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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 60 Å 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 3.5 Å 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.


Keywords: glycosyltransferase, crystallography, protein

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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 (FMIN), a wide variety of substrates bearing nitro groups to hydroxylamine or amino groups. The structure of an oxygen-insensitive NAD(P)H-dependent nitroreductase (YP_156485.1) 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 dinucleotide (FAD) cofactor bound to a nitroreductase. In addition, this is also the first structure in which both prosthetic groups, FMIN 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. FMIN, 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 FMIN bound nitroreductase.

Keywords: nitroreductase, FMIN, 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

Poster Sessions