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Keywords: protein, crystallography, glycosyltransferase

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Structural analysis of chondroitin polymerase from *Escherichia coli* K4

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Chondroitin, heparin/heparan and hyaluronan are the primary glycosaminoglycans found in humans and are linear polysaccharides consisting of an amino sugar and an uronic acid. Chondroitin chains range from 40 to over 100 repeating units of the disaccharide (GlcUA β (1–3)–GalNAc β (1–4)). Sulfated chondroitins are involved in the regulation of various biological functions such as central nervous system development, wound repair, infection, growth factor signaling, and morphogenesis, in addition to its conventional structural roles. Elongation of glycosaminoglycan chains is catalyzed by bi-functional glycosyltransferases. The bacterial chondroitin polymerase K4CP catalyzes elongation of the chondroitin chain by alternatively transferring the GlcUA and GalNAc moiety from UDP-GlcUA and UDP-GalNAc to the nonreducing ends of the chondroitin chain [1].

We previously reported the crystal structure of K4CP in the presence of UDP and UDP-GalNAc as well as with UDP and UDP-GlcUA [2]. The structures consisted of two GT-A fold domains in which the two active sites were 60Å apart. UDP-GalNAc and UDP-GlcUA were found at the active sites of the N-terminal and C-terminal domains, respectively.

Here, we present the crystal structure of K4CP complexed with chondroitin hexamer (CH6) and UDP at 3.5Å resolution. CH6 was found at the active sites of the N-terminal domain. In addition, we generated docking model of the C-terminal domain with chondroitin pentamer. These structural information revealed molecular basis of catalysis and substrate recognition, and provided the structural basis for further investigating the molecular mechanism of biosynthesis of chondroitin chain.

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FAD binding may inhibit a FMN-dependent Nitroreductase from *Idiomarina loihiensis* L2TR

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Nitroreductases are NAD(P)H-dependent flavoenzymes that catalyze the reduction of nitro groups on nitroaromatic or nitroheterocyclic compounds. The enzyme utilizes NAD(P)H to reduce, via Flavin mononucleotide (FMN), a wide variety of substrates bearing nitro groups to hydroxylamino or amino groups. The structure of an oxygen-insensitive NAD(P)H-dependent nitroreductase (YP_156458.1) from *Idiomarina loihiensis* L2TR has been determined at 1.9 Å resolution. Although very similar in structure to other nitroreductases, we report the first observation of the Flavin-adenine dinucleutide (FAD) cofactor bound to a nitroreductase. In addition, this is also the first structure in which both prosthetic groups, FMN and FAD, are bound to different molecules in the crystal asymmetric unit. There is no conformational change in the protein structure upon binding FAD vs. FMN, however, FAD adopts a novel binding mode in which the adenosine moiety folds back over its flavin moiety occupying the substrate binding pocket. The binding mode of FAD helps explain the reduced activity of FAD bound nitroreductase compared to FMN bound nitroreductase.

Keywords: nitroreductase, FMN, FAD

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Understanding the role of RecN in the DSB repair pathway of *Deinococcus radiodurans*

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Deinococcus radiodurans is well known for its ability to withstand high doses of ionizing radiations. Such treatments induce several hundreds of Double Strand Breaks in the DNA and *D. radiodurans* has therefore developed an highly efficient repair mechanism in order to restore the integrity of its genetic material. Multiple copies of the genome in concert with homologous recombination (HR) pathway lead to the complete repair of the DNA damages in only 3 hours [1].

Homologous recombination in *E. coli* is accomplished by RecBCD enzyme. In *D. radiodurans* no homologue of RecB and RecC proteins are encoded and the DNA repair is mainly carried out through the RecFOR pathway, which is responsible to load RecA protein onto the DNA.

The whole mechanism by which the RecFOR pathway repairs the DNA lesion is not fully understood yet. We are especially interested in understanding how the DSB recognition occurs inside the cell and how then the entire process takes place. In *D. radiodurans* the DNA damage could be detected by RecN protein, based on previous *in vivo* studies carried out in *Bacillus subtilis* [2].

RecN is an SMC-like protein that is made up of a "head" domain, structurally similar to the Nucleotide binding Domain (NBD) of ABC proteins, with which the protein hydrolyzes the ATP. The N- and C-termini of RecN are then connected by an unusually short coiled-coil domain, which is supposed to contain a dimerization site. We determined the crystal structures of both domains using Se-Methionine

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