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Metabolic Engineering of Bacteria

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Abstract

Metabolic glycoengineering is a technique introduced in the early 90s of the last century by Reutter *et al.*. It utilises the ability of cells to metabolically convert sugar derivatives with bioorthogonal side chains like azides or alkynes and by that incorporation into several glyco structures. Afterwards, the carbohydrates can be labelled to study their distribution, dynamics and roles in different biological processes. So far many studies were performed on mammal cell lines as well as in small animals. Very recently, bacterial glyco-structures were targeted by glycoengineering, showing promising results in infection prevention by reducing pathogen adhesion towards human epithelial cells.

Introduction

Bacteria were among the first life forms to appear on earth, and are present in most habitats on the planet, e. g., they live in symbiosis with plants and animals. Compared to human cells there are ten times as many bacterial cells in our body. Most of them are harmless or even beneficial. But some species are pathogenic and cause infectious diseases with more than 1.2 million deaths each year [1]. Those infections include cholera, syphilis, anthrax, leprosy, and bubonic plague as well as respiratory infections like tuberculosis.

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Bacterial Cell Surface Architecture

Bacteria are divided into Gram-positive and Gram-negative species. Gram-positive bacteria are surrounded by a peptidoglycan cell wall. This peptidoglycan is a polymer consisting of alternating β-(1,4) linked *N*-acetylglucosamine and *N*-acetylmuramic acids which are cross-linked by four amino acids (D-alanine, L-lysine, D-glutamine and L-alanine) to form a mesh of 20 – 80 nm diameter (Figure 1).

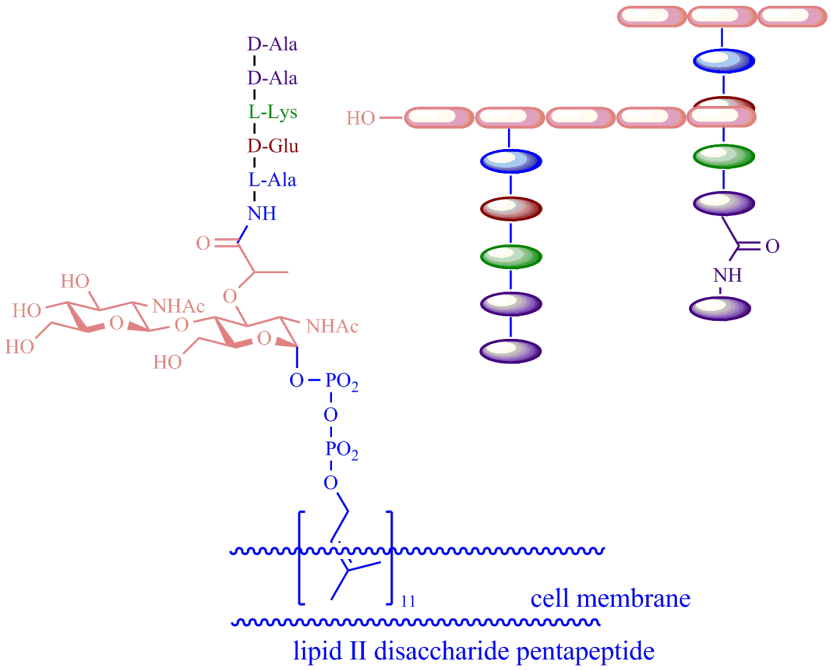


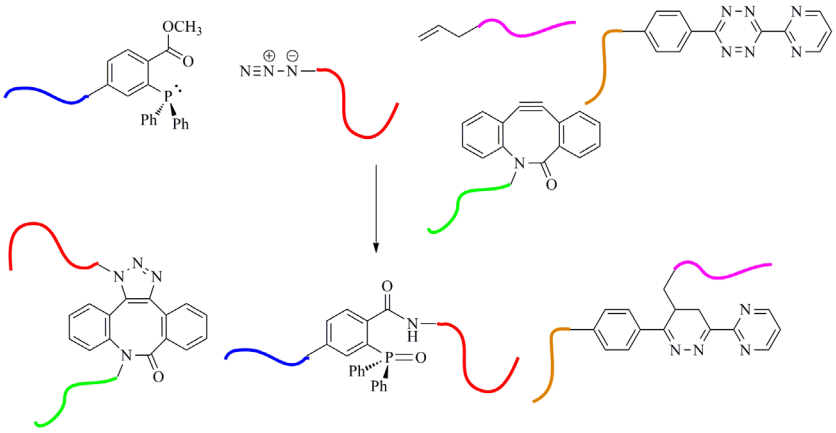
Figure 1. Structure of bacterial peptidoglycan, anchored in the cell membrane.

