays only about 30% identity with the two other known mesophilic PTEs,[7] but its topology is similar. The *Sso*Pox structure could be described as a distorted $(\beta/\alpha)_8$ barrel. The structures superimpose well with a root-mean-square deviation (rmsd) for α-carbon atoms between *Sso*Pox and *P. diminuta* PTE (over 268 atoms) and *Sso*Pox and *A. radiobacter* PTE (over 271 atoms) of 1.05 Å and 1.11 Å, respectively.