Organophosphates (OP) constitute the largest class of known insecticides and several of them are potent nerve agents, like the sarin that was used for terrorism in the Tokyo’s subway in 1995. Consequently, organophosphate-degrading enzymes are of interest as bioscavengers. Recently, a phosphotriesterase (PTE)(known as SsoPox) was isolated from the hyperthermophilic archcoon Sulfolobus solfataricus [1]. In addition to its PTE activity, SsoPox possesses a high lactonase activity, particularly a quorum quenching lactonase involved in the bacterial quorum sensing [2]. This activity has been shown to reduce drastically the pathogen’s virulence of some bacteria. Thus, the use of this kind of enzymes is considered as a promising alternative to antibiotics. Structures of SsoPox in its apo form (2.6 Å resolution) [3] and in complex with a lactone mimic compound (2.0 Å resolution) have been solved and revealed evidences explaining the thermal stability of SsoPox. We have also proposed two catalytic mechanisms, both for its PTE and lactonase activities. Combined with biochemical data, this work strongly suggests that SsoPox is a natural lactonase with promiscuous PTE activity [4]. Illustrated by several examples of convergent and divergent evolution, this shows how a promiscuous activity in a natural lactonase was used as a seed by the Nature to diverge to optimized PTEs. Finally, this work provides a base in biotechnology to achieve efficient countermeasures against OP poisoning and represents a promising alternative to antibiotics against pathogens like Pseudomonas species.

Keywords: phosphotriesterase, quorum sensing, enzyme evolution

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Crystal structure of α-carbonic anhydrase from Chlamydomonas reinhardtii

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Carbonic anhydrase (CA) plays important roles in biological processes such as photosynthesis, respiration, secretion of HCO3-, pH homeostasis and ion exchange. The proteins commonly contain a zinc ion in the active site for catalyzing the hydration of CO2 and vice versa. It is known that there are three classes of CA, designated α-, β- and γ-CAs, depending on the amino acid sequence similarities. The α-class is different from others in the structural architecture. Furthermore, even in the α-class, the enzyme from unicellular green alga, Chlamydomonas reinhardtii (chCA) is unique in post-translational modifications that it is glycosylated and spliced into two peptides. Such glycosylations are found in only mammalian CAs but they are not spliced. To reveal the structural details and the role of N-glycosylation, an X-ray analysis of chCA has been performed. chCA is a homodimeric protein, the two subunits being crystallographically independent. In each subunit, residues from Ser298 to Asn345 are spliced to separate into long and short peptides. The two subunits are, however, linked together by a disulfide bond. In the catalytic site, a zinc ion is bound to the three conserved His163, His165 and His182 in a tetrahedral configuration. A water molecule is trapped at the fourth position of the Zn atom. The electron density maps indicate that N-glycosylations occur at the three sites, Asn101, Asn135 and Asn297. This structure is the first example of CA attached to N-glycosides. chCA molecules are interacted to each other with a six-fold screw symmetry to form a long column. Furthermore, they are fused through the lateral interactions like a beehive. Each catalytic site is exposed to the central tunnel. It suggests that chCA in the crystalline state also catalyze the reaction.

Keywords: plant enzymes, glycosylation, zinc compounds

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Mechanistic role of the catalytic residue D300 in human pancreatic alpha-amylase

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Human pancreatic alpha-amylase (HPA) catalyzes the hydrolysis of alpha-1, 4 glycosidic linkages in starch and other ingested carbohydrates. In order to understand the catalytic mechanism and substrate binding characteristics of HPA, we have prepared variants with substitutions at D300, one of the three putative active site residues. Subsequent kinetic and crystallographic studies have been carried out to examine the variant enzymes in complex with the carbohydrate inhibitor acarbose and the substrates G4 and G6. The solved structures of D300N/acarbose and D300A/G4 show that these variant enzymes do not catalyze the wild type enzyme hydrolysis, elongation and transglycosylation reactions expected of acarbose and G4. Indeed, intact acarbose and G4 are bound without alteration at subsites -4 to -1 in their complex crystal structures. Nonetheless, distortion of the sugar moiety bound at subsite -1 was observed in both the D300N/acarbose and D300A/G4 structures. Surprisingly, in the structure of the D300A/G6 variant complex, two hydrolysis products (G3) and two intact G6 molecules are observed. This indicates that the D300A variant is catalytically active but at very slow rate. One G6 molecule was found bound to subsites -4 to +2 at the HPA active site while the other was bound close to the calcium site, implying that this latter region may play a role in starch binding. Notably, sugar distortion was not observed at subsite -1 when G6 was bound across the active site. Overall our studies show D300 in HPA plays key roles in both substrate binding and hydrolysis/transglycosylation catalytic reactions. Supported by the Canadian Institutes of Health Research.

Keywords: amylase, glucosidase, catalytic mechanism