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CRYSTAL STRUCTURE ANALYSIS OF SEPTUM SITE-DETERMINING PROTEIN MIND FROM *PYROCOCCUS HORIKOSHII*

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Rod-shaped bacteria have three potential division sites in a cell. One of them is at mid-cell position, while the others are adjacent to the cell poles. Thus the precise placement of the FtsZ ring at the cell center is prerequisite for the accurate cell division. In *Escherichia coli*, the cell division site is determined by MinC, MinD, and MinE. MinD is a membrane-associated ATPase and is a septum site-determining factor through the activation and regulation of MinC and MinE. MinD is also known to undergo a rapid pole-to-pole oscillation movement in vivo. We have determined the three-dimensional structure of MinD from *Pyrococcus horikoshii* at 2.3Å resolution using the Se-Met MAD method. The crystal structure consists of a β-sheet with seven parallel and one antiparallel strands and eleven peripheral α-helices. Although we made no attempt to add ATP or ADP molecules in the purification or crystallization step, the electron density clearly shows that MinD contains bound ADP and magnesium-ion at the pocket close to the edge of the b-sheet on the surface of the MinD molecule. Structure analysis shows that MinD is most similar to nitrogenase iron protein, which is a member of the family of the P-loop containing nucleotide triphosphate hydrolase superfamily of proteins. Moreover MinD has a limited structural similarity with family of motor proteins. Although the tertiary structure of ATPase activity site is similar in these proteins, the overall topology is different. This fact suggests that MinD may work as a molecular switch in bacterial cell division.

Keywords: MAD CELL DIVISION ATPASE

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INSIGHT INTO THE ENZYMATIC MECHANISM OF ARGININOSUCCINATE LYASE

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Structural and functional analysis of wild type and site-directed mutants of duck δ1 and δ2 crystallin are being used to investigate the enzymatic mechanism of argininosuccinate lyase (ASL). ASL catalyses the reversible breakdown of argininosuccinate to arginine and fumarate, a reaction involved in arginine biosynthesis and in the urea cycle. During evolution, overexpression of ASL in the avian eye lens, followed by gene duplication has resulted in two delta crystallins: δ2, the ASL orthologue, and the enzymatically inactive delta1. The crystal structures of duck δ1 and δ2 crystallin have been solved. Structural comparisons indicate that both intra- and inter-species conformational changes occur in two regions of the N-terminal domain. As the residues implicated in the catalytic mechanism of δ2/ASL are conserved in δ1, we postulate that amino-acid substitutions in these two regions of δ1 are important for substrate binding and hence catalysis. The crystal structure of δ1 crystallin revealed the presence of a sulfate anion in the active site region that may mimic the fumarate moiety of the argininosuccinate substrate. This induced a large conformational change in the 280's loop and a rigid body motion in domain 3. The results suggest that Ser 281 may play the role of the acid catalyst in the enzymatic mechanism of δ2/ASL. The crystal structure of the inactive S281A δ2 mutant with argininosuccinate bound to all four active sites was solved and together with extensive mutagenesis mapping of the active site region, provide further insight into the catalytic mechanism of ASL/δ2 crystallin.

Keywords: **ARGININOSUCCINATE LYASE, DELTA CRYSTALLIN, ENZYME MECHANISM**

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STRUCTURE-FUNCTION STUDIES OF EPSP SYNTHASE FROM *PSEUDOMONAS AERUGINOSA*

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The increase and spread of multidrug resistant strains of pathogenic bacteria has led to a growing need for new antimicrobial agents. The shikimate pathway, which leads to the biosynthesis of ring-containing compounds, is an important drug target. This pathway is present in bacteria, microbial eukaryotes, plants and apicomplexan parasites, but absent in mammals. The aroA-encoded 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase catalyses the sixth step of the pathway, converting shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) to EPSP and phosphate, and is the target for the broad-spectrum herbicide glyphosate.
This study aims to structurally and functionally characterise EPSP synthase from the bacterial pathogen *Pseudomonas aeruginosa*, which is known to be tolerant to glyphosate. *P. aeruginosa* EPSP synthase has been overproduced and purified to homogeneity, and subsequent crystallisation trials yielded large numbers of crystals which diffracted to high resolution. Data were collected to at least 1.6- Å resolution, solved by molecular replacement and refined using CNS and SHELX. Protein crystal structures were obtained of *P. aeruginosa* EPSP synthase in the uncomplexed state, and in complex with both S3P and glyphosate and S3P alone. These crystal structures were compared to understand the conformational changes induced by substrate and inhibitor. In addition, the interaction of glyphosate in the crystal structures of *P. aeruginosa* EPSP synthase and the glyphosate-sensitive *E. coli* EPSP synthase were compared to understand the origins of resistance in the former enzyme.

Keywords: **EPSP SYNTHASE GLYPHOSATE CONFORMATIONAL CHANGE**

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CRYSTALLIZATION AND PRELIMINARY CRYSTALLOGRAPHIC CHARACTERIZATION OF RECOMBINANT SUCROSE PHOSPHORYLASE FROM *STREPTOCOCCUS MUTANS*

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Sucrose phosphorylase(SP) is a central enzyme in the sucrose and starch metabolism. SP catalyze the reaction: sucrose + orthophosphate = D-Fructose + α-D-glucose 1-phosphate. SP is, based on sequence alignments, placed in the retaining glycoside hydrolase (GH) family 13 {Coutinho, P.M. & Henrissat, B. (1999)}. The family has an alpha amylase fold with a (β/α) 8 -barrel and a C-terminal β-sandwich domain. Crystals of recombinant SP from the oral bacteria *Streptococcus mutans*{Russell, 1988}, have been obtained by the vapor-diffusion method in the presence of polyethylenglycol 6000, with the additives Mg^{2+} and Ca^{2+}. The crystals diffract to a 2 and 3 Å resolution and they all belonged to the space group $P2_1$. The cell parameters are not constant, and we are currently working on a protocol to get reproducible cell parameters. Selenomethionine substituted SP has been produced and crystals grew under the same conditions as the native protein, but seeding was necessary. Preliminary results from MAD experiments on the selenomethionine variant will be presented.

Keywords: **PROTEIN CRYSTALLIZATION,SUCROSE-METABOLISM, ENZYME**