# New UDP-GlcNAc C4 Epimerase Involved in the Biosynthesis of 2-Acetamino-2-deoxy-L-altruronic Acid in the O-Antigen Repeating Units of *Plesiomonas shigelloides* O17[†]

Przemyslaw Kowal and Peng George Wang*

*Department of Chemistry, Wayne State University, Detroit, Michigan 48202*

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ABSTRACT: *Plesiomonas shigelloides* is a ubiquitous waterborne pathogen responsible for diseases such as diarrhea and bacillary dysentery, commonly afflicting infants and children. This bacterium is endowed with an O-antigen gene cluster consisting of 10 consecutive reading frames. One of these, designated *wbgU* (*orf3*), has been overexpressed and biochemically characterized to show that it encodes a uridine diphosphate−*N*-acetylglucosamine (UDP-GlcNAc) C4 epimerase, only the second microbial enzyme characterized to have this activity. Epimerization is an equilibrium reaction resulting in a 70:30 ratio of UDP-GlcNAc to uridine diphosphate−*N*-acetylgalactosamine (UDP-GalNAc), irrespective of the initial substrate. The $K_m$ values for UDP-GalNAc and UDP-GlcNAc are 131 $\mu$M and 137 $\mu$M, respectively. WbgU is also capable of converting nonacetylated derivatives but with much lower efficiency. It contains a tightly bound nicotinamide adenine dinucleotide [NAD(H)] molecule and requires no other cofactors for activity. We propose here that this enzyme catalyzes the first of the three transformations in the biosynthetic pathway of 2-acetamino-2-deoxy-L-altruronic acid, an unusual sugar present in the O-specific side chains of lipopolysaccharide of *P. shigelloides* O17 and its close relative *Escherichia coli* Sonnei.

*Plesiomonas shigelloides* is a Gram-negative flagellated bacterium common to virtually all environments. It has been isolated from organisms such as humans, birds, fish, and water insects (*1*) and is associated with diarrheal diseases (*2*). *P. shigelloides* serotype O17 and its close genetic relative *Escherichia coli* Sonnei (*Shigella sonnei*) possess virtually identical O-antigen biosynthetic gene clusters (*3*). The O-antigen genes in *E. coli* Sonnei are uncommonly located on a plasmid, the invasion plasmid Pinv. The plasmid is 180 kb in size and is necessary for penetration of host epithelial cells (*4*−*6*). Genetic analyses have shown that *E. coli* Sonnei obtained its plasmid-borne cluster from *P. shigelloides* through a recent horizontal gene transfer event (*7*). The cluster includes 10 open reading frames (*orf1*−*orf10*) that have been assigned putative functions on the basis of sequence homology.

The O-antigen, composed of many repeating oligosaccharide units, is part of the lipopolysaccharide (LPS)[1] in the outer membrane of Gram-negative bacteria. It contributes antigenic variability to the cell surface and confers O-serotype specificity. *E. coli* Sonnei has an O-antigen identical to that of serotype 17 *P. shigelloides* (*8*, *9*). The repeating unit consists of two unusual sugars: 2-acetamino-4-amino-2,4,6-trideoxy-D-galactose (4n-FucNAc) and 2-acetamino-2-deoxy-L-altruronic acid (2Ac-AltUA) (*10*). The presence of the latter was first demonstrated in *E. coli* Sonnei lipopolysaccharide 25 years ago (*11*); however, the pathway for its biosynthesis remains to be deciphered.

Three of the 10 genes in the O-antigen biosynthetic cluster of *P. shigelloides* have a suggested involvement in the biosynthesis of 2Ac-AltUA. *Orf2*, designated *wbgT*, has significant homology to a variety of UDP-glucose/GDP-mannose dehydrogenases. *Orf3*, designated *wbgU*, shows homology to nucleotide sugar epimerases, and *orf10*, named *wbgZ*, shares homology with numerous surface polysaccharide biosynthetic genes that are likely nucleotide sugar epimerases or dehydratases.

