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Functional characterization of UDP-glucose-4-epimerase from Aspergillus nidulans

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Pathogenic fungi, namely Aspergillus spp., have gained notoriety in recent decades for posing an increased threat to human health[1]. Many challenges exist for treating fungal pathogens in human hosts due to the close evolutionary relationship between the eukaryotic systems[2]. In addition to the limited number of viable drug targets within fungi, most treatments are inhibited by either host toxicity or reduced potency due to the recent emergence of fungal drug resistance[3]. Antifungal drugs which block cell-wall synthesis are believed to be the most promising candidates for clinical treatment[4].

In this context, we are interested in the catalytic pathway of Aspergillus nidulans UDP-glucose-4-epimerase (GALE) which produces precursor building blocks for fungal lipopolysaccharide (LPS) biosynthesis. Specifically, GALE is responsible for reversibly inverting the 4'-hydroxyl configuration of UDP-glucose to form UDP-galactose. Given that GALE exhibits interspecies variation, targetable differences between GALE of the host and pathogen can be exploited for rational drug design. In order to identify such differences, detailed structural characterization of A. nidulans GALE is required. Ultimately, our goal is to elucidate the structure-function relationship responsible for galactose metabolism in A. nidulans GALE.

Crystals of A. nidulans GALE grown in the presence of UDP-Galactose via microbatch were diffracted to 2.4 Å resolution at the Canadian Light Source (CLS) 01HD-1 beamline. Examination of the data revealed non-merohedral twinning from which a single lattice was ultimately extracted for data processing. The final space group was C2 with unit-cell parameters a = 66.13Å, b = 119.15Å, c = 161.42Å, and β = 98.48°. The structure was solved by molecular replacement with human GALE (PDB code 1h2j) as the template model and subsequently refined through PHENIX. The electron density revealed the presence of NAD(H) co-factor and a UDP-sugar moiety bound within the active site. The structure is similar to human GALE and exhibits two distinct regions: an N-terminal domain characterized by a modified Rossman fold of six strands of parallel β-sheets flanked on either side by α-helices believed to be responsible for co-factor positioning and a C-terminal motif composed of six β-strands and five α-helices postulated for UDP-sugar binding. The characteristic XXXXX motif of the short chain dehydrogenase-reductase superfamily is contained within the N-terminal domain.

Inspection of the A. nidulans structure revealed nearly identical positioning of equivalent active-site residues which were shown to be responsible for NAD(H) anchoring and UDP-sugar binding in human GALE. Site-directed mutagenesis and kinetic studies on active-site residues indicate the A. nidulans mechanism is similar to that observed in the human form. Interestingly, while we have found A. nidulans GALE to be active with larger N-acetyl substrate derivatives, mutations which should inhibit the conversion of these substrates, based on previous biochemical studies of E. coli GALE, do not follow the expected trend.

Keywords: crystallography, enzymology, mutagenesis

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Structural study of the intertwined dimers of SH3 domains: biological implications of the domain swapping

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The structural study of the intertwined dimers of SH3 domains: biological implications of the domain swapping

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Domain swapping has been proposed as a potential mechanism for the protein aggregation which would acts as a starting point in the amyloid fibers formation. Sometimes, domain swapping yields intertwined oligomers. Up to date this phenomenon has been described in more than 50 proteins where the appearance of domain swapping has been characterized from their crystallographic structures and there is not additional information about its occurrence in solution and how takes place its formation. The crystallographic structure of the c-Src-SH3 domain shows an intertwined dimer in the asymmetric unit and its formation takes place in the solution upon addition of low molecular weight polyethylene glycol (PEG) [1]. The presence of this domain swapped oligomer was described before in molecular dynamics simulations studies of amyloidogenesis conducted with the monomeric NMR structure of the c-Src SH3 domain. These studies predicted the formation of two different types of aggregates: an open aggregate that propagates upon packing of the –RT loops of the monomers by swapping of the two strands in the loop and a closed aggregate consisting of an intertwined dimeric structure in which both monomers exchange their respective –RT loops [2]. Our results offer experimental validation to this model proving the existence of a closed dimeric structure in solution that is stabilized by the interactions provided by the PEG molecule. The crystallographic structure shows that the PEG molecule forms multiple hydrogen bonds with residues of both chains of SH3 domains either directly or through various molecules of water buried in the interface. Besides, interactions among the residues present in both chains of the intertwined structure would be implied in the stabilization of this dimer structure. The c-Src-SH3 intertwined structure is of special interest as the three hydrophobic shallow pockets that form the binding site is composed by residues of both chains and it is exposed to the solvent, providing two binding sites. However the titration of the intertwined dimer with polyproline rich peptides (VSL12, N55A and APP12) performed by means of fluorescence seems to indicate the presence of a single site of binding. These results might have some biological implications as the activity of the Src family tyrosine kinases are modulated by the intramolecular interaction of polyproline sequences with the SH3 domain. Besides the mutations of the oncogenic v-Src tyrosine kinase appears just in some loops of the SH3 domain and these mutations would have some effect in the propensity of this SH3 domain to form intertwined structures. We have performed a systematic study of mutation of several residues in the loops of the protein in order to characterize the formation of this intertwined dimer and their effect in the interaction of the SH3 domain with polyproline rich sequences.

