

Activated Sugar Precursors: Biosynthetic Pathways and Biological Roles of an Important Class of Intermediate Metabolites in Bacteria

Sílvia A. Sousa, Joana R. Feliciano and Jorge H. Leitão
 IBB – Institute for Biotechnology and Bioengineering, CEBQ,
 Instituto Superior Técnico,
 Portugal

1. Introduction

Activated sugar precursors are energy-rich forms of monosaccharides, mainly nucleoside diphosphate sugars, that contain the energy required for the assembly of their sugar moiety in carbohydrate sequences on appropriate carrier molecules (Fig. 1).

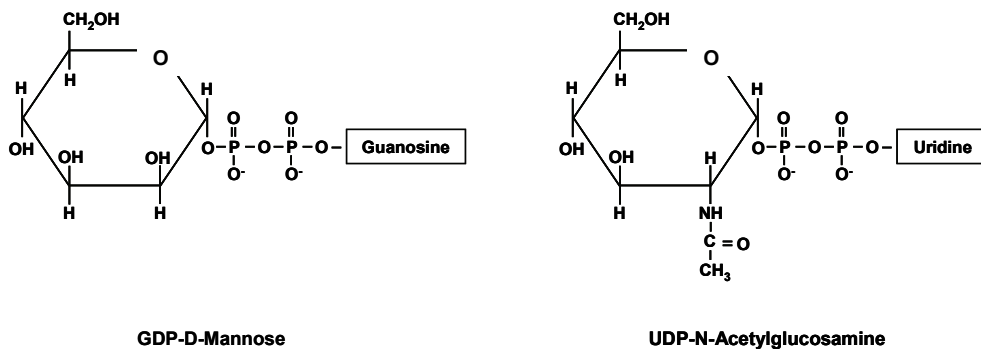


Fig. 1. Structures of the sugar nucleotides GDP-D-mannose and UDP-N-acetylglucosamine.

In bacteria, these ubiquitous metabolites are required for the synthesis of all the carbohydrate-containing polymers. Sugar nucleotides are the donors of the sugar moieties found in oligo- and polysaccharides (*e.g.* exopolysaccharides - EPS, lipopolysaccharides - LPS). Sugar nucleotides are also required for the glycosylation of proteins and lipids, for the phase 2 metabolism of xenobiotics, and for the metabolism of secondary metabolites with antibiotic activities (Gronow and Brade, 2001; Nedal and Zotchev, 2004). LPS and EPS can form highly complex structures at the bacterial outer surface, and are often involved in the molecular recognition and virulence of pathogens. Therefore, the targeting of the biosynthesis of specific carbohydrates is considered of interest for the development of new therapeutic agents (Green, 2002; Foret *et al.*, 2009).

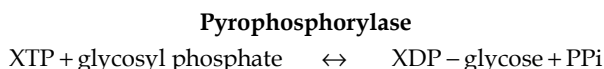
2. Methods used in sugar nucleotide analysis

Nucleotide sugars were identified for the first time almost 45 years ago (Leloir, 1971). Investigations of the metabolism of activated nucleotide sugars require rapid analytical assays that allow the separation, structural characterization and quantification of substrates, intermediates and end products. Since the late 1970s, several high-performance liquid chromatography (HPLC) methods for nucleotide analysis have been developed, including ion exchange chromatography, reversed-phase liquid chromatography and more recently the ion-pair chromatography (Ramm *et al.*, 2004). All these HPLC applications enabled the separation of nucleotide sugars and their detection was based on absorption of light of wavelengths within the UV range. This is due to the fact that all the nucleotides exhibit an absorption maximum around 260 nm. However, these methods cannot differentiate sugar nucleotides according to the nature of the nucleotide diphosphate moiety (ADP, CDP, GDP, dTDP, UDP) that is linked to C-1 of the sugar residue. For this reason, to identify the HPLC peaks, co-chromatography with reference compounds is required, although no structural information can be obtained. Currently, the analysis of the nucleotide sugars is performed by HPLC methods coupled with other methods such as diode-array detection (DAD), electrospray ionization mass spectrometry (ESI-MS) or nuclear magnetic resonance (NMR) (Ramm *et al.*, 2004). Capillary electrophoresis (CE) has also been used to resolve closely related sugar nucleotides, together with NMR spectroscopy to identify their chemical structures (Lehmann *et al.*, 2000; King *et al.*, 2009). Recently, porous graphitic carbon (PGC) liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) was successfully applied to sugar nucleotide separation and analysis (Pabst *et al.*, 2010).

3. Enzymatic synthesis of nucleotide sugars

Glucose-1P (G1P) and Fructose-6P (F6P) can be regarded as the starting materials in metabolic pathways leading to various sugar nucleotides. G1P is formed from the glycolysis intermediate G6P by the enzyme activity phosphoglucomutase (Pgm; EC 5.4.2.2) (Mehra-Chaudhary *et al.*, 2011). The glycolysis intermediate F6P is also of central importance in sugar nucleotides biosynthesis. The vast majority of sugar nucleotides can be synthesised by living organism using either G1P or F6P as starting materials. Among the few exceptions are galactose and mannose sugars. Although these two sugars can be synthesized from G1P or F6P, they can also result from the uptake of substrates like lactose found in milk, or mannose, a sugar that occurs in fruits such as cranberry.

