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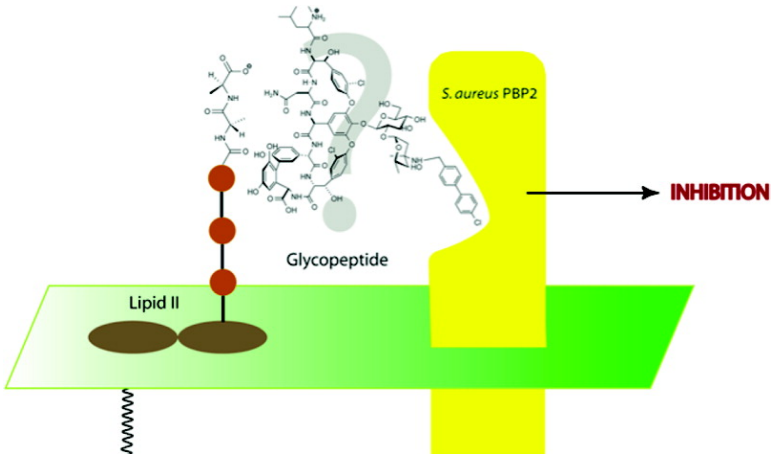

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Differential Inhibition of *Staphylococcus aureus* PBP2 by Glycopeptide Antibiotics

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have traditionally been treated with the glycopeptides vancomycin (**1a**, Figure 1) and teicoplanin (**2a**). The emergence of vancomycin-resistant bacteria has caused considerable alarm among public health providers and has prompted efforts to develop second-generation glycopeptide analogues.[1] Glycopeptide analogues such as chlorobiphenyl vancomycin (CBPV, **3a**) and dalbavancin (**4a**), which resemble teicoplanin in having a hydrophobic group on the A4-linked carbohydrate moiety, have shown particularly good activity.[2,3] In fact, dalbavancin (**4a**) is now in late-stage clinical trials.[4] However, the molecular basis for the enhanced activity of dalbavancin or CBPV is not understood.

All glycopeptide antibiotics are believed to have the same mechanism of action: they prevent maturation of the bacterial cell wall by binding to the terminal D-alanyl-D-alanine moiety of peptidoglycan precursors, thus blocking enzymes involved in the final stages of peptidoglycan synthesis.[5,6] Despite minor differences in the structures of the aglycones, the D-Ala-D-Ala binding sites are similar in all glycopeptides and the affinities for D-Ala-D-Ala are essentially identical.[7,8] Nevertheless, the potency and spectrum of various glycopeptides can differ significantly.[3] For example, dalbavancin and CBPV show superior activity against *S. aureus* strains (including MRSA) compared with vancomycin and teicoplanin (Table 1).[3] We and others have suggested that the improved activity of particular glycopeptide derivatives (e.g., **3a** and **3b**) is related to a second mechanism that does not involve D-Ala-D-Ala binding but rather direct interaction with enzymes involved in the final stages of peptidoglycan biosynthesis.[9−12] Here we test this hypothesis against the clinically relevant pathogen, *S. aureus*.

Vancomycin was proposed to inhibit bacterial transglycosylases by binding to its substrate more than 30 years ago by Strominger[5] and Perkins,[6] but this proposed mechanism of action has not been evaluated kinetically because assays to monitor the activity of purified Gram positive transglycosylases have not been available. We have recently overexpressed and purified *S. aureus* PBP2 (penicillin binding protein) in *Escherichia coli* BL21(DE3) as a C-terminal His_6 construct, and conditions were developed to monitor enzymatic activity using our C35 Lipid II analogue.[13] To determine if the glycopeptides **1a**−**4a** are substrate binders, we measured the reaction rates as a function of lipid II concentration in the presence of fixed concentrations of each inhibitor.[14] The inhibition curves display the sigmoidal shape characteristic of substrate binders, and the inflection points are consistent with a 1:1 binding mode of lipid II/antibiotic (Figure 2a).[15] Furthermore, under identical reaction conditions the IC_{50}'s are similar, reflecting the comparable affinities of all four compounds for D-Ala-D-Ala (Table 1).