WbgU belongs to the short-chain dehydrogenase/reductase (SDR) enzyme family. It is 64% identical to WbpP protein of *Pseudomonas aeruginosa* serogroup O6, the only bacterial UDP-GlcNAc C4 epimerase characterized biochemically thus far (*12*). The functional assignment of putative UDP-GlcNAc 4-epimerase genes is complicated by their close relationship to other members of this group. The SDR enzyme family possesses a characteristic GxxGxxG motif contained within an alternating $\alpha$ and $\beta$ structure of the nucleotide-binding Rossmann fold at the N-terminus. Additionally, sequence alignment experiments have revealed a conserved $S(x)_{24}$-$Y(x)_3K$ triad possibly involved in catalysis. These features are shared among enzymes of varying activities such as UDP-Glc C4 epimerase (GalE) and RFFG, a dTDP-glucose-4,6-dehydratase from *E. coli*. Therefore, thorough biochemical characterization is necessary to make an unambiguous

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* Corresponding author: Department of Chemistry, Room 329, Wayne State University, 5101 Cass Ave., Detroit, MI 48202. Phone 313-993-6759; fax 313-577-5831; e-mail pwang@chem.wayne.edu.

[1] Abbreviations: LPS, lipopolysaccharide; UDP, uridine diphosphate; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; 4n-FucNAc, 2-acetamino-4-amino-2,4,6-trideoxy-D-galactose; 2Ac-AltUA, 2-acetamino-2-deoxy-L-altruronic acid; SDR, short-chain dehydrogenase/reductase; IPTG, isopropyl 1-thio-$\beta$-D-galactopyranoside; PAGE, polyacrylamide gel electrophoresis; CE, capillary electrophoresis.

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functional assignment. In this study we present biochemical evidence that WbgU catalyzes an epimerization reaction interconverting UDP-GalNAc and UDP-GlcNAc.

## MATERIALS AND METHODS

*Materials.* Plasmid vector pET15b was purchased from Novagen Inc., Madison, WI. Ni−NTA agarose, PCR purification kit, Qiaex II gel extraction kit, QIAamp tissue kit, and DNA miniprep spin kit were from Qiagen, Santa Clarita, CA. All restriction enzymes, Taq DNA polymerase, 1 kb DNA ladder, and T4 DNA ligase were obtained from Promega, Madison, WI. UDP-GlcNAc, UDP-GalNac, UDP-Glc, UDP-Gal, and all other chemicals were from Sigma, St. Louis, MO, unless otherwise noted. Low-range protein standards were from Bio-Rad, Madison, WI. All enzymes and kits were used according to manufacturer's instructions.

*Bacterial Strains. Plesiomonas shigelloides* O17 strain C27 was purchased freeze-dried from ATCC (14030). *E. coli* DH5α (*lacZ*Δ*M15 hsdR recA*) competent cells were from Gibco−BRL Life Technology, Rockville, MD, and *E. coli* BL21(DE3) [$F^-$ *ompT hsdS*$_B$ ($r_B^-$ $m_B^-$) *gal dcm* (DE3)] competent cells were from Novagen Inc., Madison, WI.

*Cloning and Overexpression of the Enzyme. P. shigelloides* chromosomal DNA was isolated directly from the freeze-dried culture obtained from the ATCC. *WbgU* gene was amplified by polymerase chain reaction (PCR) from chromosomal DNA with 5′-GAATCTCGAG*ATG*GATATTTATATGTCTC-3′ (*Xho*I restriction site underlined, start codon in italic type) and 5′-CGCGGATCC*TTA*GCCTTTTAAAAATCTC-3′ (*Bam*HI restriction site underlined, stop codon in italic type) for the forward and reverse primers, respectively. The PCR fragment was inserted into the *Xho*I and *Bam*HI cloning site of pET15b plasmid with an N-terminal histidine tag. Integrity of the construct was confirmed by restriction analysis and sequencing. The construct pET/WbgU was subsequently transformed into DH5α for amplification and then into BL21(DE3) for protein expression. The 1-L Luria−Bertani (LB) broth culture was induced with 0.15 mM IPTG (isopropyl 1-thio-β-D-galactospyranoside) (250 rpm, 30 °C, 8 h) after it reached 1 $OD_{600}$ unit. The pelleted cells were stored at −20 °C until needed.