The majority of the nucleoside diphosphate sugars are synthesized by the condensation of a nucleoside triphosphate (XTP, where X can be any nucleoside, being uridine, guanosine, cytidine, thymidine, and adenosine the more common ones) with a sugar 1-phosphate (where the sugar can be D-glucose, D-galactose, D-mannose, 2-acetamido-2-deoxy-D-glucose, L-fucose, D-glucuronic acid, or another sugar) by a specific pyrophosphorylase enzyme activity, as shown in the following reaction:



The resulting sugar nucleotide can also be inter-converted to different monosaccharides by several mechanisms. These include epimerisation/isomerisation, decarboxylation, dehydration, dehydrogenation, oxidation or reduction reactions (Field and Naismith, 2003). For example, a common strategy is the oxidation of a hydroxyl group to a ketone, being used to activate the α protons to the ketone group, for amination and for direct epimerization. Subsequently, the nucleotide sugar will be transferred to the appropriate acceptor by specific glycosyltransferases.

In this work, we review the nucleotide sugars that are more commonly found in bacterial cells, and the enzyme activities required for their biosynthesis.

4. Sugar nucleotides: occurrence and biosynthesis

4.1 GDP-D-mannose

GDP-D-mannose is the donor of D-mannose, a sugar residue found in many bacterial extracellular polysaccharides, as is the case of xanthan, cepacian, acetan, and some sphingans (Becker et al., 1998; Cescutti et al., 2000; Richau et al., 2000; Griffin et al., 1997; Fialho et al., 2008). The synthesis of GDP-D-mannose from F6P requires the enzyme activities phosphomannose isomerase (PMI; EC 5.3.1.8), phosphomannose mutase (PMM; EC 5.4.2.8) and GDP-D-mannose pyrophosphorylase (GMP; EC 2.7.7.13) (Fig. 2). In many bacteria, the PMI and GMP enzyme activities are carried out by a bifunctional protein that belongs to the type II PMIs family of proteins (Sousa et al., 2007; Griffin et al., 1997). These proteins have two separate conserved domains: the mannose-6-phosphate isomerase family 2 domain in the C-terminus, and the nucleotidyl transferase domain in the N-terminus (Jensen and Reeves, 1998). These enzymes catalyse the reversible isomerization of fructose-6-phosphate into mannose-6-phosphate and transfer mannose-1-phosphate to GTP forming GDP-D-mannose, respectively (Fig. 2; Wu *et al.*, 2002).

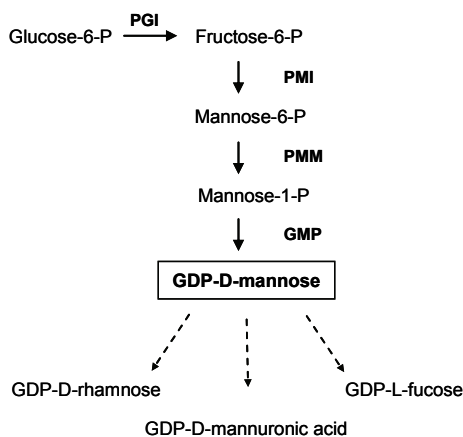


Fig. 2. Metabolic pathway leading to GDP-D-mannose. PGI, phosphoglucose isomerase; PMI, phosphomannose isomerase; PMM, phosphomannose mutase; GMP, GDP-D-mannose pyrophosphorylase.

For example, GDP-D-mannose is one of the sugar nucleotides necessary for the synthesis of the exopolysaccharide Cepacian (Richau et al., 2000). This EPS is composed of a branched

acetylated heptasaccharide repeating unit with D-glucose, D-rhamnose, D-mannose, D-galactose, and D-glucuronic acid, in the ratio 1:1:1:3:1 (Cescutti et al., 2000). Cepacian is produced by environmental, and human, animal and plant pathogenic isolates belonging to several *Burkholderia* species (Ferreira et al., 2010). In *B. cepacia* IST408, a clinical isolate from a cystic fibrosis patient, the lack of the type II PMI BceA significantly affected the ability of the mutant strain to form biofilms (Sousa et al., 2007).

4.2 GDP-D-Rhamnose

D-Rhamnose is a relatively rare deoxyhexose. This sugar is mainly found in the LPS of pathogenic bacteria, where it is involved in host-bacterium interactions and in the establishment of infection (Webb et al., 2004). For example, it is a constituent of the opportunistic pathogen *Pseudomonas aeruginosa* A-band of the O polysaccharide of LPS (Rocchetta et al., 1999). This glycan is also present in the S-layer of the Gram positive thermophile *Aneurinibacillus thermoaerophilus* (Kneidinger et al., 2001). Due to the D-rhamnose association with bacterial structures related to virulence, enzymes leading to its biosynthesis have been studied as promising targets for the development of novel antibacterial agents.

Biosynthesis of GDP-D-rhamnose, the precursor for D-rhamnose, starts with the dehydration of GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose, in a reaction catalyzed by the GDP-D-mannose-4,6 dehydratase (GMD; EC 4.2.1.47) (Fig. 3). The mechanism of this reaction has been proposed to involve a protein-bound pyridine dinucleotide (NAD⁺ or NADP⁺) as responsible for the transfer of a hydrogen from the C-4 to the C-6 position of the deoxy-monosaccharide (Sturla et al., 1997). The 4-keto moiety of the intermediate is then reduced to GDP-D-rhamnose by the enzyme activity GDP-4-keto-6-deoxy-D-mannose reductase (RMD; EC 1.1.1.281) (Fig. 3). The joint GMD and RMD enzyme activities are also known as GDP-rhamnose synthase (GRS).

