

# Kinetic Analysis of Teicoplanin Glycosyltransferases and Acyltransferase Reveal Ordered Tailoring of Aglycone Scaffold to Reconstitute Mature Teicoplanin

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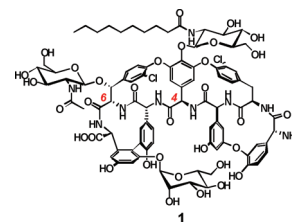
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The glycopeptide antibiotics, teicoplanin, vancomycin, and chloroeremomycin, are clinically important compounds used as a treatment of last resort for methicillin-resistant *Staphylococcus aureus* and other Gram-positive pathogens.<sup>1–3</sup> Clinically administered teicoplanin comprises a naturally occurring mixture of five differentially acylated scaffolds, including teicoplanin A2-3 **1** (Figure 1).<sup>9</sup> The heptapeptide scaffolds of these molecules are assembled by nonribosomal peptide synthetases,<sup>4</sup> with extensive oxidative crosslinking giving rigidity to the antibiotic cores.<sup>5,6</sup> Importantly, after release from the enzymatic assembly lines, all of the family members are modified through glycosylation, and some through acylation,<sup>7</sup> modifications that are important in conferring biological activity.<sup>8</sup> We have been interested in characterizing the postassembly line-tailoring processes of glycopeptides to understand the enzymatic properties of the respective catalysts and to construct frameworks for the rational design of new analogues.

The glycosyltransferases, tGtfA and tGtfB, are able to act separately on the teicoplanin aglycone **2** and also in tandem to produce the bisglycosylated heptapeptide **6**.<sup>6</sup> The acyltransferase, tAtf, is known to mediate condensation of an acyl CoA with the teicoplanin scaffold glycosylated at position 4<sup>6,10</sup> and also with the UDP sugar, UDP-2-aminoglucose.<sup>6</sup> For transfer to the glycosylated aglycone **3**, a range of acyl chains could successfully be transferred, with decanoyl CoA giving the highest activity;<sup>10</sup> decanoyl CoA has hence been used as the preferred acyl donor throughout this work, thus constituting the pathway to teicoplanin A2-3 **1**. In the absence of any kinetic data, however, it was impossible to postulate which would be the preferred acyl and glycosyl acceptors for these enzymes in vivo. Building on our prior work to establish the kinetic order of action of the glycosyltransferases of the vancomycin and chloroeremomycin families,<sup>11,12</sup> we report here the first detailed kinetic characterization of the teicoplanin glycosyltransferases (tGtfA and tGtfB) and acyltransferase (tAtf).

Initially, we sought to determine the relative order of addition of the glycosyl moieties at positions 6 and 4 of the aglycone, by the action of tGtfA and tGtfB, respectively. Thorough kinetic analysis revealed that, although tGtfA is able to catalyze addition of UDP-GlcNAc to the aglycone **2**, it does so with 56-fold lower catalytic efficiency relative to addition of the same sugar to 4-(2'-



**Figure 1.** Chemical structure of glycopeptide, teicoplanin A2-3 **1**. Positions 4 and 6 are indicated in red.

aminoglucose)-AGT **3**, and 35-fold lower  $k_{cat}/K_m$  relative to the parameters for 4-GlcNAc-AGT **4** (Table 1). Hence, tGtfA prefers a scaffold monoglycosylated at position 4, but is fairly indiscriminate regarding the N-acylation state of the sugar moiety at this position (Scheme 1).

The AGT scaffold preglycosylated at position 6 (6-GlcNAc-AGT) is not accepted by tGtfB at all, regardless of whether UDP-GlcNAc, UDP-2-aminoglucose, or UDP-(*N*-decanoyl)-2-aminoglucose is used as the glycosyl donor (data not shown). However, AGT **2** is readily accepted by this enzyme (Table 1). Overall, these data suggest that tGtfB acts first on the naked aglycone **2** to glycosylate at position 4. tGtfA acts subsequently to condense a second GlcNAc moiety onto either the 4-GlcNAc-AGT **4** or 4-(2'-aminoglucose)-AGT **3** scaffolds at position 6, giving bisglycosylated heptapeptides **5** and **6** (Scheme 1).