*WbgU Purification by Chromatography*. All chromatographic steps were carried out at 4 °C. The cells were lysed with lysozyme and then sonicated (Branson sonifier 450, Danbury, CT) to shear the nucleic acids. The sample was centrifuged for 30 min at 31000*g* and the clear lysate was loaded onto a Ni−NTA (nickel−nitrilotriacetic acid) agarose affinity column (15 mL bed volume) equilibrated with 50 mM Tris-HCl, pH 8.7, 400 mM NaCl, and 5 mM imidazole. The column was washed with 5 column volumes of 20 mM imidazole in the same buffer, and the protein was eluted with 200 mM imidazole.

Protein fraction was diluted with 50 mM Tris-HCl buffer, pH 8.9, loaded onto a 5 mL Mini-Q anion-exchange cartridge (Bio-Rad, Madison, WI), and eluted with a linear gradient of 40−400 mM NaCl over 10 column volumes. WbgU was concentrated by use of centrifugal concentrators with a 10K molecular weight cutoff (Millipore, Bedford, MA) and then dialyzed against 50 mM Tris-HCl buffer, pH 9.0, with an 8000 molecular weight cutoff membrane.

*Determination of Oligomerization Status by Gel Filtration.* A 60 × 1.6 cm Superdex 200 column (Amersham Pharmacia Biotech) was used for oligomerization status determination of WbgU. The column was equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 100 mM NaCl. Molecular mass standards (14−158 kDa, 200 μg each in 400 μL) were injected and the column was run at 0.5 mL/min. WbgU was applied to the column under identical conditions.

*Enzyme Activity Assay*. The reaction mixture consisted of 1 mM substrate (UDP-GlcNAc or UDP-GalNAc), 20 mM Tris-HCl buffer, pH 8.0, and varying amounts of freshly prepared enzyme in a total volume of 50 μL. The reaction was carried out at 37 °C for 15 min and was quenched by boiling for 6 min. Analysis was performed by capillary electrophoresis (CE) with Isco model 3850 electropherograph. Electrophoresis conditions were as follows: 75 μm i.d., bare fused silica capillary (MicroSolv Technology Corp., Eatontown, NJ), 50 cm total length, 40 cm to detector, 250 V/cm, 4 s vacuum injection, with monitoring at 262 nm and 25 mM sodium tetraborate running buffer, pH 9.4.

*Determination of Physicochemical Properties: Temperature and pH Range*. Temperature experiments were conducted with 250 ng of WbgU in 50 μL of buffer containing 50 mM Tris-HCl, pH 8.5, and 0.5 mM UDP-GalNAc for 5 min. Activity was checked between 10 and 60 °C in 10 °C increments as well as at 37 °C. Activity under differing pH conditions was checked at 37 °C.

*Determination of Cofactor Requirement*: *NAD*(*H*) *and Divalent Metals*. To assess the need for external cofactors, reactions were set up as described above with the addition of $NAD^+$ (1 mM) and divalent metal (5 mM $MgCl_2$, $MnCl_2$, and $CaCl_2$) and compared to WbgU activity in reactions lacking these components. In addition, activity of the enzymes was evaluated in the presence of 10 mM β-mercaptoethanol.

*Extraction of NAD*(*H*) *from Purified WbgU*. NAD(H) extraction was performed as described previously (*12*). Spectra were recorded on a DU 530 spectrophotometer (Beckman Instruments, Fullerton, CA) equipped with a 50-μL microcell.

*Determination of Kinetic Parameters for UDP-GlcNAc and UDP-GalNAc*. Reactions were performed at 37 °C in 20 mM Tris-HCl, pH 8.5, and a total volume of 40 μL. Substrate concentrations were varied from 0.05 to 1.2 mM with 75 and 150 ng of enzyme used for UDP-GalNAc and UDP-GlcNAc, respectively. Time course studies were performed at 0.05 and 1.2 mM and quenched at 0, 1, 2, 3, 5, 7, 10, and 15 min. Substrate concentrations between 0.05 and 1.2 mM were used to determine the $K_m$ and $V_{max}$ parameters with reactions quenched after 2 min by boiling for 6 min. Samples were analyzed by capillary electrophoresis as described under Enzyme Activity Assay.

*Determination of Kinetic Parameters for UDP-Glc and UDP-Gal.* The reactions consisted of 20 mM Tris-HCl, pH 8.5, with substrate varying from 0.05 to 1.2 mM in a total volume of 40 μL. Thirty micrograms of enzyme was used for both substrates. Reactions were quenched after 3 min for UDP-Gal and 5 min for UDP-Glc by boiling for 6 min. The analysis was carried out by CE as described.