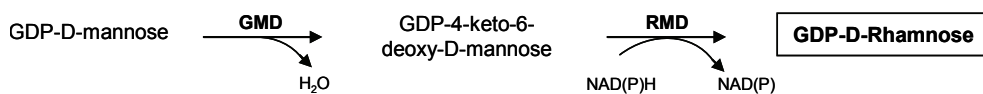


Fig. 3. Metabolic pathway leading to GDP-D-rhamnose. GMD, GDP-D-mannose-4,6 dehydratase; RMD, GDP-4-keto-6-deoxy-D-mannose reductase.

The two proteins involved in this pathway are members of the nucleotide diphosphate (NPD)-sugar modifying subfamily of the short-chain dehydrogenase/reductase (SDR) superfamily (Kavanagh et al., 2008). This family share low sequence identity, but their three-dimensional structures are quite conserved (Fig. 4). The most conserved feature of these proteins is the Rossman-fold motif, involved in dinucleotide binding (Webb et al., 2004). The Rossman fold is composed of a α/β folding pattern, with 7 β -strands flanked by 6-7 α -helices on each side (Fig. 4). The glycine-rich Wierenga motif, GXXGXXG, is also present in the N-terminus region. The Wierenga motif is the specific region for the binding of the cofactor NADP(H) (Fig. 4). These proteins also share the conserved triad Tyr-XXX-Lys and Ser/Thr in their catalytic centers (Fig. 4).

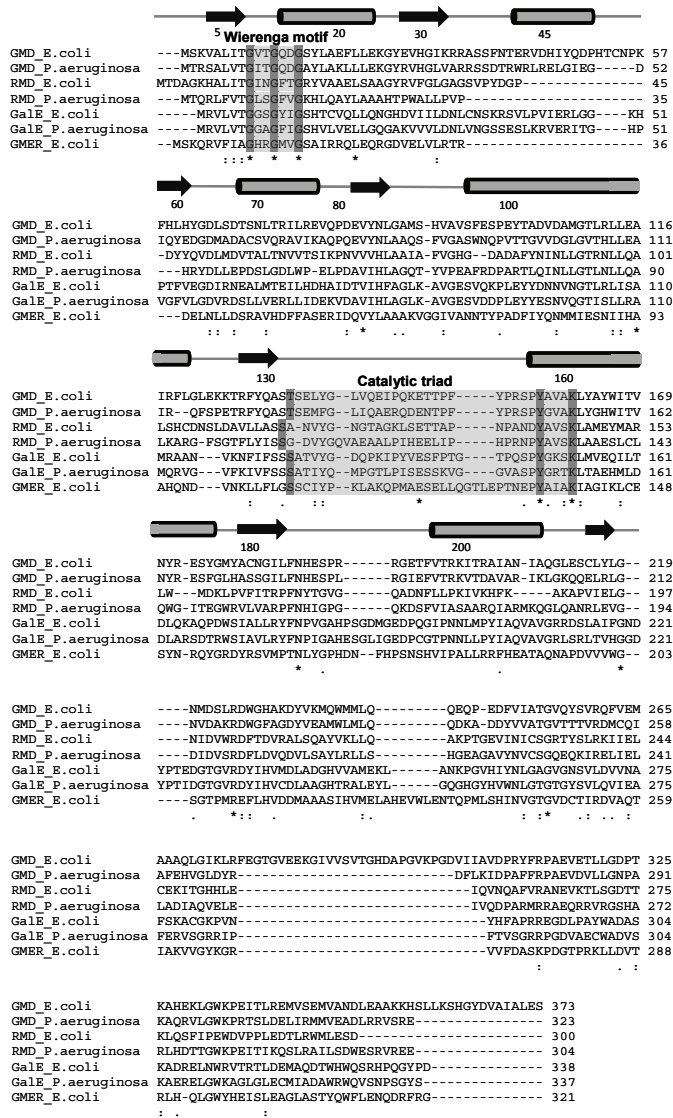


Fig. 4. Amino acid sequence alignment of *E. coli* GMD (AAC77842), *P. aeruginosa* GMD (AAG08838), *E. coli* RMD (ACV53840), *P. aeruginosa* RMD (AAG08839), *E. coli* GalE (AAC73846), *P. aeruginosa* GalE (AAG04773), and *E. coli* GMER (AAC77843). The Wierenga motif and the catalytic triad typical of the SDR family of proteins are highlighted. Asterisks indicate the amino acid residues that are identical in all proteins. One or two dots indicate semi-conserved or conserved substitutions, respectively. The conserved secondary structure elements are shown above the alignment segments, where cylinders represent α -helices and arrows represent β -sheets. Alignments and the secondary structure predictions were performed with ClustalW2 and the PSIPRED software, respectively.

The GMD enzyme activity is widespread in nature, and also catalyzes the first step in the biosynthesis of other sugars, including L-fucose, D-talose and D-perosamine (King *et al.*, 2009). Bioinformatic analysis suggests that the closest paralog of RMD is GMD (Fig. 4). This conclusion is also supported by the existence of GMD proteins with bifunctional activity, which catalyzes the dehydration of GDP-D-mannose and the reduction of the 4-keto sugar nucleotide to a 6-deoxysugar nucleotide (King *et al.*, 2009).

4.3 GDP-L-Fucose

L-Fucose is a 6-deoxy-sugar widely distributed in nature, occurring in glycoconjugate compounds in microorganisms, plants and animals. This sugar nucleotide is commonly found in complex carbohydrates that are constituents of the cell wall and of LPS of some Gram-negative bacteria. The presence of L-fucose in these polysaccharides has been shown to play an important role on the interaction between bacteria and the host tissues. For example, *Helicobacter pylori* fucosylated glycoconjugates are involved in adhesion mechanisms and in evasion of bacteria from the host immune system (Moran, 2008). This *H. pylori* fucosylated glycoconjugate is closely related to antigens of the Lewis system that are commonly present on the surface of human cells (Rosano *et al.*, 2000). In 1960, Ginsburg identified the highly conserved metabolic pathway leading to the synthesis of GDP-L-fucose via GDP-D-mannose (Ginsburg, 1960; Fig. 5).



