Bioorganic Chemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Prospects for novel inhibitors of peptidoglycan transglycosylases

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Peptidoglycan
Transglycosylase
Inhibitor
Antibiotic
Discovery

ABSTRACT

The lack of novel antimicrobial drugs under development coupled with the increasing occurrence of resistance to existing antibiotics by community and hospital acquired infections is of grave concern. The targeting of biosynthesis of the peptidoglycan component of the bacterial cell wall has proven to be clinically valuable but relatively little therapeutic development has been directed towards the transglycosylase step of this process. Advances towards the isolation of new antimicrobials that target transglycosylase activity will rely on the development of the enzymological tools required to identify and characterise novel inhibitors of these enzymes. Therefore, in this article, we review the assay methods developed for transglycosylases and review recent novel chemical inhibitors discovered in relation to both the lipidic substrates and natural product inhibitors of the transglycosylase step.

1. Introduction

In the search for new treatments of bacterial infections and to combat the increasing threat of resistance to existing antimicrobials, there is renewed interest in the exploitation of existing validated targets with novel approaches. With respect to bacterial cell wall biosynthesis, the validity of the peptidoglycan biosynthetic apparatus is well established, particularly in consideration of the fact that many of these antimicrobial targets exist at or beyond the extra-cytoplasmic surface of the cell membrane and are well conserved across all bacterial species [1,2]. The biosynthetic pathway leading to peptidoglycan precursor lipid II and the generalised scheme for its polymerisation into the peptidoglycan layer is well documented. Briefly, uridine 5′-pyrophosphoryl-N-acetyl muramyl-L-alanyl-γ-D-glutamyl-meso-diaminopimelyl-D-alanyl-D-alanine (UDP-Mur*N*Ac-L-Ala-D-Glu-L-(Lys/meso-DAP)-D-Ala-D-Ala) or its L-lysine derivative (UDP-Mur*N*Ac-L-Ala-D-Glu-L-(Lys)-D-Ala-D-Ala) is produced in the cytoplasmic pathway before linkage at the cytoplasmic membrane surface to an undecaprenyl (C55) carrier lipid, prior to the addition of Glc*N*Ac, forming lipid II [3]. This peptidoglycan precursor is then transferred to the outer surface of the cytoplasmic membrane where it is polymerised by monofunctional transglycosylases and class A bifunctional Penicillin Binding Proteins (PBPs) into long glycan chains [4] (Fig. 1). The transpeptidase activity of Class A and B PBPs then produce inter-strand peptide cross-links from pentapeptides emanating from adjacent glycan chains. The resulting polymer has the mechanical strength and rigidity required to resist cytoplasmic osmotic stress and forms a scaffold for a number of extracellular structures and functions.

Both academic and industrial effort over many decades has been directed towards the transpeptidase function of the penicillin binding proteins (PBPs) in this context, with the development of many generations of β-lactam-based antibiotics [5]. However, there has been relatively little development directed towards the essential transglycosylase function required to provide the polymeric transpeptidase substrate, which can also be the product of the same bifunctional peptidoglycan biosynthetic enzyme [6]. Dual inhibition of both transglycosylase and transpeptidase functions would be a powerful antimicrobial strategy providing therapeutic options in a variety of scenarios, including those currently untreatable. Since the active site of the transglycosylase enzymes exist at the membrane surface where peptidoglycan intermediates are presented to the enzymes, this has been viewed as a difficult interface to target [7]. In addition, consideration of the catalytic function of the enzymes leads to the conclusion that the transglycosylase enzymes have long extended active sites, which traditionally have been viewed as more difficult to target [8]. Nevertheless, nature has already provided an exemplar solution to this issue in the form of the moenomycin group of antimicrobials, which appear to mimic the polymerised form of

Abbreviations: Glc*N*Ac, *N*-acetyl-glucosamine; Mur*N*Ac, *N*-acetyl-muramic acid; Gal*N*Ac, *N*-acetyl-galactosamine; UDP, uridine diphosphate; PBP, penicillin binding protein.

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http://dx.doi.org/10.1016/j.bioorg.2014.05.007
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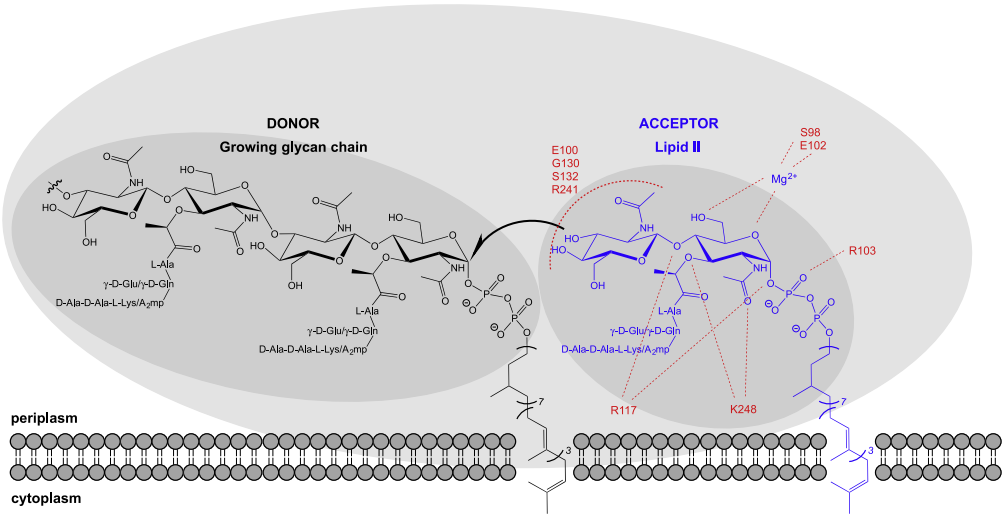