## RESULTS

*Protein Expression and Purification*. WbgU is a 38.4 kDa protein with a neutral isoelectric point (p$I$ = 7.1). The

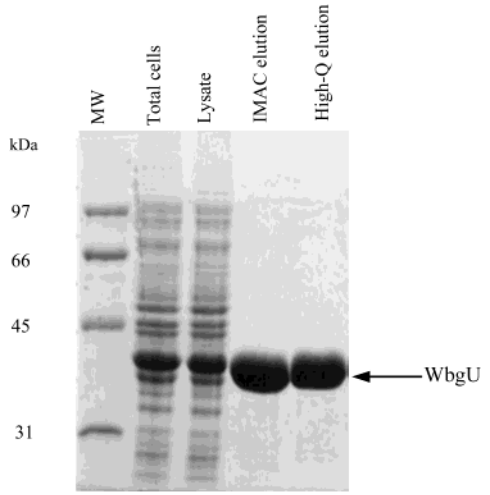


FIGURE 1: SDS−PAGE analysis of WbgU expression and purification. Aliquots were removed from each step of purification as described under "Experimental Procedures and loaded onto an SDS−15% polyacrylamide gel. Protein was detected with Coomassie Brilliant Blue.

enzyme was expressed in the pET system as an N-terminally polyhistidine tagged protein. Target protein expression was very high and comprised about 30% of the total cellular protein. WbgU expressed almost entirely in soluble form provided that the expression was carried out at 30 °C with a significantly lowered IPTG concentration of 0.15 mM (Figure 1). Immobilized Ni affinity column was used as the first chromatographic step, resulting in 90−95% pure protein. Most of the remaining contaminants could be removed with anion-exchange chromatography, giving >95% purity. Therefore only 3-fold purification was required to reach homogeneity. On average, 60 mg of WbgU could be obtained per 1 L of culture.

Gel-filtration experiments produced a single peak corresponding to an approximately 80 kDa protein, indicating that WbgU is a dimer under the experimental conditions. No monomer or polymeric forms were detected.

*Characterization of Activity by Capillary Electrophoresis.* Activity assay indicated that the protein is very active toward UDP-GlcNAc and UPD-GalNAc substrates. Capillary electrophoresis was used to confirm the identity of the reaction products by comparison to standard compounds. The epimers could be baseline-resolved with 12.2 and 12.7 min retention times for UDP-GlcNAc and UDP-GalNAc, respectively (Figure 2). The epimerization reaction, when complete, consistently resulted in a 70:30 ratio of UDP-GlcNAc to UDP-GalNAc, irrespective of the initial substrate. However, the reaction with UDP-GalNAc was approximately twice as fast as that with UDP-GlcNAc. Therefore, to keep the reaction rates the same, half as much enzyme was used with UDP-GalNAc in comparison with UDP-GlcNAc (Figure 3). To achieve reasonable conversion rates for the UDP-Glc and UDP-Gal derivatives, the amount of enzyme used was increased 200-fold. Substrate conversions reached 20% for UDP-Glc and 65% for UDP-Gal (Figure 4); hence the final equilibrium reached was dependent on the initial substrate, unlike the case with N-acetylated substrates.

*Biophysical Properties and Cofactor Requirements.* WbgU exhibits the highest activity at 37 °C. Twenty percent maximal activity could be detected at temperature as low as 10 °C and as high as 50 °C. The enzyme is active in a very broad pH range and in a variety of buffers (Tricine, Bis-tris propane, Tris-HCl) with highest activity between pH 7.0 and 9.0. The activity is independent of divalent metals, and no external $NAD^+$ needs to be added. The addition of $\beta$-mercaptoethanol has been found to stabilize the enzyme, increasing its lifetime in the reaction mixture by 50%, but had no effect on the rate of the reaction.

*NAD(H) Extraction.* NAD(H) extraction was performed to ascertain the stoichiometric ratio of cofactor to protein. The bound cofactor could be extracted by proteinase K degradation of WbgU. The released cofactor was reduced with sodium borohydrate ($NaBH_4$). Yields of 0.65 to 0.75 mol of NAD(H)/mol of protein were obtained, indicating tight binding of the nucleotide to the protein during its synthesis.

