derivatives and performing SAD experiments on the MX beamlines at the ESRF in Grenoble. Biophysical and biochemical studies have also been performed in order to dissect the role of RecN and its various domains so as to propose a much more accurate mechanism of DSB recognition.

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Keywords: DNA repair, homologous recombination, RecN

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Structural insight into modes of substrate selectivity and catalysis in the shikimate dehydrogenase superfamily

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Shikimate dehydrogenase (SDH) catalyzes the reversible, NADPH-dependant reduction of dehydroshikimate to shikimate, a key step in the biosynthesis of the aromatic amino acids in plants, fungi and bacteria. The absence of the enzyme in animals makes it an attractive target for antibiotics. SDH belongs to an enzyme superfamily, the members of which utilize a common structural scaffold to catalyze reactions involving a range of substrates. We are exploring the diverse substrate preferences of the members of the SDH superfamily by x-ray crystallographic analysis of the enzymes. Our structural characterization of two SDH homologs has identified a complement of active site residues that appear to be important determinants of substrate preference. We investigate the biochemical role of these residues by site-directed mutagenesis. We further explore the significance of these residues by attempting to reengineer the substrate preference of one SDH homolog. In addition, we show by mutagenesis and kinetic analysis that an invariant pair of ionizable active site residues, a lysine and an aspartate, act as a catalytic dyad in two functionally distinct SDH homologs, providing evidence for a conserved catalytic mechanism across the SDH superfamily. Structural and mechanistic characterization of the members of the SDH superfamily will aid in the rational design of drugs targeting the enzymes.

Keywords: Dehydrogenase, enzyme structure, enzyme catalysis

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Crystal structure of Cytosine Deaminase complexed with a mimic of the tetrahedral intermediate

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Cytosine Deaminase from E.Coli. is a member of the amidohydrolase superfamily. The crystal structure of the zinc-activated enzyme was solved in the presence of a mimic of the tetrahedral intermediate. This compound inhibits the deamination of cytosine with Ki of 52 nM. The zinc and iron containing enzymes were characterized to determine the effect of the divalent cations on activation of the hydrolytic water.

Mutation of Gln-156 decreases the catalytic activity by more than 5 orders of magnitude, supporting its role in substrate binding. Mutations of Glu-217, Asp-313, and His-246 significantly decrease catalytic activity, supporting the role of these three residues in activation of the hydrolytic water molecule and facilitation of proton transfer reactions.

A chemical mechanism for substrate deamination by cytosine deaminase is proposed.

Keywords: enzyme, crystal, structure

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Crystal structure of flavin reductase from *Rhizobium* sp. strain MTP-10005

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Resorcinol hydroxylase from *Rhizobium* sp. strain MTP-10005 is a two-component enzyme system. The small component (GraD) is an an oxidoreductase containing a flavin molecule as a cofactor. GraD catalyzes the NADH-dependent reduction of free FAD according to a ping-pong bisubstrate-biproduct mechanism. The reduced FAD is then used by the large component GraA to hydroxylate resorcinol to hydroxyquinol.

GraD was crystallized at 293 K by the sitting-drop vapour-diffusion method using a precipitant solution containing 13 - 14% (w/v) PEG 2000, 6 - 9% (v/v) 2-propanol, 100 mM sodium citrate pH 5.6, 100 mM DTT and 200 μM FAD. The approximate dimension of the obtained crystals was 0.1 × 0.1 × 0.15 mm^3. The crystal diffracted to 1.8Å and belongs to space group $P4_12_12$ with unit cell parameters of $a = b =$ 77.7 Å and c = 124.2 Å. The asymmetric unit contains two molecules of GraD with a corresponding crystal volume per protein mass (V_M) of 2.35 $Å^3$/Da and a solvent content of 47.6% by volume. The crystal structure has been determined by molecular replacement and refined at 1.8 Å resolution. The current model was refined to an R-factor of 16.1% (R_{free} = 19.2%). GraD exists as a homodimer, and each monomer was found to contain an FAD.

Keywords: flavin, reductase, protein crystallography

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Unexpected reactions resulting from mutating catalytic residues in an amidase

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Nitrilase superfamily amidases catalyze the conversion of various amides to their corresponding acids and ammonia using highly conserved Cys, Glu, Glu, Lys (CEEK) catalytic residues. They find applications as potential biocatalysts in the fine chemical industry; as tools for drug synthesis; while those from prokaryotic organisms are attractive drug targets. Although the catalytic mechanism for these