Keywords: protein, structure, oligomer

Crystal structure of arabinose 5-phosphate isomerase from Bacteroides fragilis NCTC 9343 – a novel target for Gram-negative antibacterial drug development

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Lipopolysaccharide (LPS) is an essential outer membrane glycolipid located on the cellular surface of almost all gram-negative bacteria. LPS contains three moieties: lipid A, core oligosaccharide and O-antigen. The inner oligosaccharide core primarily consists of 2-keto-3-deoxy-D-manno-octulosonate (Kdo). Previous studies have shown that Kdo2-lipid A provides the minimal LPS requirement to sustain cell growth of the bacteria. Bacteria containing compromised LPS are less pathogenic and more susceptible to antibiotics. D-arabinose 5-phosphate isomerase (API) catalyzes the reversible 1,2-keto/aldohexose tautomeration of D-ribose 5-phosphate (Ru5P) to D-arabinose 5-phosphate (ASP) in the first step of Kdo biosynthesis. API is highly conserved among many Gram-negative bacteria, and is the main source of ASP in these bacteria. Thus, API provides an effective target for Gram-negative antibacterial drug development and a potent inhibitor will likely offer broad protection against many bacteria.

The crystal structure of arabinose 5-phosphate isomerase from Bacteroides fragilis NCTC 9343 (BFAPI) at 1.7 Å resolution contains a sugar isomerase domain (SIS) with an endogenous ligand, CMP-Kdo, bound at the active site. Here CMP-Kdo is the end product of Kdo biosynthesis and could act as a feedback inhibitor on BFAPI. BFAPI forms a tetramer both in solution and in the crystal. The active site in each monomer is located in a surface crevice at the tetramer interface and consists of highly conserved residues K50, S95, S97, T100 and D165 from the parent monomer and H79 and H186 from two separate adjacent monomers. BFAPI displays sequence similarity to KdsD, KpsF, GutQ and polysialic acid capsule expression protein. KdsD, KpsF and GutQ proteins are all API’s that catalyze the same chemical reaction, but are involved in different biological functions (KdsD in LPS biosynthesis, gutQ in hexitol D-glucitol metabolism, and KpsF in polysialic capsule biosynthesis). Structure and sequence analysis show that two highly conserved residues, H79 and H186, could play important catalytic roles in the isomerization reaction.

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Keywords: arabinose 5-phosphate isomerase, 2-keto-3-deoxy-D-manno-octulosonate (Kdo), structural genomics

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Structural basis for the wobbler mouse degeneration disorder caused by mutation in the subunit of the GARP complex

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Keywords: protein, structure, oligomer

The multisubunit Golgi-associated Retrograde Protein (GARP) complex is required for tethering and fusion of endosome-derived transport vesicles to the trans-Golgi network (TGN). Mutation of leucine-967 to glutamine in the Vps54 subunit of GARP is responsible for spinal muscular atrophy in the wobbler mouse, an animal model of amyotrophic lateral sclerosis (ALS). The crystal structure at 1.7Å resolution of the mouse Vps54 C-terminal fragment harboring leucine-967, in conjunction with comparative sequence analysis, reveals that Vps54 has a continuous β-helical bundle organization similar to that of other multisubunit tethering complexes. The structure shows that leucine-967 is buried within the β-helical bundle through predominantly hydrophobic interactions that are critical for domain stability and folding in vitro. Mutation of this residue to glutamine does not prevent integration of Vps54 into the GARP complex but greatly reduces the half-life and levels of the protein in vivo. Severely reduced levels of mutant Vps54 and, consequently, of the whole GARP complex therefore underline the phenotype of the wobbler mouse.

Keywords: golgi apparatus, vesicle trafficking, X-ray.