It has been shown that the vancomycin and chloroeremomycin glycosyltransferases, GtfB, GtfC, GtfD, and GtfE and the acyltransferase, aTf, will accept both vancomycin and teicoplanin scaffolds.<sup>13,14,10</sup> In light of this promiscuity, particularly in the related Gtfs, it is interesting that neither tGtfA nor tGtfB can catalyze glycosylation of the vancomycin aglycone core (data not shown). Both teicoplanin Gtfs are highly selective for their cross-linked peptide scaffold, implying that their active sites are capable of sensing chemical groups distal to the reactive functionalities as well as the overall conformation of the glycosyl acceptor.

tGtfB is relatively promiscuous in terms of the sugar donor that it will utilize: UDP-GlcNAc, UDP-2-aminoglucose, and UDP-(*N*-decanoyl)-2-aminoglucose are all accepted with similar catalytic efficiencies, effecting transfer onto the AGT aglycone **2** (Table 1). The concentration of UDP-GlcNAc is likely much higher in the cell relative to the other glycosyl donors, so the probable glycosylation pathway in vivo involves tGtfB-mediated glycosylation with UDP-GlcNAc. The teicoplanin biosynthetic cluster contains a dedicated deacylase,<sup>15</sup> which has been shown to carry out deacetylation of 4-GlcNAc-AGT **4** to afford 4-(2'-aminoglucose)-AGT **3**.<sup>15</sup> Deacylated scaffold **3** or **5** would be required to enable the action of acyltransferase tAtf (Scheme 1).

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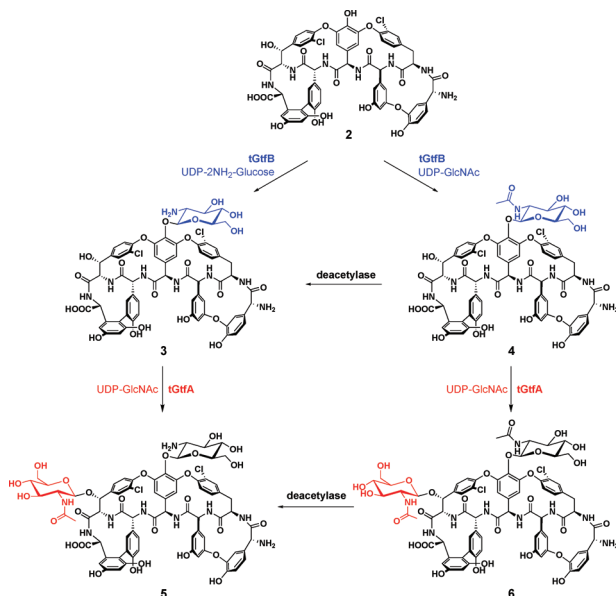
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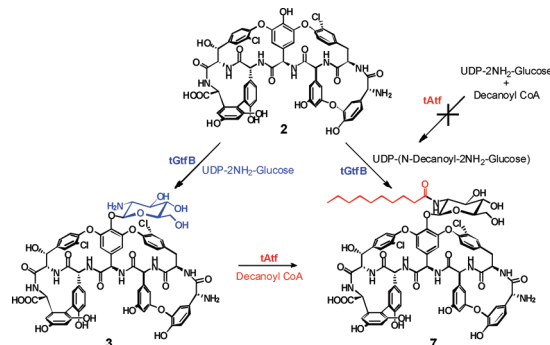
**Table 1.** Kinetic Data for Glycosyl and Acyl Transfer by tGtFA, tGtFB, and tAtf

