

### CRYSTAL STRUCTURE OF PHOSPHOENOLPYRUVATE CARBOXYLASE: THE REACTION MECHANISM AND REGULATION

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Phosphoenolpyruvate carboxylase (PEPC) catalyzes the irreversible carboxylation of phosphoenolpyruvate (PEP) to form oxaloacetate (OAA) and phosphate in the presence of the divalent cation such as Mg<sup>2+</sup> or Mn<sup>2+</sup> during C4 and crassulacean acid metabolism (CAM) photosynthesis. Despite the knowledge of the structure of the *E. coli* PEPC (EcPEPC) complexed with L-aspartate, there has been no understanding of the overall mechanism for the carboxylation reaction and the allosteric regulation, since no structural information on the activated R state of the enzyme and on the substrate binding site has been available. We determined the crystal structure of active state (R-state) C4-form maize PEPC (ZmPEPC) at 3.0 Å resolution by molecular replacement using EcPEPC as a probe. The structure includes sulfate ion at the plausible binding site of an allosteric activator, glucose-6-phosphate. The crystal structure of inactive state (T state) EcPEPC has also been determined as the quaternary complex of Mn<sup>2+</sup>, PEP analogue (DCDP; 3,3-dichloro-2-dihydroxyphosphinoylmethyl-2-propenoate), and an allosteric inhibitor, L-aspartate, at 2.35 Å resolution. In the complex, PEP analogue and Mn<sup>2+</sup> were tightly bound in the active site of the enzyme in the similar mode as we have proposed previously. From the structure comparison between R and T states PEPCs, the dynamic movements were revealed in ZmPEPC around two loops near the C-terminal side of the β-barrel where the catalytic site locates. Based on these molecular structures, we will propose the mechanisms for carboxylation reaction and for the allosteric regulation of PEPC.

**Keywords:** CARBOXYLASE, ACTIVE FORM STRUCTURE, REACTION MECHANISM

### CRYSTAL STRUCTURE OF 4-HYDROXYPHENYLACETATE 3-MONOOXYGENASE LARGE CHAIN (HPAA) FROM *THERMUS THERMOPHILUS* HB8

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The regulation of the pg promoter controls the expression of the meta operon of the 4-hydroxyphenylacetic acid (4-hpa) catabolic pathway of *thermus thermophilus* hb8 in the course of the nutritive adaptation. These proteins expressed by the hpa gene clusters cooperatively mediate the degradation of aromatic energy sources. Such energetic catabolism process is first mediated by hydroxylphenylacetate monooxygenases that catalyze hydroxyl groups of phenolic substrates to catechol products. 4-hydroxyphenylacetate 3-monooxygenase large chain (hpa: mw = 54000) from *t. Thermophilus* hb8 is one of the monooxygenases and mediates the biodegradation of the aromatic compounds to generate living energy. We determined crystal structure of hpa to establish structure-function relationships of the bacterial energy metabolism. Purified hpa (by "structurome project" of Riken Harima Institute) was crystallized by vapor diffusion method. The crystal belongs to the space group I222, with cell dimensions of a = 91.8, b = 99.6 and c = 131.1 Å. Diffraction data were collected at the bl44b2 and bl45xu stations, spring-8, japan. Phase information was determined by the mad method using one crystal of a pt derivative at 2.5 Å resolution. Additional high resolution data set was collected at 1.6 Å resolution using a native crystal. Hpa molecules form homo-dimer, with approximately dimensions of 90\*100\*130 Å. One subunit of hpa molecule comprises of 22 α-helices and 13 β-strands. This is the first crystal structure for bacterial phenolic monooxygenases. Preparation of the complex with substrate analogues, and functional analysis are now underway.

**Keywords:** HPA GENE OPERON, PHENOLIC MONOOXYGENASE, BIODEGRADATION

### TRAPPING REACTION INTERMEDIATES: A TRANSITION STATE ON THE R61 DD-PEPTIDASE CATALYTIC PATHWAY

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A tetrahedral transition state analog has been trapped crystallographically by reacting a novel tripeptide phosphonate inhibitor with a penicillin-binding protein (PBP), the *Streptomyces* strain R61 D-alanyl-D-alanine carboxypeptidase/transpeptidase (DD-peptidase). DD-peptidases are bacterial enzymes that are the targets for β-lactam antibiotics (penicillins and cephalosporins). These drugs inhibit PBPs because they mimic the D-alanyl-D-alanine portion of the natural cell wall peptidoglycan substrate of these enzymes. When PBPs are inhibited, growing bacteria cannot cross-link their cell wall, which leads to cell death.

The 1.1 Å X-ray crystal structure reveals a complex formed between the R61 DD-peptidase (37.5kDa) and a highly specific phosphonate inhibitor, synthesized by Rex Pratt and John Anderson at Wesleyan University. The data were collected at BNL, NSLS Beamline X12B; refinement results: R = 0.11, Rfree = 0.14. The phosphonate molecule, glycyl-L-α-amino-ε-pimelyl-D-alanyl-phosphonate, corresponds to a portion of the peptide bridge of *Streptomyces* R61 peptidoglycan. The structure of this complex reveals the interactions involved in the short-lived transition state formed with the natural substrate, which is identical to the tripeptide portion of the phosphonate. Comparisons of this structure with previously determined structures of non-covalent reaction intermediates of the R61 DD-peptidase indicate that the present structure represents the tetrahedral transition state in the deacylation step of the DD-peptidase reaction. This structure provides further insight into the catalytic mechanism of PBPs.

**Keywords:** PENICILLIN-BINDING PROTEINS, TETRAHEDRAL TRANSITION STATE, HIGH-RESOLUTION ENZYMATIC COMPLEX

### CRYSTAL STRUCTURE OF THE COMPLEX BETWEEN CALYCUIN A AND PROTEIN PHOSPHATASE 1 CATALYTIC SUBUNIT

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The crystal structure of the catalytic subunit of protein phosphatase 1 (PP1), PP1γ, in complex with a marine toxin, calyculin A, was determined at 2.0 Å resolution. The metal binding site contains the phosphate group of calyculin A and forms a tight network via the hydrophilic interactions between PP1 and calyculin A. Calyculin A is located in two of the three grooves, namely, in the hydrophobic groove and the acidic groove.

This is the first observation to note that the inhibitor adopts not a pseudocyclic conformation but an extended conformation in the hydrophobic groove and acidic groove on the PP1γ surface in order to form a complex with the protein. The conformation of β12-13 loop, which is considered to play an important role for binding inhibitors, is similar to that of PP1γ-okadaic acid complex, and not similar to that of PP1α-microcystin-LR complex. In PP1γ-calyculin A β12-13 loop, only the Tyr272 residue interacted with the inhibitor. The crystal structure indicates that the amino acid terminus of calyculin A contributes in a limited manner to the binding to PP1γ.

This result is consistent with findings from the studies of dose-inhibition analysis, which showed that the inhibitory activity is largely retained in hemicalyculin A, a derivative which lacks the C29-C37 component. The crystal structure also shows the importance of two salt bridges formed between calyculin A and two arginine residues (Arg96 and Arg221) in the substrate recognition site.

**Keywords:** PROTEIN PHOSPHATASE 1, PP1C CALYCUIN A COMPLEX, PP1C INHIBITOR COMPLEX