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Positive cooperativity between acceptor and donor sites of the peptidoglycan glycosyltransferase

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ABSTRACT

The glycosyltransferases of family 51 (GT51) catalyze the polymerization of lipid II to form linear glycan chains, which, after cross linking by the transpeptidases, form the net-like peptidoglycan macromolecule. The essential function of the GT makes it an attractive antimicrobial target; therefore a better understanding of its function and its mechanism of interaction with substrates could help in the design and the development of new antibiotics.

In this work, we have used a surface plasmon resonance Biacore® biosensor, based on an amine derivative of moenomycin A immobilized on a sensor chip surface, to investigate the mechanism of binding of substrate analogous inhibitors to the GT. Addition of increasing concentrations of moenomycin A to the *Staphylococcus aureus* MtgA led to reduced binding of the protein to the sensor chip as expected. Remarkably, in the presence of low concentrations of the most active disaccharide inhibitors, binding of MtgA to immobilized moenomycin A was found to increase; in contrast competition with moenomycin A occurred only at high concentrations. This finding suggests that at low concentrations, the lipid II analogs bind to the acceptor site and induce a cooperative binding of moenomycin A to the donor site. Our results constitute the first indication of the existence of a positive cooperativity between the acceptor and the donor sites of peptidoglycan GTs.

In addition, our study indicates that a modification of two residues (L119N and F120S) within the hydrophobic region of MtgA can yield monodisperse forms of the protein with apparently no change in its secondary structure content, but this is at the expense of the enzyme function.

1. Introduction

The glycosyltransferases of family 51 (GT51) are essential enzymes found in bacteria with peptidoglycan cell wall [1]. They exist in two forms: as a monofunctional domain or linked to the N-terminal end of penicillin-binding (PB) domain in bifunctional PB proteins (PBPs) [1]. Both forms catalyze the polymerization of lipid II (undecaprenyl pyrophosphate-MurNAc(pentapeptide)-GlcNAc) precursor to form linear glycan chains which, after cross linking by the transpeptidases, form the net-like peptidoglycan macromolecule that encases bacteria and protects them from rupture under their high cytoplasmic pressure. Inhibition of the GT blocks peptidoglycan synthesis and leads to bacterial lysis and death, therefore the GT is a promising target in the search for new antibacterial agents to counter the arising antibiotic resistant strains.

Two main strategies are being followed to identify novel pharmacophore lead molecules for the design and synthesis of new GT inhibitors that could be developed as antibiotics: high throughput screening of chemical libraries [2–4] and rational synthesis of substrate and moenomycin (GT inhibitor) analogs [5–8]. The second strategy requires a deep understanding of the GT mechanism and structural data on protein–ligand complexes. Substantial progress has been made in the biochemistry of the GT [9–14] and several crystal structures have been determined, both

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in the apo form and in complex with moenomycin A and a lipid II analog [15–20]. These structures show that the GT is composed of a lysozyme-like globular domain and a small hydrophobic domain (called jaw domain) with a deep cleft between them containing the active site (Fig. 1). The extended active site is divided into two subsites, separated by a mobile region (composed of an alpha helix and a beta-hairpin), a donor site for the growing glycan chain binding and an acceptor site for lipid II binding. Elongation of the growing chain is achieved by the addition of disaccharide unit MurNAc-GlcNAc of lipid II in a processive way. Comparison of the apo and moenomycin A bound structures shows that the head domain remains unchanged after moenomycin A binding whereas a major conformational change occurs in the jaw subdomain with partial restructuration of the mobile region [15].

At the initiation phase of polymerization, the lipid II substrate molecules bind to the donor and acceptor sites which makes binding studies to determine the affinity of the substrate for each site particularly complex. Differences in affinity would determine the order of binding in the initiation stage and might consequently affect the affinity for the second site by an allosteric effect. We have used lipid II mimic inhibitors and moenomycin A, which is proposed to mimic the growing chain, to investigate these questions. An assay was developed by a modification of the SPR method described by Welzel and colleagues [21]. This technique is based on the interaction of an immobilized moenomycin A amino derivative with peptidoglycan glycosyltransferase and was originally used to test moenomycin derivatives for their inhibitory activity. We improved the sensitivity of this assay and used it to characterize several lipid II analog inhibitors as well as enzymatically inactive GT mutants as to their donor site functionality. Moenomycin A necessary for the synthesis of the amino derivative is not commercially available. Hence we also developed a method to isolate moenomycin A in good purity and yields from a Flavomycin® standard containing a mixture of moenomycin congeners.

We show that in the presence of low concentrations of lipid II analogs, supposedly bound to the acceptor site, moenomycin A binding to the donor site of the GT increased, indicating an allosteric activation of the donor site. At higher concentrations of these analogs, lower GT binding levels were observed, indicating competition with moenomycin A at the donor site.