*Determination of Kinetic Parameters for UDP-GlcNAc and UDP-GalNAc.* Time course experiments performed at different enzyme dilutions indicated that the rate of conversion of UDP-GlcNAc is slower than that of UDP-GalNAc. To ensure initial rate conditions, enzyme dilutions were selected such that substrate conversion of 10% or less was achieved after a 2 min reaction. The kinetic parameters obtained are listed in Table 1. The $K_m$ values are 137 $\mu$M and 131 $\mu$M for UDP-GlcNAc and UDP-GalNAc, respectively.

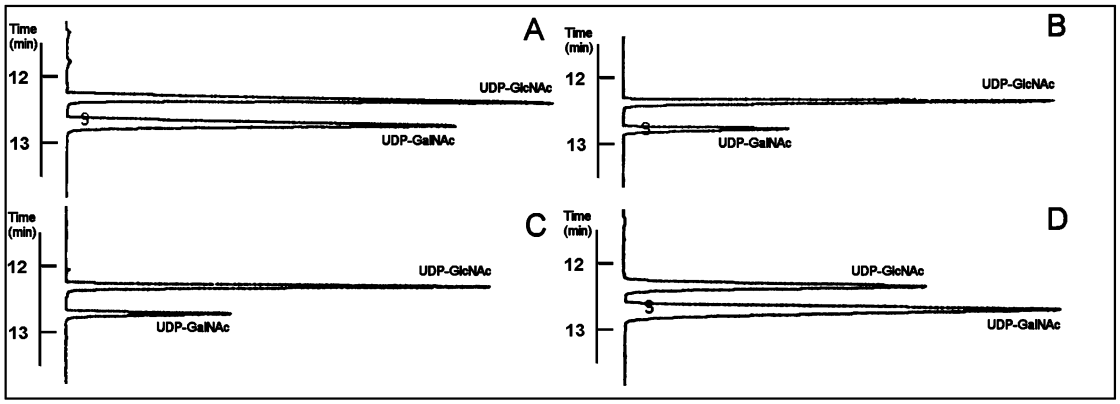


FIGURE 2: Capillary electrophoretic traces of the UDP-GlcNAc C4 epimerase reaction after reaching equilibrium. (a) Standard mixture of UDP-GlcNAc and UDP-GalNAc, 1 mM each; (b) UDP-GlcNAc reaction; (c) UDP-GalNAc reaction; (d) spiked UDP-GlcNAc reaction (1 mM UDP-GalNAc added after quenching). In each case, the UDP-sugar substrate was used at 1 mM. UDP-GlcNAc has a retention time of 12.2 ± 0.1 min and UDP-GalNAc has a retention time of 12.7 ± 0.1 min.

Table 1: Kinetic Parameters for WgbU and Its Four Substrates as Determined by Capillary Electrophoresis[a]

| substrate | $K_m$ ($\mu$M) | $V_{max}$ (pmol/min) | enzyme (pmol) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($mM^{-1}$ $min^{-1}$) |
|---|---|---|---|---|---|
| UDP-GlcNAc | 137 ± 17 | 1707 ± 122 | 3.7 | 461 ± 33 | 3443 ± 403 |
| UDPGalNAc | 131 ± 10 | 1920 ± 60 | 1.85 | 1038 ± 32 | 7924 ± 871 |
| UDP-Glc | 153 ± 16 | 167 ± 14 | 740 | 0.226 ± 0.020 | 1.78 ± 0.28 |
| UDPGal | 160 ± 6 | 455 ± 55 | 740 | 0.615 ± 0.074 | 3.83 ± 0.22 |

[a] Values listed in this table were obtained from capillary electrophoresis and are the average of five independent experiments for each UDP-GlcNAc and UDP-GalNAc and three independent experiments in the case of UDP-Glc and UDP-Gal. All parameters are of the same magnitude as those reported for the WbpP protein (*12*), though, for all substrates, WbgU exhibits slightly higher activity.