enzyme	glycosyl donor	glycosyl acceptor	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$K_{\text{m}}$ (donor) ( $\mu\text{M}$ )	$K_{\text{m}}$ (acceptor) ( $\mu\text{M}$ )	$k_{\text{cat}}/K_{\text{m}}$ (acceptor) ( $\text{min}^{-1} \text{mM}^{-1}$ )
tGtFA	UDP-GlcNAc	<b>2</b>	$0.38 \pm 0.01$	$807 \pm 66$	$225 \pm 19$	1.6
		<b>3</b>	$7.6 \pm 0.1$	$32 \pm 6$	$85 \pm 22$	89
		<b>4</b>	$10.2 \pm 0.3$	$151 \pm 21$	$175 \pm 14$	58
		<b>5</b>	$12.4 \pm 0.7$	$1900 \pm 200$	$281 \pm 37$	44
tGtFB	UDP-GlcNAc	<b>2</b>	$2.3 \pm 0.1$	$1600 \pm 200$	$104 \pm 15$	22
	UDP-2-aminoglucose	<b>2</b>	$4.1 \pm 0.5$	$250 \pm 30$	$1400 \pm 300$	2.9
	UDP-( <i>N</i> -decanoyl)-2aminoglucose	<b>2</b>				
enzyme	acyl donor	acyl acceptor	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$K_{\text{m}}$ (donor) ( $\mu\text{M}$ )	$K_{\text{m}}$ (acceptor) ( $\mu\text{M}$ )	$k_{\text{cat}}/K_{\text{m}}$ (acceptor) ( $\text{min}^{-1} \text{mM}^{-1}$ )
tAtf	decanoyl CoA	<b>3</b>	$6200 \pm 400$	$1.5 \pm 0.4$	$10 \pm 2$	620000
		UDP-2-aminoglucose	$43 \pm 4$	$<10$	$6500 \pm 1800$	6.6

**Scheme 1.** Order of Glycosylation of AGT **2** by Glycosyltransferases, tGtFA and tGtFB

tAtf was previously demonstrated to mediate condensation of an acyl CoA with both 4-(2'-aminoglucose)-AGT **3**<sup>6,10</sup> and with UDP-2-aminoglucose.<sup>6</sup> Our detailed kinetic evaluations have shown that these two transformations are indeed both catalyzed by tAtf, but with vastly different kinetic profiles (Table 1). Since the catalytic efficiency of tAtf for the glycosylated scaffold **3** is 100000-fold higher than for the free UDP sugar, it is clear that tAtf's preferred acyl acceptor is the glycosylated heptapeptide **3**. Because of substrate limitations, we were unable to characterize the activity of tAtf with bisglycosylated AGT, **5**. However, given the very high  $k_{\text{cat}}$  and low  $K_{\text{m}}$  of tAtf for **3**, we believe it highly likely that this is the native substrate for the enzyme. Therefore, not only do our kinetic data confirm an ordering in the glycosylation steps of teicoplanin maturation, but also in the acylation process. Thus, tGtFB acts first on the AGT aglycone **2** to produce **3**, which is then the preferred substrate for acylation by tAtf (Scheme 2).

This study represents the first systematic evaluation of the ordering of postassembly tailoring modifications on the teicoplanin scaffold. In particular, we have demonstrated that acylation is rapid, occurring preferentially after tGtFB-mediated attachment of the 2-aminoglucose moiety to position 4 of the aglycone rather than on the free UDP-sugar. Furthermore, action of the two glycosyltransferases, tGtFB and tGtFA, directs ordered addition of glycosyl units to the 4 and 6 positions of the aglycone **2**. This information on the kinetic order of scaffold tailoring will be indispensable in designing biosynthetic routes to novel derivatives of teicoplanin **1**

**Scheme 2.** Order of Tailoring of Aglycone **2** by tGtFB and tAtf

with unusual sugar and acyl appendages. As vancomycin and teicoplanin resistance becomes increasingly widespread,<sup>1,2</sup> novel glycopeptide analogues produced in this manner may prove highly useful in the clinic.

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**Supporting Information Available:** Experimental procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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