illuminate probable mechanisms of catalysis and suggest how GlpD shuttles electrons into the respiratory pathway. Homologs of GlpD are found in practically all organisms, from prokaryotes to humans, with over 45% consensus protein sequences, signifying that these structural results on the prokaryotic enzyme may readily be applied to the eukaryotic GlpD enzymes.

Keywords: membrane, proteins, mechanism

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The crystal structure of capsule synthesis protein CapD, a major virulence factor in *B. anthracis*

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The capBCADE genes are responsible for synthesis and transport of the poly-gamma-D-glutamic acid capsule known to protect *Bacillus anthracis* from phagocytic killing during infection. Here, we present the crystal structures of CapD with and without the dipeptide alpha-L-Glu-L-Glu in the active site of the enzyme. CapD shares the structure of N-terminal nucleotide hydrolases and in particular *Escherichia coli* and *Helicobacter pylori* gamma-glutamyltranspeptidases. Unlike bacterial gamma-glutamyltranspeptidases, CapD displays transpeptidation activity and its structure reveals a wide open active site for poly-gamma-glutamate binding and processing. Based on sequence and structure comparison, we propose that Pro427, Gly428, Gly429 contribute to CapD activity by activating Thr352 and stabilizing an oxyanion hole via classical main chain amides hydrogen bonds. Structural observations are corroborated by mutagenesis and functional studies. Modeling of a di-gamma-D-Glu substrate in the active site suggests that Thr352 could form an acyl-enzyme intermediate with gamma-carboxylate of poly-gamma-glutamate chains. Resolution of this intermediate by a nucleophile would result in transpeptidation activity.

Keywords: CapD, PDGA, Ntn-hydrolase family

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Crystal structure of adenosine kinase from *M. tuberculosis* in complex with nucleoside analogs

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Adenosine kinase (AK) is a key enzyme in the purine salvage pathway of *Mycobacterium tuberculosis*, an intracellular pathogen that causes tuberculosis (TB). Mtb AK, a unique bacterial adenosine kinase catalyzes the phosphorylation of adenosine to adenosine monophosphate and is involved in the bioactivation of some nucleoside analogs that have demonstrated selective activity against *M. tuberculosis*. The mechanism of action of these adenosine analogs is likely to be different from those of current TB treatments; therefore, specific activation of nucleoside analogs by Mtb AK may prove to be a novel therapeutic intervention for TB, particularly for multi-drug resistant TB. Specific inhibition of this key enzyme in the purine salvage pathway may also be exploited for therapeutic development. The crystal structures of the enzyme in complex with adenosine or one of three selected nucleoside analogs have been determined at 1.9 Å resolution with *R* factor of 0.19 and R_{free} of 0.25. The structure reveals a tightly associated homo-dimer, which is different from the known human and T. gondii AK, but rather resembles the structure of ribokinases. The monomer consists of a small domain that is responsible for the dimer formation and a large catalytic domain. The active sites reveal the protein-ligand interaction and significant structural differences between the human and Mtb AK. The structural information provides the structural basis for the specific activation of nucleoside analogs by Mtb AK and should aid in the design of more potent and selective antimycobacterial agents. We thank SBC-CAT and NE-CAT at APS for access to beam lines 19-BM and 8-BM. This research is supported in part by NIH grants, AI55344 to RL.

Keywords: nucleoside analogs, tuberculosis, drug design

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Structure and mechanism of rhamnogalacturonan lyases

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We present the first experimental evidence of rhamnogalacturonan lyase (RGL), belonging to family PL4 according to CAZy classification (www.CAZy.org,[1]), with bound substrate in the active site. *Aspergillus aculeatus* RGL (AARGL), which native structure is published as 1NKG in the PDB [2], is involved in the degradation of rhamnogalacturonan-I, an important plant cell wall polysaccharides. Through enzyme variants H210A and K150A, characterized kinetically and structurally, we have shown that His210 and Lys150 are key active site residues. X-ray diffraction data collected at MaxLab, Lund, Sweden, after soaking the K150A variant with a rhmanogalacturonan digest shows evidence of bound substrate at the -3/+3 subsites (Fig.1). Considering our results and structural and sequence comparisons to PL family enzymes, we propose a beta-elimination mechanism on [,2)-a-L-Rhamno-(1,4)-a-D-Galacturonic acid (1,]. The lack of calcium in the mechanism significantly differs from other characterized lyases, also supporting a diffent mechanism.

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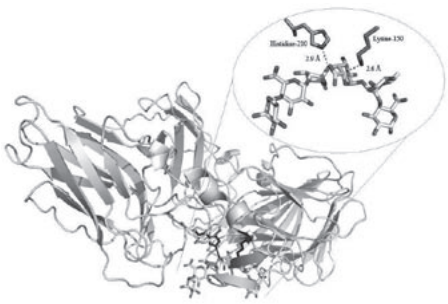

Keywords: biological macromolecular crystallography, carbohydrate degradation, pectate lyases substrate binding