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Minireview

UDP-hexose 4-epimerases: a view on structure, mechanism and substrate specificity

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ABSTRACT

UDP-sugar 4-epimerase (GalE) belongs to the short-chain dehydrogenase/reductase (SDR) superfamily of proteins and is one of enzymes in the Leloir pathway. They have been shown to be important virulence factors in a number of Gram-negative pathogens and to be involved in the biosynthesis of different polysaccharide structures. The metabolic disease type III galactosemia is caused by detrimental mutations in the human GalE. GalE and related enzymes display unusual enzymologic, chemical, and stereochemical properties; including irreversible binding of the cofactor NAD and uridine nucleotide-induced activation of this cofactor. These epimerases have been found active on UDP-hexoses, the N-acetylated and uronic acid forms thereof as well as UDP-pentoses. As they are involved in different pathways and functions, a deeper understanding of the enzymes, and their substrate promiscuity and/or selectivity, could lead to drug and vaccine design as well as antibiotic and probiotic development. This review summarizes the research performed on UDP-sugar 4-epimerases' structure, mechanism and substrate promiscuity.

1. General introduction

Uridine diphosphate galactose 4-epimerase, or UDP-Gal 4-epimerase (GalE, EC 5.1.3.2), is one of the enzymes in the Leloir pathway.[1] This pathway is responsible for the conversion of galactose into glucose 1-phosphate with the help of four enzymes (Fig. 1). First, galactose mutarotase converts β-galactose into its α-anomer.[2,3] Next, this α-galactose is phosphorylated by galactokinase to produce galactose 1-phosphate.[4,5] In the third step, galactose 1-phosphate uridylyltransferase exchanges the phosphate group with UDP-glucose (UDP-Glc) to form UDP-galactose (UDP-Gal) and glucose 1-phosphate.[6,7] The latter is then further metabolized through glycolysis, while the former is epimerised by UDP-Gal 4-epimerase to regenerate UDP-Glc for the next cycle.[8,9] In case of detrimental mutations in one of these enzymes, carriers will suffer from the inherited metabolic disease type III galactosemia. Vomiting, jaundice, and lethargy are early symptoms of this rare but potentially deadly disease. Delayed complications include mental retardation, liver cirrhosis, renal failure, and cataract.[1] Fortunately, progression of the disease can be prevented by a galactose- and lactose-free diet. Just as for the other enzymes of the Leloir pathway, new insights in human GalE's conformation, stability, dynamics and ligand binding are often obtained via the study of disease-associated variants.[10] Over 20 of such variants of the protein have been described in the literature.[11] It has also been found that UDP-galactose 4-epimerase plays an essential role in development and homeostasis of *Drosophila* that extends beyond the Leloir pathway.[12]

On the other hand, UDP-sugars are also important as precursors in lipopolysaccharides (LPS). LPS is involved in several aspects of cell-cell interactions, such as host-pathogen interactions[13] and biofilm formation.[14,15] Apart from non-modified sugars, also N-acetylated and carboxylated sugars are found in LPS.[16,17] Consequently, the corresponding UDP-sugars have to be made by the cells and GalE-like UDP-sugars 4-epimerases are used for this purpose. UDP-galactose 4-epimerase is also found to be important for the biosynthesis of other polysaccharide structures, such as capsular polysaccharide (CPS),[15] extracellular polysaccharide (EPS) from *Streptococcus thermophiles*[18]—one of the most widely used bacteria in the dairy industry—and proteoglycans (PGs) of articular chondrocytes.[19] Together with UDP-glucose pyrophosphorylase (GalU), GalE is known to be an important virulence factor in a number of

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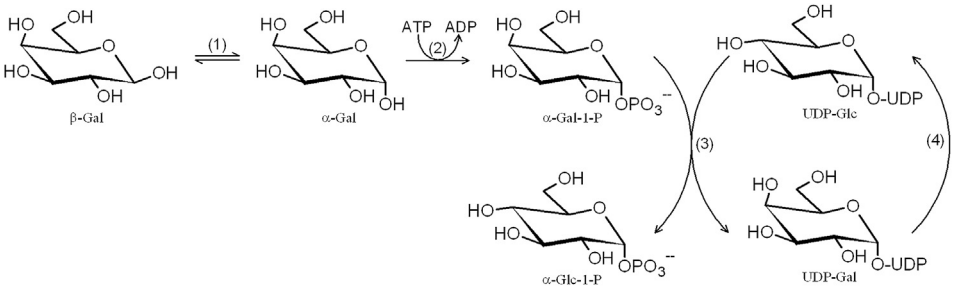

Fig. 1. The different enzymes involved in the Leloir pathway: (1) galactose mutarotase, (2) galactokinase, (3) galactose 1-phosphate uridylyltransferase and (4) UDP-galactose 4-epimerase.

Gram-negative pathogens[20] as well as essential for the expression of a serum-resistant phenotype of *Haemophilus parasuis*, the etiological agent of Glässer's Disease.[15] Swine herds infected by *H. parasuis* show increased nursery mortality resulting in substantial economic losses in the pig industry.