Fig. 1. Schematic diagram of the transglycosylase active site showing doner and acceptor sites. Residue numbers in the acceptor sites refer to those determined for *S. aureus* monofunctional transglycosylase in relation to lipid II analogue as described by Huang et al. [28].

the substrate within the transglycosylase active site. Poor pharmacokinetics prohibits the use of moenomycins in humans, yet this group of antibiotics has been used for decades in agriculture, principally in animal husbandry applications [9]. Remarkably, there is almost no incidence of resistance to these compounds, which implies that the transglycosylase activity may have significant attraction for future targeting.

Understanding the active site architecture of the transglycosylase through X-ray crystallographic analysis along with advances in biochemical study through the provision of native substrate and chemically defined probes, and the development of assay technologies that can support industry standard screening techniques, provide a new prospect for inhibitor discovery for new generation chemotherapy (Fig. 1). In this review article we provide a perspective of the assay technologies available and compounds recently discovered, that are pertinent in that context.

2. Assays for transglycosylase activity

Bacterial transglycosylases have been studied for over 50 years [10]. The discovery and development of novel transglycosylase inhibitors has been highly dependent on appropriate activity assays. However, progress has been hampered by the lack of quantitative and high throughput approaches capable of fast, accurate enzyme activity measurement. In addition, such efforts have been affected by the relative chemical complexity and lack of availability of the transglycosylase substrate, lipid II. Chemical and chemi-enzymatic approaches to overcome this hurdle have been reported by several groups, [11–22]. In addition, lipid II and other peptidoglycan intermediates have become available from the UK Bacterial Cell Wall Biosynthesis Network (UK-BaCWAN). Since both the transglycosylase enzymes and substrate are within a lipid membrane environment, assay conditions and design needs to factor in these chemical properties and physical limitations. The solution of several X-ray crystal structures of mono-functional and bifunctional enzymes has enhanced structure based drug design efforts [7,23–28], an advance which has depended upon the design and implementation of reliable and accurate high-throughput assays. The following sections discuss the main assay types currently available, whilst Fig. 2 and Table 1 provide concise summaries.

2.1. Paper and thin layer chromatography

Paper chromatography was first used to study the full polymerisation of peptidoglycan using particulate enzyme preparations isolated from *Staphylococcus aureus* with radiolabelled UDP-*N*-acetylmuramyl-pentapeptide and UDP-*N*-acetylglucosamine as substrates [29]. The assay was adapted to use [^{14}C]-labelled lipid II with membrane protein preparations [22]. The use of penicillin to inhibit transpeptidase and carboxypeptidase activities of bifunctional PBPs facilitated analysis of the transglycosylation reaction, and the assay has been used for several studies [30–34]. Whilst scintillation counting allows collection of quantitative data in a stopped assay format, paper chromatography remains a cumbersome technique that is low throughput and lacks the ability to rapidly characterise the product post reaction.

Thin layer chromatography has also been used to study polymerisation, using fluorescent lipid II for detection [35]. With the fluorescent substrate, a dansyl reporting group was linked to the ε-amino group of the lysine side chain of the pentapeptide via a sulfonamide linkage to generate fluorescent dansyl lipid II. Transglycosylase kinetic parameters have been shown to be unaffected by the presence of this group (see Section 2.6 for further discussion).

The presence of a dansyl group prevents transpeptidation from occurring on this molecule, which results in a transglycosylation-specific assay when used as the sole substrate in a stopped assay format. Paper and thin layer chromatography are both highly sensitive techniques, allowing very small amounts of material to be detected. However, the assay remained inherently low throughput and qualitative.

2.2. Polyacrylamide gel based techniques

Transglycosylase activity can also be studied using a polyacrylamide gel based assay developed from a technique initially used in the late 1980s. Tricine–SDS–PAGE [36,37] is a variation on the more commonly used glycine SDS-PAGE that has been optimised for low molecular weight proteins. Glycan products made of repeating disaccharide units have a net negative charge, allowing their separation by electrophoresis, and shorter chain lengths in particular are within the optimum separation size range. The system was modified to separate the polymeric products of isolated transglycosylase domains using [^{14}C]-lipid II and lipid IV as