All GTs contain a conserved hydrophobic surface (Fig. 1) that mediates their interaction with the cytoplasmic membrane and renders the purified proteins polydisperse [18,22]. This property makes a thorough quantitative binding study of the MtgA by SPR complex. The possibility to improve the solubility (monodispersity) of the protein by modification of residues in the hydrophobic surface was investigated. A monodisperse form of the protein was obtained without detergent and characterized.

2. Materials and methods

2.1. Materials

Surface plasmon resonance (SPR) biosensor analyses were performed on a Biacore Q® (Biacore AB, Uppsala, Sweden). Semi-preparative HPLC (high performance liquid chromatography) was performed using an HPLC consisting of a Merck-Hitachi LaChrom® Pump L7100 (Hitachi Ltd., Tokyo, Japan), a manual sample injector valve model 7125 (Rheodyne Inc., Cotati, USA), a NUCLEODUR® C18 HTec column (dimensions: 250 × 10 mm, particle size: 5 μm) (Macherey-Nagel GmbH & Co. KG, Düren, Germany) in a thermostated column compartment model ERC 125 (ERC GmbH, Riemerling, Germany), a Merck-Hitachi LaChrom® UV–vis Detector L-7420 with semi-micro flow cell (Hitachi Ltd., Tokyo, Japan), and a 162 chromatography signal interface (Autochrom Inc., Milford, USA). Analytical HPLC was performed using an HPLC consisting of a Merck-Hitachi LaChrom® Pump L7100 (Hitachi Ltd., Tokyo, Japan), a Merck-Hitachi LaChrom® Programmable Autosampler L-7250 (Hitachi Ltd., Tokyo, Japan), a NUCLEODUR® C18 HTec column (dimensions: 250 × 4 mm, particle size: 5 μm) (Macherey-Nagel GmbH & Co. KG, Düren, Germany) in a Merck LaChrom® Column Oven L-7350 (Merck KGaA, Darmstadt, Germany), a Merck-Hitachi LaChrom® Diode Array Detector L-7450 (Hitachi Ltd. Tokyo, Japan), and a Merck-Hitachi Interface D-7000 (Hitachi Ltd., Tokyo, Japan). HPLC eluents and SPR running buffers were filtered through a Whatman® OE 67 cellulose acetate membrane filter (pore size 0.45 μm; Whatman GmbH, Dassel, Germany) and degassed prior to use.

High-purity water was produced from deionized water using a Milli-Q® Gradient A10 (Millipore, Molsheim, France). Acetonitrile (Fisher Scientific UK Limited, Loughborough, UK), methanol (VWR International, Fontenais-Sous-Bois, France), acetone (VWR International, Fontenais-Sous-Bois, France), and chloroform (Fisher Scientific UK Limited, Loughborough, UK) were HPLC grade. Acetonitrile (puriss.) for semi-preparative HPLC, dimethyl sulfoxide (p.a.), 25% ammonia, sodium hydroxide (≥99%), sodium chloride (p.a.), HEPES (p.a.), TRIS (≥99.9%), and EDTA (disodium

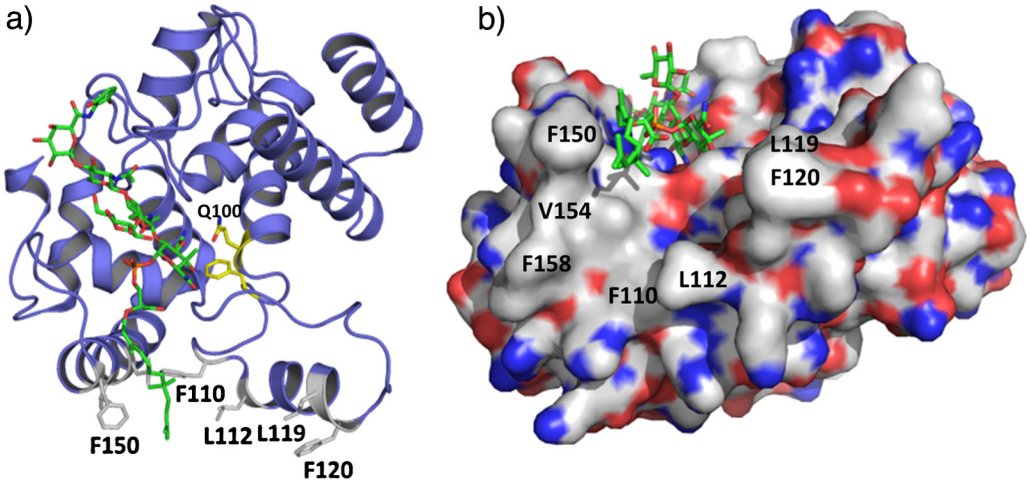

Fig. 1. (a) Ribbon representation of the structure of MtgA-E100Q in complex with moenomycin A bound to the donor site (PDB 3HZS). The modified residues L119N, F120S are shown in stick. (b) Electrostatic surface representation of MtgA with positively charged residues in blue, negatively charged residues in red and neutral residues in grey. The residues of the hydrophobic surface are indicated. Figures were generated by The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC).