



Study of capsule synthetic enzyme of *E.coli*

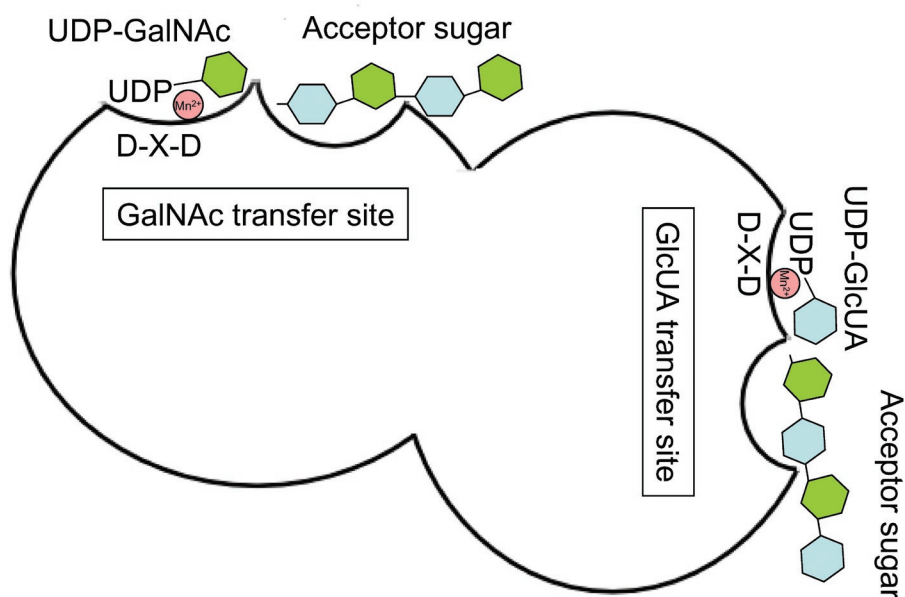
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Dr. Kakuta received his Ph.D from the Hokkaido University in 1995. His work with Dr. M. Negishi at NIH about crystal structure of sulfotransferases led to an interest in biosynthesis of sugar chain. He moved to the Osaka University in 1999 as Assistant Professor in the Department of Biological Sciences and moved to the Kyushu University in 2001 as Associate Professor in the Department of Bioscience and Biotechnology and Laboratory of Biochemistry.

Schematic representation of Chondroitin Polymerase



Escherichia coli strain K4 produces the K4 antigen, a capsule polysaccharide consisting of a chondroitin backbone (GlcUA (1-3)-GalNAc (1-4))_n to which -fructose is linked at position C-3 of the GlcUA residue. The region 2 of the K4 capsular gene cluster essential for biosynthesis of the polysaccharide, and we further identified a gene encoding a bifunctional glycosyltransferase that polymerizes the chondroitin backbone. The enzyme, containing two conserved glycosyltransferase sites, showed homologous at the amino acid level to class 2 hyaluronan synthase and chondroitin synthase from *Pasteurella multocida*. The soluble enzyme expressed in a bacterial expression system transferred GalNAc and GlcUA residues alternately. Based on these results, we have concluded the enzyme is K4 Chondroitin Polymerase (K4CP).

We have determined the crystal structure of K4CP in the presence of the donor substrate product UDP, the catalytic Mn(2+) ion. The enzyme is a alpha/beta protein with two domains that constitute the two glycosyltransferases. The conserved DXD motifs has direct interaction with the ribose of the UDP molecule as well as with the Mn(2+) ion. We will discuss about the mechanism of the biosynthesis of chondroitin chain based on the crystal structure.

Keywords : capsule synthesis, Chondroitin Polymerase, crystal structure