Conformational change upon product binding to *Klebsiella pneumoniae* UDP-glucose dehydrogenase: A possible inhibition mechanism for the key enzyme in polymyxin resistance

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ABSTRACT

Cationic modification of lipid A with 4-amino-4-deoxy-L-arabinopyranose (L-Ara4N) allows the pathogen *Klebsiella pneumoniae* to resist the antibiotic polymyxin and other cationic antimicrobial peptides. UDP-glucose dehydrogenase (Ugd) catalyzes the NAD$^+$-dependent twofold oxidation of UDP-glucose (UPG) to produce UDP-glucuronic acid (UGA), a requisite precursor in the biosynthesis of L-Ara4N and bacterial exopolysaccharides. Here we report five crystal structures of *K. pneumoniae* Ugd (*Kp*Ugd) in its apo form, in complex with UPG, UPG/NADH, two UGA molecules, and finally with a C-terminal His$_6$-tag. The UGA-complex structure differs from the others by a 14° rotation of the N-terminal domain toward the C-terminal domain, and represents a closed enzyme conformation. It also reveals that the second UGA molecule binds to a pre-existing positively charged surface patch away from the active site. The enzyme is thus inactivated by moving the catalytically important residues C253, K256 and D257 from their original positions. Kinetic data also suggest that *Kp*Ugd has multiple binding sites for UPG, and that UGA is a competitive inhibitor. The conformational changes triggered by UGA binding to the allosteric site can be exploited in designing potent inhibitors.

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1. Introduction

The opportunistic pathogen *Klebsiella pneumoniae* can cause urinary tract and blood stream infections, as well as severe pneumonia with a high rate of mortality and morbidity (Podschun and Ullmann, 1998). It is emerging as a major causative agent of multi-drug resistant infections and pyogenic liver abscess frequently complicated by metastatic meningitis or endophthalmitis (Fang et al., 2004; Wu et al., 2009). Expression of two essential virulence factors, capsular polysaccharide (CPS) and lipopolysaccharide (LPS), in *K. pneumoniae* clinical isolates (Huh et al., 2004) allows it to escape from host innate immune responses (Easley et al., 2007). The LPS modifications with 4-amino-4-deoxy-L-arabinopyranose (L-Ara4N) further confer *K. pneumoniae* resistance to the antibiotic polymyxin (Helander et al., 1996), a member of cationic

antimicrobial peptides (CAMPs). Functioning as a key component of the innate immune system (Sommer et al., 2004), CAMPs act by binding to the negatively charged lipid A of LPS on the bacterial cell surface, leading to membrane permeabilization and cell death (Burtnick and Woods, 1999; Gatzeva-Topalova et al., 2005; Princivalle and de Agostini, 2002). In order to evade the bactericidal action of CAMPs, some Gram-negative bacteria have developed mechanisms to modify the phosphate group of lipid A with the positively charged sugar L-Ara4N (Burtnick and Woods, 1999; Guo et al., 1997; Princivalle and de Agostini, 2002). By reducing the surface negative charge, such modification decreases the binding affinity and results in the resistance to CAMPs (Ordman and Kirkwood, 1977a). In *K. pneumoniae* CG43, polymyxin B (PXB) resistance was demonstrated to be correlated to the expression of UDP-glucose dehydrogenase (Ugd; EC 1.1.1.22), a LPS modification enzyme involved in L-Ara4N biosynthesis (Cheng et al., 2010).

Ugd belongs to a small group of NAD$^+$-dependent 4-electron oxidoreductases and catalyzes the conversion of UDP-glucose (UPG) to UDP-glucuronic acid (UGA), the initial step of the L-Ara4N biosynthesis pathway (Fig. S1) (Breazeale et al., 2005; Huh et al., 2004). UGA also serves as a precursor for synthesis of bacterial antiphagocitic CPS, colanic acid (M-antigen), plant cell walls, and mammalian glycosaminoglycans (Hung et al., 2007; Lacour et al.,

2008), and it is critical to bacterial virulence (Jiang et al., 2010) as required for the biosynthesis of extracellular polysaccharide. Mutations in the *ugd* gene of *Xanthomonas campestris* lead to loss of pathogenicity (Burtnick and Woods, 1999). In other pathogenic bacteria like *Burkholderia cenocepacia* (Gatzeva-Topalova et al., 2005) and *B. pseudomallei* (Burtnick and Woods, 1999), insertion mutagenesis studies showed that *ugd* is essential for viability and PXB resistance. Moreover, *ugd* deletion in *Pseudomonas aeruginosa* increased its susceptibility to PXB, chloramphenicol, cefotaxime, and ampicillin (Hung et al., 2007). A recent report on *Proteus mirabilis* showed that the expression of Ugd can be induced by PXB (Jiang et al., 2010). Therefore, limiting UGA availability by inhibiting Ugd activity may represent a strategy against bacterial infections.