The UDP-Gal 4-epimerases (and the other GalE-like UDP-sugar 4-epimerases) belong to the short-chain dehydrogenase/reductase (SDR) superfamily of proteins. These enzymes show great functional diversity and despite their lower sequence identities (typically only 15–30%) specific sequence motifs are detectable, reflecting their common folding patterns. SDRs are widely spread in nature and involved in different physiological processes such as normal and metastatic growth, hypertension and fertility.[1] GalE and related enzymes display unusual enzymologic, chemical, and stereochemical properties; including irreversible binding of NAD and uridine nucleotide-induced activation of NAD,[21] which will be further addressed below. The UDP-sugars 4-epimerase family consists of epimerase active on UDP-Glc/UDP-Gal, N-acetylated forms (UDP-GlcNAc/UDP-GalNAc),[16] uronic acid forms (UDP-GlcUA/UDP-GalUA)[17] as well as UDP-pentoses (UDP-L-Ara/UDP-Xyl)[22] or on combinations thereof.[13,14,23,24] Attempts have been made to elucidate the determinants for substrate specificity of UDP-sugars 4-epimerases as this can render information to use these epimerases as targets for new antibiotics and drugs.[25] Substrate specificity will be further discussed at the end of this review.

2. Structure

UDP-Gal 4-epimerases are formed as homodimers, and the *E. coli* monomer, for example, contains 338 amino acids resulting in a molecular mass of 37.3 kDa.[26] The human epimerase is slightly longer (348 amino acids) and its dimer weighs 76.6 kDa (twice 38.3 kDa). Given that UDP-Gal 4-epimerase is part of the big SDR superfamily, it displays some characteristics that are typical for its members. For example, the YxxxK motif and the GxxGxxG motif are two of the encountered signature sequences.[27,28] The former contains the characteristic Tyr/Lys couple that plays a key role in catalysis. The repetitive glycine motif is located in the Rossmann fold,[29] which is typical for nucleotide binding enzymes, and the first 2 glycines participate in NAD^+ binding whereas the third glycine facilitates close packing of the helix to the beta-strand.[30]

Crystal structures of several GalE homologues (and variants) have been determined using X-ray crystallography, including that of *E. coli*,[26,31–34] human GalE[28,35,36] and the group 3 WbpP from *Pseudomonas aeruginosa*.[13] In the yeast *Saccharomyces cerevisiae*, UDP-galactose 4-epimerase and galactose mutarotase are contained within a single polypeptide chain referred to as Gal10p. The structure of this dual function enzyme has also been determined.[37]

The structure of the *E. coli* GalE[26] is discussed here in more detail. In Fig. 2, the overall structure is shown of its Y299C variant in complex with UDP-N-acetylglucosamine (PDB code 1LRK), which was chosen because it highlights the importance of the gate keeper residue for substrate specificity.[31] Two different domains are distinguished; an N-terminal nucleotide binding domain and a smaller C-terminal domain that is responsible for the correct positioning of its substrate, a UDP-sugar. The N-terminal domain comprises seven parallel β-strands that are flanked on both sides by α-helices and shape the Rossmann fold.[38,39] Two paired Rossmann folds tightly bind one NAD^+ cofactor per subunit. In *E. coli* GalE, the NAD^+ interacts more extensively with the protein than was observed with other SDR enzymes. A total of 35 protein-NAD^+ contacts were observed with distances up to 3.2 Å, of which seven were mediated by ordered water molecules, while other SDR enzymes showed 22–27 contacts. This results in irreversible denaturation of the enzyme after removal of the cofactor.[32] In contrast, the NAD^+ cofactor could be removed from human GalE without denaturation. Here, fewer protein-NAD^+ contacts were observed in the crystal structure, which explains the reversible character of cofactor binding.[35] The C-terminal domain is built from five β-strands and four α-helices. As the domains are necessary for the binding of the cofactor and the substrate, respectively, the active site is located between these two domains.[38,39] Determination of the structure of human UDP-Gal 4-epimerase, revealed an active site that was 15% larger than that of the *E. coli* enzyme. A possible explanation was found in the secondary role of the human enzyme, which is epimerization of UDP-N-acetylgalactosamine (UDP-GalNAc). Activity on the larger acetylated substrates would require a larger active site.[28,40]

3. Mechanism

The GalE mechanism was already studied and broadly outlined in the 1970s.[41,42] The first step after substrate binding is the abstraction of the hydroxylic proton at C4 by an enzymatic base and the transfer of a hydride from the C4 position of the sugar to NAD^+ to form NADH and a transient keto-sugar. The characteristic tyrosine residue in the active site can act as enzymatic base responsible for deprotonation, since it occurs in a phenolic form stabilized by the nearby lysine residue.[43] Initially, it was believed that the distance between this tyrosine and the substrate was too large for direct proton abstraction[33] and had to be bridged by a proton shuttle, with the conserved serine in the structure of *E. coli* GalE being suggested for that function.[34] Based on the human GalE structure, however, the hypothesis of a proton shuttle by the conserved serine (or threonine) was disproved. It was found that the serine facilitates both the removal of the 4′-hydroxyl hydrogen of the sugar by the phenolic tyrosine and the transfer of the hydride