Fig. 5. Metabolic pathway leading to GDP-L-fucose. GMD, GDP-D-mannose-4,6 dehydratase; GMER, GDP-4-keto-6-deoxy-D-mannose epimerase/reductase.

The first step of this pathway is the dehydration of GDP-D-mannose by GMD, leading to the formation of the unstable intermediate GDP-4-keto-6-deoxy-D-mannose. This intermediate undergoes subsequent epimerization at C-3 and C-5 hexose ring centers that changes the D- to L-configuration of the monosaccharide. This results in the production of GDP-4-keto-6-deoxy-L-galactose. A NADPH-dependent reduction of the keto group at C-4 occurs subsequently, leading to the formation of GDP-L-fucose. The enzyme responsible for these two last steps is the bifunctional enzyme with both GDP-4-keto-6-deoxy-D-mannose epimerase/reductase activities (GMER; EC 1.1.1.271) (Rosano *et al.*, 2000). Amino acid sequence analysis indicated that the protein also belongs to the SDR family. The GDP-L-fucose formed is the substrate for various fucosyltransferases that are responsible for the incorporation of L-fucose in glycoproteins, glycolipids and oligosaccharides (Ma *et al.*, 2006).

4.4 GDP-D-mannuronic acid

GDP-D-mannuronic acid is the precursor of the acidic sugars mannuronic acid and guluronic acid, mainly found in bacterial alginates. GDP-D-mannuronic acid is synthesized from GDP-D-mannose, in a redox reaction catalyzed by GDP-mannose dehydrogenase (GMdh; EC 1.1.1.132). The reaction involves the irreversible oxidation of GDP-D-mannose via a 4-electron transfer using NAD⁺ as the cofactor (Fig. 6).

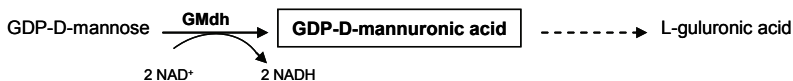


Fig. 6. Metabolic pathway leading to GDP-D-mannuronic acid and L-guluronic acid. GMdh, GDP-mannose dehydrogenase.

GMdh is a member of the NAD-dependent 4-electron transfer dehydrogenases. This protein family also includes UDP-glucose dehydrogenases (UGD) (Snook et al., 2003). Both the GMdh and UGD enzyme activities are mechanistically similar, using a unique active site to catalyze the two-step conversion of an alcohol group to the corresponding acid, via a thiohemiacetal intermediate.

GDP-D-mannuronic acid is the activated sugar precursor for alginate polymerization in *P. aeruginosa*, which is a partially O-acetylated linear polymer of D-mannuronic acid and L-guluronic acid, linked via β -1,4 glycosidic bonds (Shankar et al., 1995). In the case of the *P. aeruginosa* alginate, after polymerization, some D-mannuronic acid residues can be further converted to L-guluronic acid by the extracellular enzyme activity polymannuronic acid C-5-epimerase (Jerga et al., 2006). *P. aeruginosa* is able to cause severe and life-threatening infections in immunosuppressed patients, such as burn and cancer chemotherapy patients (Wagner and Iglewski, 2008), as well as in patients suffering from cystic fibrosis (CF). Alginate allows the bacterium to resist to antipseudomonal antibiotics and to the host immune system (Wagner and Iglewski, 2008). In addition, long-term infection with *P. aeruginosa* leads to lung tissue damage of CF patients, to which contribute, among others, extracellular proteases and lipases produced by the bacterium. Tavares et al. (1999) demonstrated that the step catalyzed by GMdh is critical for the control of the alginate pathway in *P. aeruginosa*, channelling GDP-D-mannose into the alginate pathway instead of A-band LPS biosynthesis. Therefore, inhibition of GMdh activity may lead to the prevention of alginate biosynthesis by *P. aeruginosa*. Recently, Li and colleagues (2008) demonstrated that ambroxol (2-amino-3,5-dibromo-N-[trans-4-hydroxycyclohexyl] benzylamine) was able to partially inhibit the production of alginate by *P. aeruginosa* strains via the reduction of the activity of the GMdh enzyme.

4.5 dTDP-L-rhamnose

L-rhamnose is a fundamental constituent of the O-antigen of LPS in several gram-negative bacteria. For example, in *Shigella* and *Salmonella* species, the O-antigen repeating unit is mainly constituted by L-rhamnose (Van den Bosch et al., 1997). The L-rhamnosyl residue has also an essential structural role in the cell wall of *Mycobacterium tuberculosis* (Ma et al., 2001). The donor of the L-rhamnose moiety found in bacterial structures is deoxythymidine-diphosphate (dTDP)-L-rhamnose. dTDP-L-rhamnose is synthesized from glucose-1-phosphate and deoxythymidine triphosphate (dTTP) in a four-step pathway (Fig. 7). The first-step is catalysed by glucose-1-P deoxythymidyl transferase (RmlA; EC 2.7.7.24) that leads to dTDP-D-glucose from glucose-1-P and dTTP. In bacteria, dTDP-D-glucose is a key metabolite for the production of several monosaccharides that are components of the cell wall polysaccharides, or that are components of some antibiotics, such as the macrolide erythromycin A (Vara and Hutchinson, 1988). The second-step is the dehydration of dTDP-D-glucose to dTDP-4-keto-6-deoxy-D-glucose, a reaction catalysed by dTDP-D-glucose 4,6

