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CRYSTAL STRUCTURE OF GALDIERIA RUBISCO REVEALS A NOVEL CLOSURE MECHANISM OF THE ACTIVE SITE

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the rate-limiting step of photosynthesis and thus limits plant growth. In order to improve crop productivity, an understanding of the structure/function relationships of the enzyme is extremely important. Rubisco from a red alga, *Galdieria partita* has the highest specificity for carboxylation reaction among the Rubiscos reported so far. Crystal structure of unactivated Galdieria Rubisco has been determined at 2.6 Å resolution. Surprisingly, the enzyme bound a sulfate only in the *P*1 anion-binding site of the active site and the flexible loop6 was closed. This result is inconsistent with a fact that the loop6s of other unactivated Rubiscos complexed with sulfate or phosphate are open. Galdieria Rubisco has a unique hydrogen bond between Val-332 on the loop6 and Glu386, and this interaction seems to stabilize the closure of the loop6. The stabilization of the loop6 is likely to concern the highest specificity for the carboxylation of Galdieria Rubisco. We will also discuss a high resolution (1.7 Å) structure of activated Galdieria Rubisco complexed with 2-carboxyarabinitol-1,5-bisphosphate.

Keywords: RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE (RUBISCO), PHOTOSYNTHESIS, GALDIERIA PARTITA

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CRYSTAL STRUCTURE OF SPLICEABLE PRECURSORS OF YEAST VMA1-DERIVED HOMING ENDONUCLEASE

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Protein splicing precisely excises out an internal intein segment from a protein precursor, and concomitantly ligates flanking N- and C-extein polypeptides of the precursor. A series of precursor recombinants bearing 11 N-extein and 10 C-extein residues is prepared for the 50-kDa intein of the Saccharomyces cerevisiae VMA1-derived homing endonuclease that is referred to as VDE and also as PI-SceI. Dissolved crystals of the X10SSS recombinant with replacements of C284S, H362N, N737S, and C738S showed a single 53-kDa band in the SDS-PAGE analysis. The crystal structure of X10SSS showed that the peptide bonds between the intein and introduced extein polypeptides are not in cis conformation. The C284 SG atom in the N-terminal junction is capable of nucleophilically attacking the G283 C atom so as to form a tetrahedral intermediate containing a five-membered thiazolidine ring. The tetrahedral thiazolidine intermediate is then resolved into thioester acyl and amino groups upon the cleavage of the linkage between the G283 C and C284 N atoms. This thioester acyl formation completes the initial steps of N→S acyl shift at the N-terminal junction, and is followed by the transesterification to the C738 thiol in the C-terminal junction. Crystals of another recombinant X10SNS with replacements of C284S, H362N, and C738S gave 50-kDa and 53-kDa bands, indicating that the splicing reaction proceeded partially during the crystallization. The X10SNS structure gave continuous electron densities in the vicinity of the splicing site, which is attributable to the ligated peptide.

Keywords: **PROTEIN-SPLICING INTEIN THIAZOLIDINE**

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CRYSTALLISATION AND PRELIMINARY X-RAY ANALYSIS OF THE RECOMBINANT ARTHROBACTER GROBIFORMIS INULIN FRUCTOTRANSFERASE

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Inulin is a linear β-2,1 linked fructose polymer terminated by a sucrose which is found abundantly in special plants such as Jerusalem artichoke, dahlia and so on. We have reported an inulin fructotransferase [EC 2,4,1,93] from Arthrobacter globiformis which can convert inulin to di-D-1,2':2,3'dianhydride (DFAIII) and a small amount of oligosaccharides. Although the enzymatic properties and the molecular cloning have been reported, the structural basis of DFAIII forming by the enzyme is not clear. Here, we report crystallization and preliminary X-ray studies as a first step towards determining the structure and catalytic mechanism. The mature form of enzyme, a polypeptide of 410 amino acids with a molecular weight of 43,400 Da, was over-expressed and purified. Crystallization was performed by the hanging-drop vapor-diffusion method by 0.1 M HEPES-Na (pH = 7.5) and 1.5 M lithium sulfate at 293 K. The diffraction data set better than 1.5 Å resolution have been collected under the cryogenic conditions at the KEK photon factory synchrotron-radiation source The crystal belongs to the space group *R*32, with unit cell parameters a = b = 92.2 Å, c = 229.3 Å and γ = 120° in the hexagonal axes. Assuming three molecules in the asymmetric unit, the Vm value for the crystals is 2.15Å^3/Da indicating a solvent content of 44.1%. We are currently in the process of screening for heavy atom derivatives screening.

Keywords: **INULIN FRUCTOTRANSFERASE CRYSTALLISATION**

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THE STRUCTURE OF C-C BOND HYDROLASE MhpC REVEALS AN UNUSUAL CATALYTIC MECHANISM

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MhpC is a C-C bond hydrolase of the α/β-hydrolase fold that is a key enzyme in the bacterial degradation pathway of phenylpropionic acid. It contains the catalytic triad characteristic of the serine proteases but does not operate via an acyl-enzyme intermediate. Instead, it is proposed to operate by the base-catalyzed attack of water, forming a gem-diol intermediate. The native structure was determined by MAD phasing, and the three-dimensional structures of MhpC in complex with substrate analogue inhibitors has led to the identification of the substrate binding moieties. The invariant serine residue of the catalytic triad is thought to function differently to the nucleophilic serine of the typical serine proteases and thus leads to the proposal of a novel mechanism for this class of enzyme.

Keywords: MAD, HYDROLASE, MECHANISM