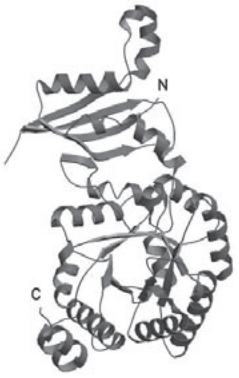
Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) catalyzes the addition of gaseous CO_2 into ribulose 1,5-bisphosphate (RuBP). RuBisCOs are classified into four forms based on sequence similarity: forms I, II and III are bona fide RuBisCOs, and form IV is called the RuBisCO-like protein (RLP). RLP lacks several residues involved in the substrate binding, and it does not catalyze RuBP-dependent CO_2 fixation in vitro. We have demonstrated that RLP from *Bacillus subtilis* (BsRLP) catalyzed the 2,3-diketo-5-methylthiopentyl-1-phosphate enolase reaction as the fourth step in the methionine salvage pathway. The crystal structure of BsRLP in the apo form was determined at 2.3 Å resolution. The structural comparison with other homologous proteins reveals that an induced fit of Lys150 to the active site plays a crucial role in the substrate recognition. Two loops of RLP, which corresponds to the catalytic flexible loops (60's loop and Loop6) in RuBisCO, are structurally ordered in the apo (open) and substrate binding (closed) conformations, respectively. Thus, the structural analysis sheds light on its functional evolution to RuBisCO.



Keywords: crystal structure, enzyme evolution, enzyme function

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Molecular mechanism of the redox-dependent interaction between ferredoxin reductase and ferredoxin

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Redox-dependent affinity regulation is critical to fast and efficient electron transfer (ET) between ET proteins. The molecular mechanism of the affinity regulation, however, remains elusive due to the lack of tertiary structures of the ET proteins in every redox state relevant to the ET reaction. BphA4 and BphA3 are, respectively, an FAD-containing NADH-dependent ferredoxin reductase and a Rieske-type [2Fe-2S] ferredoxin from a biphenyl dioxygenase BphA derived from *Acidovorax* sp. strain KKS102. Our biochemical study showed that the reduction of the FAD in BphA4 increases the affinity between BphA3 and BphA4 approximately 20-fold. In order to reveal the molecular mechanism of this redox-dependent affinity regulation, we determined the crystal structure of BphA4 in oxidized, hydroquinone, semiquinone, and reoxidized forms; the crystal structure of BphA3 in oxidized and reduced forms; and the crystal structure of the ET complex of BphA3 and BphA4 (Senda, M *et al.* J. Mol. Biol. (2007)). A comparative analysis of the seven crystal structures obtained revealed that the conformational changes of BphA4 upon reduction of FAD are required for the formation of the high-affinity BphA3-binding site in BphA4. The complex formation between BphA3 and BphA4 induces a conformational change of His66(A3), forming an ET pathway composed of Trp320(A4) and His66(A3). After accepting an electron, BphA3 is likely to undergo a flip of the peptide bond between Gly46 and Glu47. This peptide flip seems to break the electrostatic interaction between Glu47(A3) and Arg327(A4), inducing the dissociation of BphA3 from BphA4. The interplay of ET and induced conformational changes seems to be critical to the sequential reaction of the ET from NADH to BphA3.

Keywords: electron transfer mechanism, macromolecular interactions, flavoproteins

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Crystal structures of GAR transformylase 1 (PurN) from *A. aeolicus*, *S. toebii* and *G. kaustophilus*

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Glycinamide ribonucleotide (GAR) transformylase 1, PurN, catalyzes the 3rd reaction in the purine nucleotide biosynthetic pathway; a formyl transfer from 10-formyl tetrahydrofolate (10fTHF) to β-GAR. This enzyme is one of the targets of anti-cancer drug and it is important to elucidate the structural insight of the reaction catalyzed by PurN. The crystal structures of PurN from *E. coli* and human have been determined and are subjected to the structure-based drug design. Here, we determined crystal structures of PurN from three thermophilic eubacteria, *Aquifex aeolicus* VF5, *Symbiobacterium toebii* and *Geobacillus kaustophilus* HTA426. The space group, maximum resolution and *R*-value (free *R*-value) for each structure are as follows: *A. aeolicus* (2YWR), $P4_12_12$, 1.77 Å, 19.2% (22.8%), *S. toebii* (2YZP), $P3_121$, 2.07 Å, 20.8% (24.6%), *G. kaustophilus*, $C222_1$, 1.85 Å, 20.0% (22.6%). Multiple alignments of the five sequences corresponding to *A. aeolicus*, *S. toebii*, *G. kaustophilus*, *E. coli* and human shows that the three proteins studied here have an extra residue at the N-terminus. Overall structure of the three PurN are similar to each other and the conformation of the three conserved residues in the catalytic center, Asn107, His109, Asp145 for the three protein are well overlapped to Asn106, His108, Asp144 for *E. coli* (1C2T, 1C3E, 1CDE) and human (1MEO, 1NJS, 1RBM, 1RBQ, 1RBY, 1RBZ, 1RC0, 1RC1, 1ZLX, 1ZLY). Detail of structural analysis as well as the molecular dynamics analysis will be presented.

Keywords: nucleoside metabolism, transformylase, thermophilic proteins

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Structural basis for natural lactonase and promiscuous phosphotriesterase activities

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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 *Sso*Pox) was isolated from the hyperthermophilic archeon *Sulfolobus solfataricus* [1]. In addition to its PTE activity, *Sso*Pox 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 *Sso*Pox 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 *Sso*Pox. We have also proposed two catalytic mechanisms, both for its PTE and lactonase activities. Combined with biochemical data, this work strongly suggests that *Sso*Pox 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.

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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 HCO_3^-, pH homeostasis and ion exchange. The proteins commonly contain a zinc ion in the active site for catalyzing the hydration of CO_2 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* (*ch*CA) 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 *ch*CA has been performed. *ch*CA 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. *ch*CA 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 *ch*CA 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