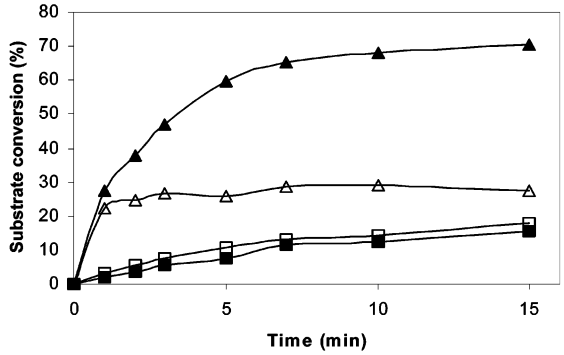


FIGURE 3: Time course for the epimerization of UDP-GlcNAc and UDP-GalNAc by WbgU using capillary electrophoresis. Reactions consisted of 0.075 mM substrate (triangles) and 1.5 mM substrate (squares). UDP-GlcNAc, open symbols; UDP-GalNAc, solid symbols. The amounts of enzyme used were 125 and 250 ng for UDP-GalNAc and UDP-GlcNAc, respectively.

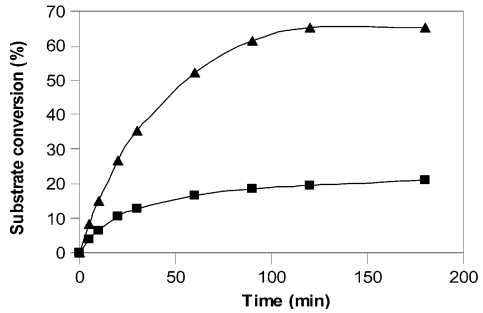


FIGURE 4: Time course for the epimerization of UDP-Glc and UDP-Gal by WbgU, from capillary electrophoresis. Reactions consisted of 30 $\mu$g of enzyme and 0.4 mM substrate in a total volume of 40 $\mu$L. (▲) UDP-Gal; (■) UDP-Glc.

*Determination of Kinetic Parameters for UDP-Glc and UDP-Gal.* The kinetic parameters determined under initial rate conditions are summarized in Table 1. The $K_m$ values were 153 $\mu$M and 160 $\mu$M for UDP-Glc and UDP-Gal, respectively. The rates of reactions with these two substrates were much slower than with UDP-GlcNAc and UDP-GalNAc, requiring substantially larger amount of enzyme and longer reaction times.

## DISCUSSION

UDP-GlcNAc is a common precursor of a variety of surface carbohydrates in bacteria (*13*). Structural analyses of surface carbohydrates have revealed epimers and deoxy derivatives of this amino sugar, thus inferring the presence of the corresponding activities. However, information about the enzymes responsible for these reactions is scarce.

WbgU is a small protein with hitherto unknown function. It is encoded by the third open reading frame (*orf3*) in the O-antigen cluster of *Plesiomonas shigelloides* O17 as well as in the Pinv plasmid of *E. coli* Sonnei (*3*). It is a member of the short-chain dehydrogenase/reductase (SDR) family of proteins that encompasses enzymes such as dehydrogenases, dehydratases, and epimerases. WbgU primary structure contains characteristic sequences common to the SDR family, including the GxxGxxG nucleotide binding motif and the proposed $S(x)_{24}Y(x)_3K$ catalytic triad (*12*). However, these features are widespread among the members of this family and their presence alone is insufficient to make reliable homology-based functional assignments.

The activity was first described in crude extracts from *Bacillus subtilis* (*14*) and later reported or inferred in a variety of prokaryotic and eukaryotic species (*15*−*21*). The potential location of the gene encoding UDP-GlcNAc 4-epimerase activity has been reported by Estrela et al. (*22*). The authors identified a new locus, *gneA*, associated with the UDP-GlcNAc activity following characterization of a *B. subtilis* 168 mutant incapable of synthesizing the so-called minor teichoic acid [poly(3-*O*-$\beta$-D-glucopyranosyl)-*N*-acetylgalactosamine 1-phosphate]. Further inquiries into the matter revealed that *galE* encoding a UDP-Glc C4 epimerase is most likely responsible for the epimerization of the *N*-acetylated derivatives (personal communication). The first thorough demonstration of a UDP-GlcNAc 4-epimerase activity appeared recently (*12*), and *wbgU* is the second gene encoding this activity to be characterized thus far. In addition, the genes for a putative UDP-GlcNAc C4 epimerases have been found in *E. coli* O55:H7 (*23*) and *Yersinia enterocolitica* serotype O:8 (*24*). In both cases, cell extracts could interconvert UDP-GlcNAc and UDP-GalNAc, but the activity responsible has not been purified or characterized further.