Because the compounds **1a**−**4a** share a common mechanism of inhibition (i.e., blockage caused by substrate binding), differences in the behavior of compounds may be obscured in the transglycosylase assays. To determine whether any of compounds **1**−**4** retain inhibitory activity when D-Ala-D-Ala binding is abolished, we prepared and tested compounds **1b**−**4b** in which the peptide

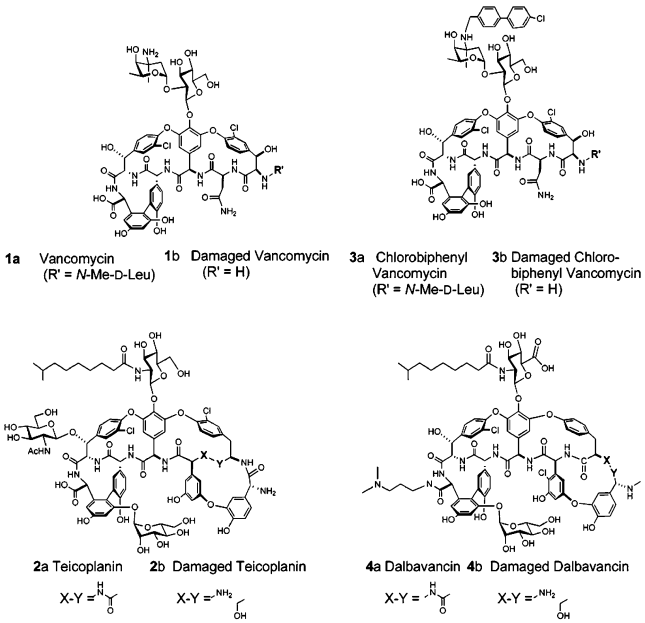

Figure 1. Glycopeptide antibiotics.

Table 1. Biological Activity and Transglycosylase Inhibition for Glycopeptides

glycopeptide	MIC[a] *S. aureus*[b]	IC_{50}[c] (μm) *S. aureus*
1a	3.2	1.7
2a	3.2	1.2
3a	0.1	2.7
4a	0.1	1.1
1b	>264	>500
2b	>100	>500
3b	4.8	3.5
4b	50	70

[a] MIC values (μg/mL) were obtained using a standard microdilution assay. The MIC is defined as the lowest antibiotic concentration that resulted in no visible growth after incubation at 35 °C for 22 h. [b] Bacterial strain 29213. [c] IC_{50} values were obtained against *S. aureus* PBP2.[22]

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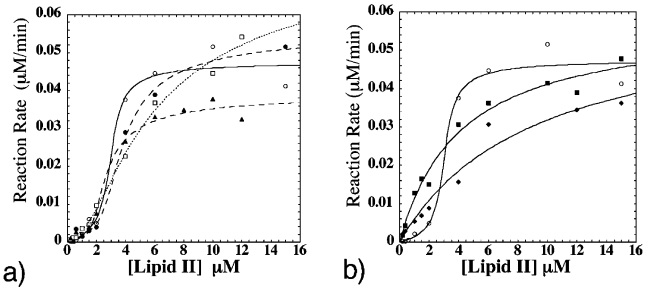

Figure 2. Inhibition curves for glycopeptides. (a) Vancomycin [3 μM (●)], CBPV [3 μM (○)], teicoplanin [2 μM (□)], and dalbavancin [2 μM (▲)] have curves characteristic of substrate binding. (b) Damaged CBPV [5 μM (◆)], unlike CBPV (○), does not exhibit substrate binding, and the control [0 μM (■)] shows no inhibition.

binding pockets are damaged.[16,17] Both the CBPV derivative (**3b**) and the dalbavancin derivative (**4b**) have measurable IC_{50}'s. The IC_{50} of **3b** is low enough that we were able to evaluate the mode of inhibition. The inhibition curve is not sigmoidal like that of the parent compound, consistent with our presumption that these damaged compounds do not bind the lipid II substrate (Figure 2b).[18] Because neither teicoplanin nor vancomycin inhibits PBP2 when their substrate binding pockets are damaged, we have concluded that compounds **3b** and **4b** contain structural elements that enable them to interact with the enzyme itself. Unlike **1b** and **2b**, compounds **3b** and **4b** also retain some biological activity against *S. aureus* (29213) (Table 1). In fact, the biological activity of the damaged compounds correlates with their ability to inhibit *S. aureus* PBP2, the major PBP in this organism and essential enzyme for the expression of vancomycin resistance in VRSA.[19−21]