dehydratase (TGD or RmlB; EC 4.2.1.46). The unstable intermediate dTDP-4-keto-6-deoxy-D-glucose is the precursor for the synthesis of dideoxyhexoses and aminohexoses that are common components of antibiotic glycosides, like novobiocin and streptomycin (Nedal and Zotchev, 2004). Alternatively, this intermediate can undergo two additional conversion steps to originate dTDP-L-rhamnose. First, dTDP-4-keto-6-deoxy-D-glucose-3,5 epimerase (RmlC; EC 5.1.3.13) catalyses the C-3 and C-5 epimerization. A NADPH-dependent reduction of C-4 is followed, catalysed by dTDP-6-deoxy-L-lyxo-4-hexulose reductase (RmlD; EC 1.1.1.133).

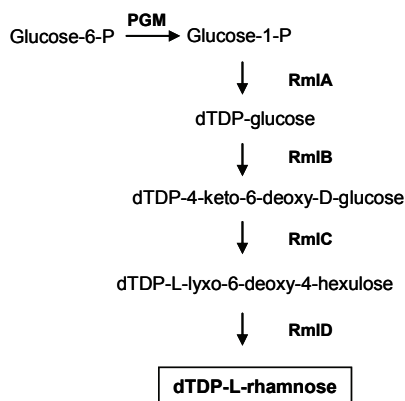


Fig. 7. Metabolic pathway leading to dTDP-L-rhamnose. PGM, phosphoglucose mutase; RmlA, glucose-1-P deoxythymidyl transferase; RmlB, dTDP-D-glucose 4,6 dehydratase; RmlC, dTDP-4-keto-6-deoxy-D-glucose-3,5 epimerase; RmlD, dTDP-6-deoxy-L-lyxo-4-hexulose reductase.

The described multi-step pathway does not exist in humans, being these four enzyme activities potential targets for the design of new therapeutic agents, as is the case of the development of new antimycobacterial agents, an area of intensive research (Ma et al., 2001).

4.6 UDP-D-glucose

UDP-D-glucose is the sugar precursor for the synthesis of several sugar-containing bacterial structures that are recognized virulence factors or determinants, such as the peptidoglycan, LPS and EPS. In gram-positive bacteria, UDP-D-glucose is the substrate for the glycosylation of teichoic acids and for biosynthesis of the glycolipid diglycosyldialcylglycerol (Glc2-DAG), the membrane anchor of lipoteichoic acids (Chassaing and Auvray, 2007). UDP-D-glucose is also an important sugar precursor for the biosynthesis of hyaluronic acid (HA) by streptococci, being the encapsulation of these bacteria by HA considered an important virulence factor (Stollerman and Dale, 2008). HA is a linear polymer of the repeating disaccharide composed of glucuronic acid (GlcA) and N-acetyl-glucosamine (GlcNAc) (Stollerman and Dale, 2008). UDP-D-glucose is the precursor for the synthesis of GlcA. UDP-glucose is the product of the enzyme activity UDP-glucose pyrophosphorylase (UGP; EC 2.7.7.9). This enzyme catalyses the reversible formation of UDP-glucose from UTP and glucose-1-phosphate (Fig. 8; Kim et al., 2010).

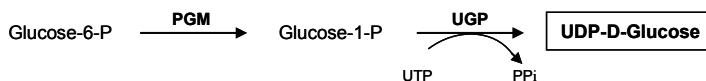


Fig. 8. Metabolic pathway leading to UDP-D-glucose. PGM, phosphoglucose mutase; UGP, UDP-glucose pyrophosphorylase; UTP, uridine triphosphate; PPi, pyrophosphate.

These enzymes have two typical domains, the N-terminal motif GXGTRXLPXTK for the activator binding site, and the VEKP motif that is essential for substrate binding (Marques et al., 2003). UGPases are present in animals, plants and microorganisms. However, prokaryotic and eukaryotic proteins are quite distinct, being the former regarded as appropriate targets for the development of novel antibacterial agents.

4.7 UDP-D-glucuronic acid

UDP-D-glucuronic acid is synthesized from UDP-D-glucose, in a NAD^+ -dependent oxidation, catalyzed by the enzyme activity UDP-glucose dehydrogenase (UGD; EC 1.1.1.22) (Fig. 9; Ge et al., 2004; Field and Naismith, 2003).

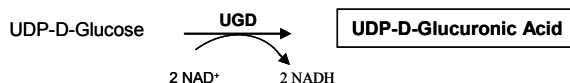


Fig. 9. Metabolic pathway leading to UDP-D-glucuronic acid. UGD, UDP-glucose dehydrogenase.

The first step in the reaction is the transfer of the *pro-R* hydride from C-6 to NAD^+ and deprotonation of O-6, generating an aldehyde. This first intermediate is converted into a covalent thioester by the transfer of a second hydride to a new NAD^+ molecule. The thioester is then hydrolyzed to liberate the free carboxylic acid, thus regenerating the protein thiol. These proteins have the three typical conserved domains of the UGD protein family, namely the NAD^+ -binding domain, the central domain, and the UDP-binding domain (Kereszt et al., 1998). In *B. cepacia* complex (Bcc) bacteria, Ara4N is present in the lipid A and in the core of LPS. Synthesis of UDP-Ara4N is essential for Bcc bacteria viability and to their high resistance to antimicrobial peptides (Ortega et al., 2007). The first step in the synthesis of UDP-Ara4N is the conversion of UDP-D-glucose to UDP-D-glucuronic acid by UGD. Recently, it was shown that the $\text{UGD}_{\text{BCAL2946}}$ and $\text{UGD}_{\text{BCAM0855}}$ of *B. cenocepacia* are essential for survival, being the $\text{UGD}_{\text{BCAL2946}}$ protein also required for polymyxin B resistance (Loutet et al., 2009). Bcc is a group of 17 phenotypically similar bacterial species that are opportunistic pathogens in cystic fibrosis (CF) patients, causing chronic and sometimes fatal pulmonary infections in these patients (Leitão et al., 2010). Treatment of these infections is difficult since Bcc bacteria are intrinsically resistant to most of the clinically relevant antimicrobial agents (Leitão et al., 2008).