Gram-negative bacteria are covered by a dense layer of lipopolysaccharides and embedded in their outer membrane. One of the essential components of lipopolysaccharides is 3-deoxy-D-manno-octulosonic acid (KDO).

Metabolic Glycoengineering

Metabolic engineering has demonstrated to be a powerful tool for the modification of eukaryotic cell surfaces. It has been applied in living organisms like human cell cultures, mice and zebrafish [2 – 7]. The principle is based on the incorporation of artificial modified monosaccharides such as derivatives of *N*-acetyl neuraminic acid, *N*-acetylmannosamine, *N*-acetylglucosamine, *N*-acetylgalactosamine and fucose in cell surface glycoproteins [8]. Starting from *N*-acetylglucosamine they are passed through the biosynthetic metabolic

pathway and finally transformed to sialic acid [9]. The incorporated modified sugars with functional groups like azides, alkynes and alkenes can bioorthogonally react with their corresponding functionalized probes [8]. Reactions like inverse Electron Demand Diels–Alder reactions of tetrazines and dienophiles such as trans-cyclooctene and cyclopropene, the Sharpless-Huisgen Mendal [3+2] cycloaddition or Staudinger type reaction and photo-crosslinking [10] have been proven to be bioorthogonal (Scheme 1) [11]. In this way, cell surfaces can be further labelled and analysed. However, human cell surfaces differ completely from bacterial ones.

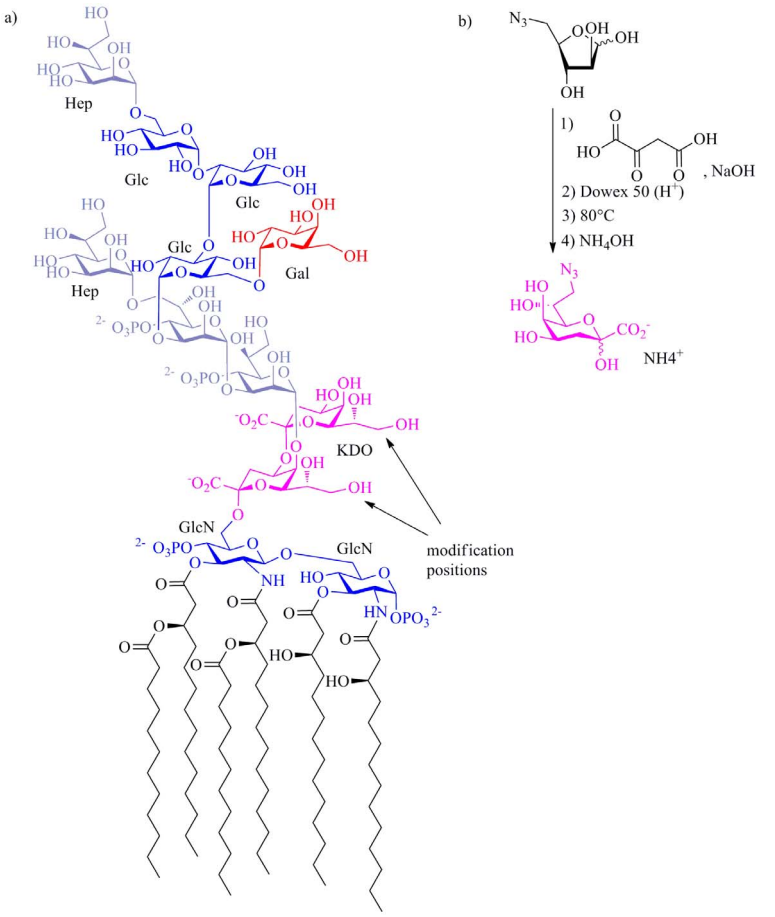


Scheme 1. Established bioorthogonal reactions: Staudinger ligation (blue-red), Huisgen-Sharpless-Mendal cycloaddition (green-red) and the inverse Electron Demand Diels–Alder reaction (iEDDA; purple-orange).

