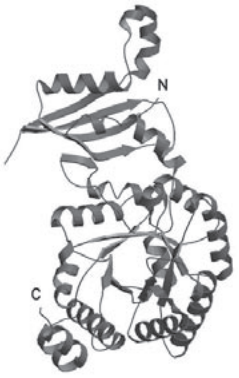
Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) catalyzes the addition of gaseous CO_2 into ribulose 1,5-bisphosphate (RuBP). RuBisCOs are classified into four forms based on sequence similarity: forms I, II and III are bona fide RuBisCOs, and form IV is called the RuBisCO-like protein (RLP). 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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