4.8 UDP-D-galactose

UDP-D-galactose is essential for the biosynthesis of the galactosyl residues found in complex polysaccharides and glycoproteins. This sugar nucleotide can be synthesized by the Leloir pathway of the galactose metabolism when bacteria grow in lactose or galactose as energy and carbon sources (Holden et al., 2003; Fig. 10). This pathway includes three enzyme activities, galactokinase (GalK, EC. 2.7.1.6), galactose-1-P uridylyltransferase (GalT,

EC 2.7.7.10) and UDP-galactose 4-epimerase (UGE or GalE; EC 5.1.3.2). First, galactose is phosphorylated by GalK forming galactose-1-P. Then, galactose-1-P is epimerized to glucose-1-P by GalT. This reaction requires the transfer of UDP from UDP-glucose, also generating UDP-galactose. UDP-galactose can be epimerized to UDP-glucose by GalE and glucose-1-P can be converted to glucose-6-P by phosphoglucose mutase (PGM).

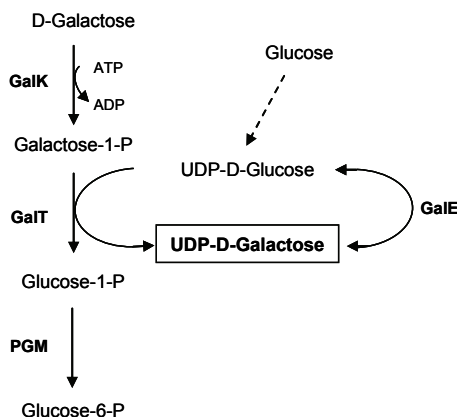


Fig. 10. Metabolic pathways leading to UDP-D-galactose. GalK, galactokinase; GalT, galactose-1-P uridylyltransferase; Gal E, UDP-galactose 4-epimerase; PGM, phosphoglucose mutase.

UDP-D-galactose can also be synthesized from UDP-D-glucose by GalE, when bacteria grow in glucose or fructose containing medium. GalE oxidizes C-4 (hydride abstraction) and then reduces the resulting ketone from the opposite face of the UDP-D-glucose, resulting in UDP-D-galactose by a free conversion between a *gluco*- to a *galacto*-configured pyranose ring (Fig. 10, Holden et al., 2003). GalE is also a member of the SDR superfamily, with the typical Tyr-X-X-X-Lys motif involved in catalysis, and the N-terminal NAD⁺-binding motif GXXGXXG (Fig. 4, Kavanagh et al., 2008).

4.9 UDP-N-acetylglucosamine

In bacteria, uridine 5'-diphospho-N-acetyl-D-glucosamine (UDP-GlcNAc) is the activated form of N-acetylglucosamine, an essential precursor for the biosynthesis of various important carbohydrate-containing structures, as is the case of the cell wall peptidoglycan, LPS and teichoic acids (Milewski, 2002). Enzymes leading to the synthesis of UDP-GlcNAc are essential for the cell wall formation, being regarded as attractive targets for the development of antibacterial compounds (Kotnik et al., 2007). Glucosamine-6-phosphate synthase (GlcN-6-P synthase or GlmS in bacteria, EC 2.6.1.16) catalyses the first step in the pathway that leads to the formation of UDP-GlcNAc (Milewski, 2002) (Fig. 11). The irreversible reaction catalyzed by this enzyme involves the transfer of an amino group from L-glutamine to D-fructose-6-phosphate (F6P), followed by an isomerisation of the sugar moiety, yielding D-glucosamine-6-phosphate. GlmS is a large ubiquitous protein present in a large number of organisms and tissues. GlmS proteins contain two typical domains, the glutamine-binding domain in the N-terminus region, and the F-6-P binding domain in the C-terminus region. However, sequence alignments revealed large differences between

prokaryotic and eukaryotic GlcN-6-P synthases, being the latter 70-90 amino acid residues longer (Milewski, 2002). This enzyme activity is also an important point of metabolic control in the biosynthesis of amino sugar - containing molecules. Several inhibitors targeting this enzyme activity have been developed, like anticapsin, tetaine, and chlorotetaine (Milewski, 2002).

