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New insights into the membrane association mechanism of the glycosyltransferase WaaG from *Escherichia coli*

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ABSTRACT

Monotopic glycosyltransferases (GTs) interact with membranes via electrostatic interactions. The N-terminal domain is permanently anchored to the membrane while the membrane interaction of the C-terminal domain is believed to be weaker so that it undergoes a functionally relevant conformational change upon donor or acceptor binding. Here, we studied the applicability of this model to the glycosyltransferase WaaG. WaaG is involved in the synthesis of lipopolysaccharides (LPS) in Gram-negative bacteria and was previously categorized as a monotopic GT. We analyzed the binding of WaaG to membranes by stopped-flow fluorescence and NMR diffusion experiments. We find that electrostatic interactions are required to bind WaaG to membranes while mere hydrophobic interactions are not sufficient. WaaG senses the membrane's surface charge density but there is no preferential binding to specific anionic lipids. However, the binding is weaker than expected for monotopic GTs but similar to peripheral GTs. Therefore, WaaG may be a peripheral GT and this could be of functional relevance in vivo since LPS synthesis occurs only when WaaG is membrane-bound. We could not observe a C-terminal domain movement under our experimental conditions.

1. Introduction

Gram-negative bacteria like *Escherichia coli* contain two membranes, an outer membrane (OM) and an inner membrane (IM). In *E. coli*, the IM and the inner leaflet of the OM consist of three types of phospholipids: zwitterionic phosphatidylethanolamines (PE, 70–80%), and the negatively charged lipids phosphatidylglycerol (PG, 10–20%), and cardiolipin (CL), the latter only found in small amounts (~5%) [1–3]. The composition of the acyl chains in *E. coli* changes in response to environmental conditions [4–8] and the outer leaflet of the OM differs from the IM in that its main constituents are lipopolysaccharides (LPS). A functioning LPS synthesis machinery is vital since LPS form a protective barrier against small molecules, such as antimicrobial compounds, while nutrients and ions enter the cell through porins located inside the OM [9]. Moreover, LPS are required for the structural integrity of the OM [10]. Several enzymes are needed for the synthesis of LPS [11,12] and here, we have examined the membrane interaction of one of them: WaaG.

WaaG has been classified as a monotopic GT-B fold glycosyltransferase (GT, see below) [13]. WaaG deficient *E. coli* strains survive under laboratory conditions but are immobile and highly sensitive to antibiotics, such as novobiocin, and, therefore, WaaG has been identified as a potential antibiotic target [10,14,15]. Detergent is used in the lysis step of the purification but can be removed in further purification steps [16]. WaaG catalyzes the transfer of a glucose moiety from the donor nucleotide sugar uridinediphosphate-α-D-glucose (UDP-Glc) to the L-glycero-D-manno-heptose II of the inner core of LPS retaining the stereochemistry of the donor. For retaining GTs, two catalytic mechanisms have been suggested [17,18]. First, catalysis may proceed via an S_N2 double-displacement mechanism, in which a covalent glycosyl-enzyme intermediate and subsequently a glycosidic bond between the sugar and the acceptor molecule is formed. However, this mechanism requires a catalytic base on the β-face of the glycoside, which is absent in many retaining GTs, e.g. for WaaG there is no obvious catalytic base close to the sugar's anomeric carbon [16,17]. Therefore, a second catalytic mechanism has been proposed for retaining GTs (see Fig. 1A). In this so called 'front-face' S_Ni-like mechanism an oxocarbenium ion intermediate is formed and the phosphate bond breakdown and glycosidic bond formation occur stepwise on the same face of the glycoside [16–18]. In contrast to the simplified depiction of Fig. 1A, the proposed

Abbreviations: CL, cardiolipin; DHPC-d_{22}, tail-deuterated 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; GT, glycosyltransferase; IM, inner membrane; LPS, lipopolysaccharide; LUV, large unilamellar vesicle; OM, outer membrane; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; TOCL, tetraoleoyl cardiolipin; UDP-Glc, uridinediphosphate-α-D-glucose