The kinetic analysis of WbgU has been conducted under initial rate conditions with the standard Michaelis−Menten model.This model assumes that the product is not a substrate of the enzyme. Therefore reactions have been kept to less than 10% substrate conversion to prevent interference from the back conversion. The kinetic parameters obtained clearly define UDP-GlcNAc and UDP-GalNAc as the preferred substrates. The conversion of UDP-GalNAc to UDP-GlcNAc is more efficient than the reverse reaction, which is also seen in the final 70:30 equilibrium favoring the Glc derivative. Since UDP-GlcNAc is a common precursor to both peptidoglycan and the surface polysaccharides, there is a competition between various pathways for this substrate. To shift the balance toward O-antigen synthesis, the enzymes utilizing UDP-GalNAc would have to be augmented, thereby pulling the equilibrium toward this epimer. Therefore, the epimerase may be part of a regulatory mechanism with an important role in balancing the distribution of UDP-GlcNAc between peptidoglycan and O-antigen synthesis.

WbgU is also capable of interconverting UDP-Glc and UDP-Gal. This observation is consistent with literature reports on other C4 epimerases, especially eukaryotic homologues of GalE and the WbpP protein that are able to accommodate substrates with modified sugar moieties. In the likely reaction mechanism, the nucleotide sugar binds the protein mainly though the UDP moiety, while the sugar is free to rotate about the oxygen−phosphate bond. Therefore, substrate derivatives would be expected to bind the enzyme as long as the UDP fragment is preserved. This fact has been the basis for the development of a uridine-based combinatorial library of compounds and the subsequent identification of an inhibitor of a human UDP-GalNAc C4 epimerase (*25*).

Although the $K_m$ values for the acetylated and nonacetylated substrates are similar, the differences are most apparent when comparing the $k_{cat}$ and $V_{max}$ values. The enzyme is 3 orders of magnitude less efficient at converting the nonacetylated substrates, indicating that it is a genuine UDP-GlcNAc 4-epimerase and not a GalE homologue.

Enzymatic activity toward all substrates is independent of exogenous $NAD^+$ and divalent metals. Addition of chloride salts of Mg, Mn, and Ca had no effect on enzymatic activity. $NAD^+$ could be extracted from an extensively dialyzed protein, suggesting that the epimerization reaction proceeds via a deprotonation−reprotonation mechanism and not via a recently reported $NAD^+$-independent mechanism (*26*). The relatively low yield of $NAD^+$ extracted from the enzyme indicated that the cofactor is very tightly bound to the enzyme during its synthesis in *E. coli*.

*N*-Acetylglucosamine is not found in the lipopolysaccharide of *P. shigelloides* and is therefore likely to serve as a precursor to other sugars. Herein we have shown evidence that WbgU is an UDP-GlcNAc 4-epimerase and may be involved in the biosynthesis of 2Ac-AltUA, a component of the disaccharide repeating unit in the O-antigen of *P. shigelloides* O17 and *E. coli* Sonnei. The proposed pathway (*7*) consists of UDP-GlcNAc epimerization at C4, oxidation to an acid at C6, and finally epimerization at C5. The C6 oxidation is likely carried out by WbgT, whose homologue, WbpO from *P. aeruginosa*, has been described recently (*27*).

The final step, a C5 epimerization, may be catalyzed by WbgZ. This enzyme contains the characteristic NAD(P)-binding Rossmann fold and belongs to the tyrosine-dependent oxidoreductase family. However, biochemical characterization of this activity in bacteria is lacking. Both WbgT and WbgZ are currently being investigated in our laboratory to clearly establish their functions.

In conclusion, this work presents biochemical evidence that *wbgU* of the O-antigen gene clusters of *P. shigelloides* and *E. coli* Sonnei Pinv plasmid encodes a UDP-GlcNAc 4-epimerase. Further, we postulate that this enzyme catalyzes the first step in the biosynthetic pathway of 2-acetamino-2-deoxy-L-altruronic acid, a sugar residue present in the O-antigen repeating unit of the two species. The functions of the remaining genes in this pathway, *wbgT* and *wbgZ*, are being investigated.

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