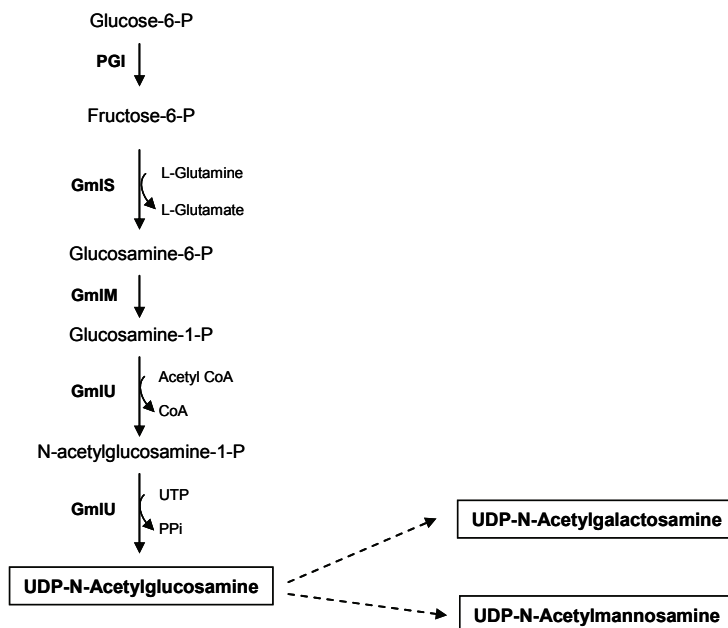


Fig. 11. Metabolic pathway leading to UDP-N-acetylglucosamine. PGI, phosphoglucose isomerase; GmIS, Glucosamine-6-phosphate synthase; GmIM, phosphoglucosamine mutase; GmIU, glucosamine-1-P acetyltransferase and N-acetylglucosamine-1-P uridyltransferase.

After its formation by GmIS, the resulting D-glucosamine-6-phosphate is further isomerised into D-glucosamine 1-phosphate by the phosphoglucosamine mutase enzyme activity (GlmM in bacteria; EC 5.4.2.10) (Fig. 11). In bacteria, the last two reactions necessary for the formation of UDP-GlcNAc are carried by the bifunctional protein with both activities of glucosamine-1-P acetyltransferase and N-acetylglucosamine-1-P uridyltransferase (GlmU in bacteria; EC 2.7.7.23 and EC 2.3.1.157) (Fig. 11). These enzyme activities first transfer an acetyl group from acetyl-CoA to form N-acetyl-glucosamine 1-phosphate, and then transfers the uridyl group to finally form UDP-GlcNAc. UDP-GlcNAc is the precursor of other sugar nucleotides, such as UDP-N-acetylgalactosamine (UDP-GalNAc) and UDP-N-acetyl-D-mannosamine (UDP-ManNAc). UDP-ManNAc is the precursor of N-acetylneuraminic acid (Sialic acid). Sialic acid is rarely found in prokaryotes, being present in certain pathogenic bacteria as a component of capsular polysaccharides (e.g. *Neisseria meningitidis*, *Escherichia coli* K1) or lipooligosaccharides (e.g. *Campylobacter jejuni*). In *C. jejuni* it is involved in evasion of the immune system by molecular mimicry of the host cells (Severi et al., 2007). In gram positive bacteria, ManNAc residues act as a bridge between the peptidoglycan and teichoic acids (D'Elia et al., 2009).

5. Biotechnological potential of nucleotide sugar metabolic pathways

The biosynthesis of the sugar moieties of the various sugar-containing cell structures starts by the synthesis of the repeating units of sugar nucleotides. The supply of the activated sugars for the biosynthesis of these polymers is dependent on the intracellular sugar nucleotide levels that are influenced by the activities of the intracellular enzymes involved in their biosynthesis. Therefore these key enzymes are potential targets for the development of new antimicrobials.

The L-rhamnose residues play an essential structural role in the cell wall of *Mycobacterium tuberculosis*. The mycobacterial cell wall core consists of three interconnected macromolecules, the mycolic acids, arabinogalactan (AG) and peptidoglycan. The outermost part is composed of mycolic acids that are esterified to the middle component, the AG. This component is connected, via the linker disaccharide α -L-rhamnosyl-(1 \rightarrow 3)- α -D-N-acetylglucosaminosyl-1-phosphate, to the 6 position of a muramic acid residue of the inner component peptidoglycan. Presently, it is known that *M. tuberculosis* strains have increased resistance to the antimicrobials agents in use, and therefore new antituberculosis drugs are necessary (Ma et al., 2001). In this context, the four enzyme activities (RmlA to RmlD) involved in the dTDP-L-rhamnose biosynthetic pathway have been studied as attractive targets for the development of new antimicrobials.

Helicobacter pylori is the causative agent of active chronic gastritis, being associated with peptic ulcer disease and increased risk for the development of gastric adenocarcinoma and primary gastric lymphoma (Edwards et al., 2000). The pathogen expresses the Lewis (Le) antigen in the O-chain of LPS (Moran, 2008). Serological and chemical structural studies have shown that *H. pylori* Le antigens mimic the human Lewis blood group determinants, having a role in gastric colonization and bacterial adhesion (Moran, 2008). Le antigen expression also affects the inflammatory response and T-cell polarization after infection. One of the factors that affect this antigen expression is the availability of activated sugar intermediates. *H. pylori* lacks galactokinase enzyme activity and is not able to use exogenous galactose. This points out that the UGE activity is an absolute requirement for the biosynthesis of UDP-galactose. In fact, *H. pylori* knockout mutants in *galE* produce truncated LPS and no Lewis antigen expression, causing a decreased ability of the mutant strain to colonize mice (Moran et al., 2000). Inactivation of *rfbM*, encoding a GMP activity that is required for GDP-L-fucose synthesis, resulted in a mutant strain with a fucose-lacking O-antigen and not able to express the Le_x antigen (Edwards et al., 2000). This mutant exhibited a reduced ability to colonize a mouse model of infection and was not able to interact with the human gastric mucosa of biopsy specimens in situ.

Some sugar polymers have applications in the food and pharmaceutical industries, like alginate, xanthan, gelan and polysaccharides from lactic acid bacteria (LAB) (Sabra et al., 2001; Becker et al., 1998; Fialho et al., 2008; Boels et al., 2001). These applications led to an increased interest in the study of the metabolic pathways leading to the formation of these polymers and their regulation, with the objective to optimize the microbial production process.