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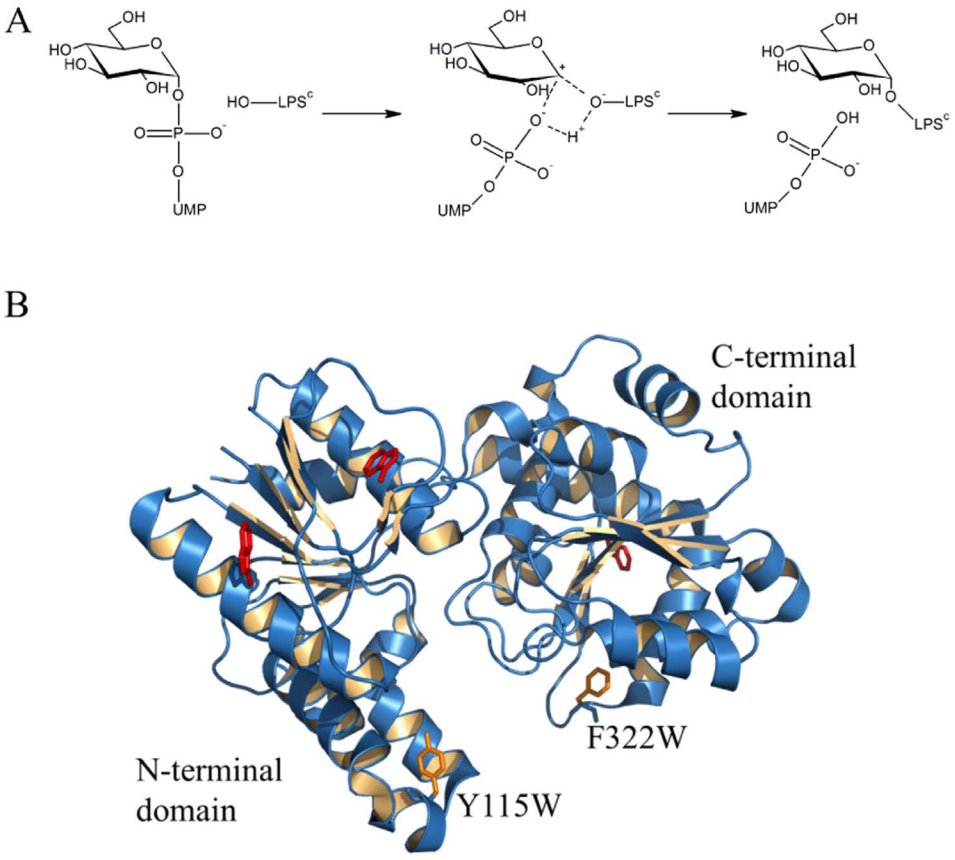

Fig. 1. A) The front-face catalytic mechanism that has been proposed for retaining GTs. UMP: uridinemonophosphate. LPS^c represents the inner core of LPS. The figure is adapted from Ref. [18]. B) Crystal structure of WaaG (PDB 2IW1). The three wildtype Trp residues are shown in red, the residues mutated into Trp residues are shown in orange and labeled according to the nomenclature used in the text.

front-face mechanism involves a number of intermediates and requires spatial rearrangements of the involved molecules relative to each other so that a glycosidic bond can eventually be formed [17]. Moreover, a carbonyl moiety, which in the case of WaaG could stem from the backbone of Ala99, plays a crucial role in stabilizing the oxocarbenium ion [16,17].

GTs constitute a diverse class of enzymes that catalyze the formation of glycosidic bonds. GTs are highly versatile as they catalyze the transfer of sugar moieties from activated donor molecules to a vast amount of acceptor molecules but are at the same time highly specific for donor and acceptor molecules [18]. As of September 2017, roughly 350,000 GTs are classified into 104 different families in the Carbohydrate Active Enzymes database (CAZy) based on significant amino acid sequence similarities [19,20]. Despite this, the majority of GTs is predicted to assume one of only three distinct folds, termed GT-A, GT-B, and GT-C but some additional isolated folds have been characterized [17,18,21].

Membrane-associated GTs of the GT-B fold contain two Rossmann-like domains which are connected by a flexible linker. The active site is located in the cleft between the two domains and the nucleotide sugar binds mainly to the C-terminal domain while the N-terminal domain interacts with the acceptor molecule. Typically, membrane-associated GT-B GTs are subdivided into monotopic GTs, that permanently anchor to one leaflet of the membrane, and peripheral GTs, that exhibit a weaker interaction with the membrane [13]. Peripheral GT-B GTs have been studied employing the glycosyltransferase PimA as a model system. It was recently shown that PimA undergoes a large conformational change upon membrane and substrate binding and membrane interactions are driven by electrostatic forces [22]. Based on studies of full-length as well as fragments of the monotopic GT-B GT *at*DGD2 a model has been developed to describe the membrane interaction of monotopic GT-B GTs [13,23]. In this model, the N-terminal domain is permanently anchored to the membrane via electrostatic interactions likely stabilized by membrane interactions of Trp, Tyr, and hydrophobic residues. The binding of the C-terminal domain to the membrane is believed to be weaker and it is hypothesized that the C-terminal domain undergoes conformational changes that are relevant for the function of the protein [18,23,24].

There are, however, substantial gaps in the understanding of the membrane binding. The classification into peripheral and monotopic proteins is based on the subcellular localization of the protein but membrane affinities are only reported for very few full-length GTs [25,26] and membrane interacting fragments thereof [27,28]. Moreover, the role of specific lipids in membrane binding is largely unclear. Finally, while evidence exists that the N- and C-terminal domains undergo conformational changes upon substrate binding in soluble GT-B GTs [29,30], it has not been conclusively shown for membrane-associated GTs that the C-terminal domain undergoes a conformational change with respect to the N-terminal domain and the membrane, as hypothesized in the model described above.

The binding of WaaG to membranes has previously been studied and electrostatic interactions with anionic lipids were found to be crucial for membrane binding [28,31]. Here, we have quantified the binding of the N- and C-terminal domains of WaaG to different types of zwitterionic and anionic lipids in the presence and absence of the donor substrate UDP-Glc employing stopped-flow fluorescence and diffusion NMR experiments. To do so, Trp point mutations were introduced at locations in either the N-terminal or C-terminal domain of the protein (Fig. 1B and Table S1). Our results demonstrate that WaaG is not a typical monotopic membrane protein, but interacts with lipids in a way more characteristic for peripheral membrane proteins.