Bacterial cell composition and targets

KDO is a specific component of the inner core of lipopolysaccharides in Gram-negative bacteria. Its biosynthesis starts with arabinose-5-phosphate (arabinose-5-P) which condenses with phosphoenolpyruvate (PEP) to yield KDO-8-phosphate (KDO-8-P). Further dephosphorylation and activation with cytidine monophosphate (CMP) allows the transglycosylation into the lipopolysaccaride core structure [12 – 14].

Dumont *et al.* targeted KDO for the glycoengineering approach in *E. coli* [15]. Referring to the biosynthesis they used 5-azido-5-deoxy-D-arabinofuranose as a precursor for the chemical synthesis of 8-azido-8-deoxy-KDO (Scheme 2). After feeding of *E. coli* with the modified KDO sugar, they were able to stain the bacterial cell surface by azide-alkyne click chemistry with a fluorescent dye (Figure 2) [15]. In general, Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* are missing KDO in their cell wall. Consequently, no labelling was observed under similar conditions.



Scheme 2. a) Structure of the major component of *E. coli* K12 lipopolysaccharide. b) Synthesis of N_3-KDO. Gal: D-galactose; Glc: D-glucose; GlcN: 2-amino-2-deoxy-D-glucose; Hep: L-glycero-D-manno-heptose.

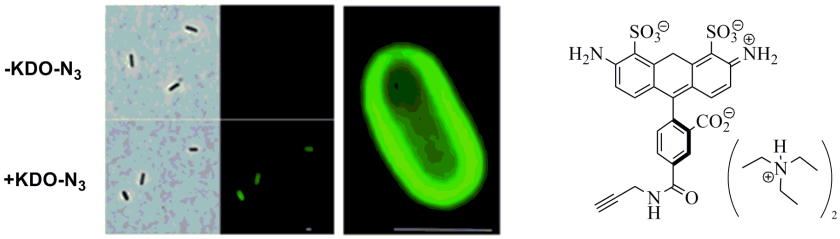


Figure 2. KDO-N_3 metabolically labels *E.coli* lipopolysaccharides. Metabolically incorporated KDO-N_3 in E. coli K12 was revealed by a Cu^I-catalyzed click reaction with alkyne modified fluoresceine (from [15]).

Cell surface labelling and adhesion decrease of S. aureus

Bioorthogonal metabolic labelling of Gram-positive *S. aureus* was instead successful when a *N*-acetylglucosamine derivative was used [16]. Bacteria fed with *N*-azidoacetylglucosamine (GlcNAz) could be labelled with alkynylated fluorescent dyes (Tetramethylrhodamine, TAMRA; and Alexa Fluor® 488) using the copper catalysed click reaction (Figure 3). Moreover, by that change the surface properties of *S. aureus*, adhesion towards a human bladder epithelic cell line (T24) could be reduced significantly.