Alginate is a polymer composed of D-mannuronic acid and L-guluronic acid residues arranged in an irregular sequence (Sabra et al., 2001). It is produced by bacterial species of the *Pseudomonas* and *Azotobacter* genera, as well as by brown algae (Sabra et al., 2001). The viscosity and gel-forming properties of this polymer have important commercial applications in the pharmaceutical industry. For example, high-quality alginates have been

studied for the reversal of type I diabetes by immobilising insulin-producing cells within alginate capsules that could be transplanted to the body of the patient (Dufrane et al., 2010). The D-mannuronic acid blocks of alginate seems to stimulate the immune cells to secrete cytokines (e.g. tumour necrosis factor, interleukin-1 and interleukin-6) (Otterlei et al., 1991). The polymer has also several applications in the food industry. For example, alginate is used to enhance foam in beer production and to help in the suspension of fruit pulp in fruit drinks (Sabra et al., 2001). The textile and paper industries also use alginates to improve the surface properties of cloth and paper, and to improve the adherence of dyes and inks (Sabra et al., 2001). Alginate-immobilised cell systems are used as biocatalysts in several industrial processes, like in ethanol production by yeast cells, and in the production of monoclonal antibodies from hybridoma cells (Sabra et al., 2001; Selimoglu and Elibol, 2010). Currently, the vast majority of the alginates commercially in use are produced from brown algae. However, environmental concerns raised due to intensive algae harvesting and processing turned the attention to bacterial alginates, which are now considered as potential commercial products.

Lactic acid bacteria (LAB) produce a wide variety of structurally different EPSs that are responsible for the rheological characteristics and texture properties of specific fermented dairy and food products (Boels et al., 2001). In addition, LAB as food additives may confer health benefits to the consumer, having immunostimulatory, antitumoral and cholesterol-lowering activities (Boels et al., 2001). LAB EPSs are preferable over presently used stabilizers, like xanthan, since they are produced by food-grade microorganisms. However, these EPSs are produced in low amounts (40 to 800 mg per liter), compared with the commercially produced EPS xanthan (10 to 25 g per liter) (Boels et al., 2003). LAB EPSs are produced from intracellular sugar nucleotides, including glucose, galactose, rhamnose, glucuronic acid, fucose, N-acetylglucosamine (GlcNAc), and N-acetylgalactosamine (GalNAc). A study of the enzymes involved in the biosynthetic pathways of these sugar nucleotides revealed some key enzyme activities, like the UDP-galactose epimerase (GalE). In a *Lactococcus lactis galE* mutant, undetectable levels of UDP-D-galactose and null EPS production were described when the organisms were cultured on glucose as the sole carbon source (Boels et al., 2001). The availability of dTDP-rhamnose that is incorporated on the side chain of EPS is also a bottleneck in EPS production by LAB (Boels et al., 2001).

Several molecules with antibacterial, antifungal, antiparasitic or anticancer activity contain sugar moieties. These are of crucial importance for the biological activity and pharmacological properties of the compound (Nedal and Zotchev, 2004). Some microorganisms, like the actinomycetes, are able to produce deoxyaminosugars. The amino group of these metabolites can be ionized under physiological pH, being involved in both electrostatic interactions with other ionisable groups or in the formation of hydrogen bonds with specific chemical groups on the target molecule (Nedal and Zotchev, 2004). For example, some macrolide antibiotics containing these metabolites bind to the peptidyl transferase ring on the ribosome and block the tunnel that channels the nascent peptide into the center of the ribosome (Schlunzen et al., 2001). Macrolide antibiotics (e.g. erythromycin and streptomycin) can be divided in two classes, being the major difference between the two classes the structure of the sugar precursors (Nedal and Zotchev, 2004) (Table 1).

<i>Deoxyaminosugar</i>	<i>Antibiotic</i>
D-Desosamine	Erythromycin Oleandomycin Pikromycin
D-Mycaminose	Tylosin
D-Mycosamine	Polyene Macrolides
D-Perosamine	Polyene Macrolides
N-methyl-L-glucosamine	Streptomycin
Dimethylforosamine	Spinosyns
α -Methylthio lincosaminide	Lincomycin
L-Daunosamine	Daunorubicin

Table 1. Deoxyaminosugars present in antibiotics (Nedal and Zotchev, 2004).

The 12- to 16- macrolide aminosugar moieties (e.g. D-desosamine and D-mycaminose) originate from TDP-D-glucose. The polyene macrolides (e.g. mycosamine and perosamine) derive from GDP-D-mannose. The study of the synthesis of the deoxyaminosugars moieties and the mechanisms of attachment to their targets is of critical importance for the elaboration of new macrolide derivatives with potential antimicrobial activity.

6. Concluding remarks

Sugar nucleotides are essential precursors for the biosynthesis of various sugar-containing bacterial cell structures. In pathogenic bacteria, some of these structures are important virulence factors involved, in the majority of the cases, in the evasion of the bacteria from the host immune system. Other sugar-containing structures, like peptidoglycan, have important roles in bacterial viability. In addition, some of these structures and their biosynthetic pathways are being regarded as attractive targets for the development of new antimicrobial drugs. Some sugar polymers also have applications in the food, pharmaceutical, textile and paper industries, having important economical significance. Therefore, the study of these metabolic pathways and their regulation is of critical importance for the optimization of the microbial production processes of carbohydrate-containing polymers. In the present work, some examples of these studies were presented and discussed.

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