The reaction catalyzed by Ugd is outlined in Fig. 1. Kinetic studies have been reported for the bovine (Ridley and Kirkwood, 1973), streptococcal (Campbell et al., 1997), *Arabidopsis thaliana* (Thompson et al., 1994) and human (Easley et al., 2007) enzymes. The streptococcal Ugd follows a bi-uni-uni-bi ping-pong mechanism in which UPG is bound first and UGA is released last

(Campbell et al., 1997; Ordman and Kirkwood, 1977b). The proposed mechanism involves an aldehyde intermediate covalently bound to a lysine residue, nucleophilic attack of an active-site cysteine on the aldehyde, and irreversible hydrolysis of the thioester intermediate (Campbell et al., 2000; Ge et al., 1998; Ordman and Kirkwood, 1977a; Ridley and Kirkwood, 1973). This cysteine residue is conserved across all species and essential for the second step of oxidation (Ge et al., 2004; Sommer et al., 2004). Mutation of strictly conserved active-site residues in human Ugd, which correspond to K197, C253, K256, D257 and K307 in *Kp*Ugd (Fig. S2), revealed their functional importance (Easley et al., 2007; Sommer et al., 2004). On the other hand, Ugd activity is competitively inhibited by UGA in a feedback regulation for making various UDP-sugars in plants (Campbell et al., 1997; Dalessandro and Northcote, 1977; Thompson et al., 1994), while UPG exhibits slight allosteric effects in *Streptococci*, *P. aeruginosa* and *B. cenocepacia* (Campbell and Tanner, 1997; Campbell et al., 1997; Hung et al., 2007).

The X-ray structures of Ugd from *Streptococus pyogenes* (*Sp*Ugd; PDB codes: 1DLI and 1DLJ) (Campbell et al., 2000), *Porphyromonas gingivalis* (*Pg*Ugd; PDB code: 3GG2), *Caenorhabditis elegans* (*Ce*Ugd;
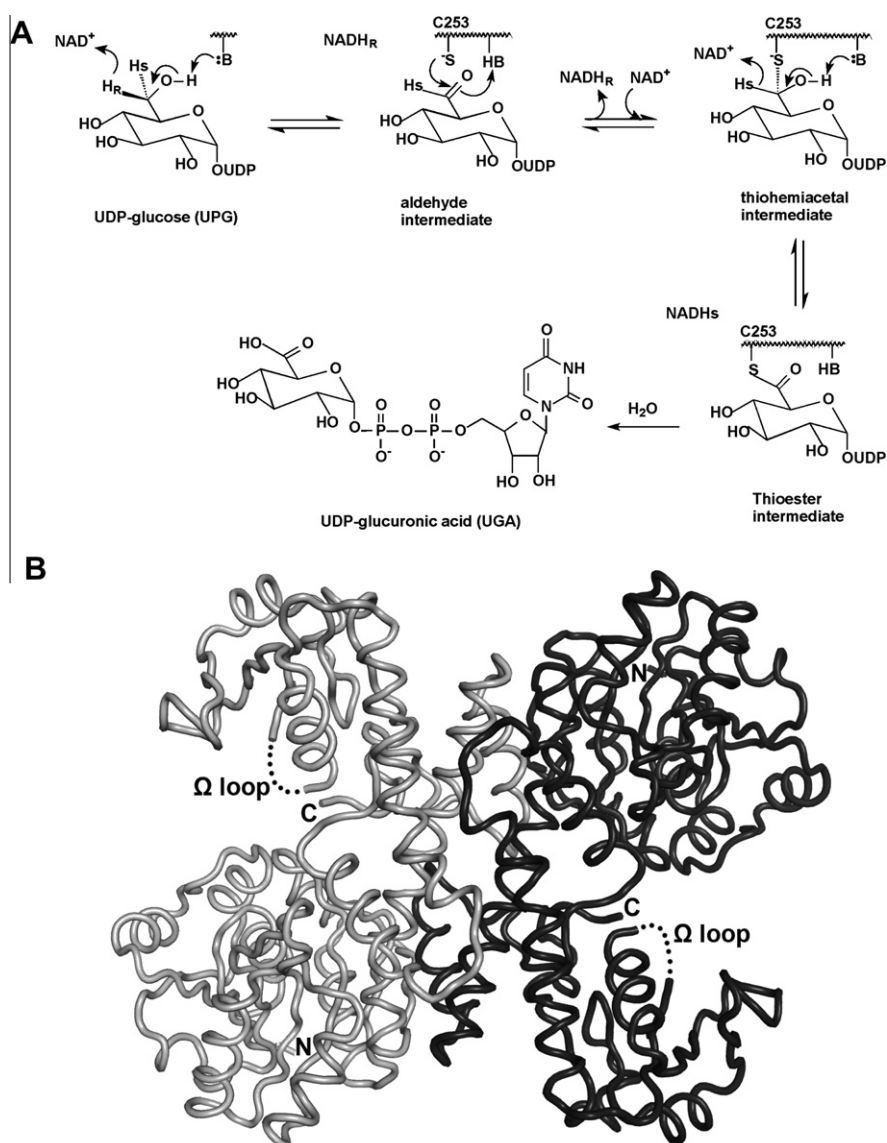

Fig. 1. The reaction mechanism and overall structure of Ugd. (A) Ugd catalyzes two successive NAD⁺-dependent oxidation reactions of UPG to yield UGA. The last step, hydrolysis of the covalent UGA-enzyme intermediate is essentially irreversible. (B) A dimer is the basic assembly unit for Ugd. For clarity, the *Kp*Ugd/6His structure is shown with the two subunits in different shades of gray.