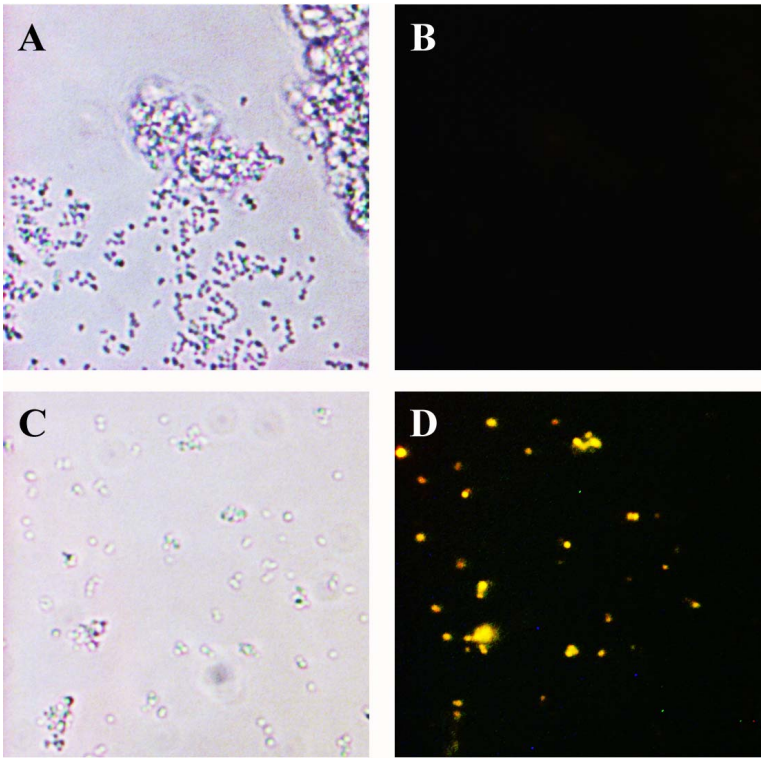
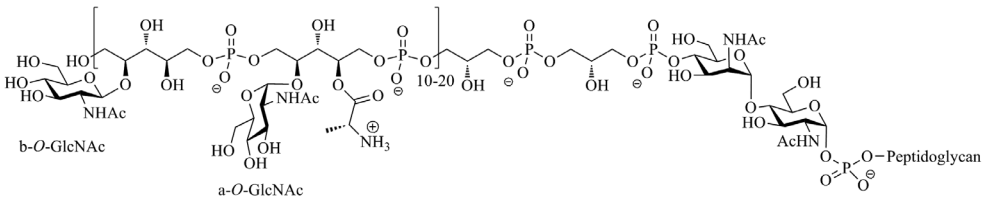
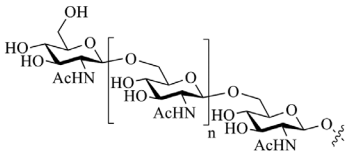


Figure 3. *S. aureus* is labelled with the fluorescent dye Tetramethylrhodamine alkyne by performing a copper catalysed [3+2] cycloaddition on the cell surface after incorporation of the unnatural sugar analogue *N*-azidoacetylglucosamine **(C, D)**. When cultivated in the presence of non-azide containing *N*-acetylglucosamine no fluorescence can be detected **(A, B)**.

Although the exact identity of the targeted glyco structures is still unknown, there are some suggestions. Based on the ability to perform the click reaction on the cell surface as well as the influence on adhesion properties, the affected components may be the peptidoglycan (see Figure 1), glycolipids (teichoic acids) and extracellular polysaccarides (EPS), especially poly-*N*-acetylglucosamine (PNAG) [17]. All of these cell surface components contain potentially accessible GlcNAc as building block (Figure 4).



Wall Teichoic Acid (WTA)



poly-*N*-acetylglucosamine (PNAG), part of the Lipopolysaccaride (LPS)

Figure 4. Structures of *S. aureus* cell surface components containing *N*-acetylglucosamine (GlcNAc) as building block.

Furthermore, teichoic acids are discussed to stimulate bacterial adhesion to human epithelial cells [18 – 20]. Glycoengineering of these and other bacterial membrane components might open a new pathway to study, and also to treat bacterial infections.

Perspectives

Complex carbohydrates on cell surfaces play an important role in many biological recognition processes like cell signalling and adhesion as well as in pathogen recognition [21]. While mammal cell surface glycans are investigated extensively, studies on bacterial carbohydrate components and their role in infection processes are still quite rare. To address crucial problems in fighting infections like emerging resistances of bacteria, pathogenesis mechanisms must be understood in more detail. Bacterial adhesion as the first step in an infection process can be influenced or even prevented by changing cell surface properties.

So far, first studies on metabolic engineering of bacterial glycostructures show the possibility to change cell surface properties and influence adhesion as one critical step in infection processes. Novel drugs based on this concept may overcome today's big problems in infection therapy.

Acknowledgements

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