The role of lipid substituents in the activity of various lipidated glycopeptides has been debated for many years. It has been suggested that secondary interactions between lipid substituents and bacterial membranes target glycopeptides to bacterial cell surfaces, which leads to enhanced D-Ala-D-Ala binding.[23] However, the assay that was used to monitor the glycosyltransferase activity of *S. aureus* PBP2 does not include membranes or detergents, which enable us to separate membrane anchoring from other effects. We have shown that there are significant biological activity differences between dalbavancin and teicoplanin, which have similar lipid chains. Moreover, damaged dalbavancin (**4b**) retains some activity and the ability to inhibit PBP2 in the absence of peptide binding, whereas damaged teicoplanin (**2b**), which contains an identical lipid chain, does not. Therefore, the activity of **4b** cannot be explained simply by nonspecific hydrophobic interactions. It has also been proposed that some lipidated glycopeptides dimerize in a manner that enhances substrate binding.[23,24] Neither dalbavancin nor damaged dalbavancin show evidence of dimerization up to concentrations of 100 μM.[25] Although CBPV and damaged CBPV have been shown to dimerize, enzyme inhibition occurs at concentrations well below the estimated K_{dim} for dimerization.[26] In addition, a covalently linked dimer of damaged CBPV has been shown not to bind peptidoglycan precursors.[27] The activity of damaged glycopeptides **3b** and **4b** is better explained by secondary interactions with *S. aureus* PBP2 itself.

This work represents the first time the mechanism of action of vancomycin has been tested kinetically using a clinically relevant transglycosylase. Using *S. aureus* PBP2, we have shown that vancomycin and other lipoglycopeptide derivatives, both natural and unnatural, inhibit the enzyme by binding its substrate. By damaging the substrate binding pocket, we revealed differences in the mechanism of action of various glycopeptides. Some of these compounds are able to inhibit the transglycosylases by a mechanism independent of peptide binding. The correlation between enzyme inhibition and biological activity for the damaged compounds suggests that activity differences between glycopeptide antibiotics reflect a combination of activity derived from peptide binding as well as secondary interactions with other targets such as the transglycosylases.

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Supporting Information Available: Structure and ^1H and ^{13}C NMR assignments of damaged dalbavancin. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lowy, F. D. *J. Clin. Invest.* **2003**, *111*, 1265−1273.
(2) Nagarajan, R.; Schabel, A. A.; Occolowitz, J. L.; Counter, F. T.; Ott, J. L.; Felty-Duckworth, A. M. *J. Antibiot.* **1989**, *42*, 63−72.
(3) Streit, J. M.; Fritsche, T. R.; Sader, H. S.; Jones, R. N. *Diagn. Microbiol. Infect. Dis.* **2004**, *48*, 137−143.
(4) Bush, K.; Macielag, M.; Weidner-Wells, M. *Curr. Opin. Microbiol.* **2004**, *7*, 466−476.
(5) Anderson, J. S.; Matsuhashi, M.; Haskin, M. A.; Strominger, J. L. *Proc. Natl. Acad. Sci. USA* **1965**, *53*, 881−889.
(6) Perkins, H. R. *J. Biochem.* **1969**, *111*, 195−205.
(7) Perkins, H. R.; Nieto, M. *Ann. N.Y. Acad. Sci.* **1974**, 348−363.
(8) Barna, J. C.; Williams, D. H. *Annu. Rev. Microbiol.* **1984**, *38*, 339−357.
(9) Ge, M.; Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. *Science* **1999**, *284*, 507−511.
(10) Chen, L.; Walker, D.; Sun, B.; Hu, Y.; Walker, S.; Kahne, D. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5658−5663.
(11) Kerns, R.; Dong, S. D.; Fukuzawa, S.; Carbeck, J.; Kohler, J.; Silver, L. L.; Kahne, D. *J. Am. Chem. Soc.* **2000**, *122*, 12608−12609.
(12) Roy, R. S.; Yang, P.; Kodali, S.; Xiong, Y.; Kim, R. M.; Griffin, P. R.; Onishi, R.; Kohler, J.; Silver, L. L.; Chapman, K. *Chem. Biol.* **2001**, *8*, 1095−1106.
(13) Barrett, D.; Leimkuhler, C.; Chen, L.; Walker, D.; Kahne, D.; Walker, S. *J. Bacteriol.*, in press.
(14) Reaction conditions used to measure glycopeptide inhibition: ^{14}C-GlcNAC-labeled lipid II analogue (Ye, X. Y.; Lo, M. C.; Brunner, L.; Walker, D.; Kahne, D.; Walker, S. *J. Am. Chem. Soc.* **2001**, *123*, 3155−3156) and glycopeptide in DMSO were mixed with 1000 units/mL of penicillin G and buffer (50 mM HEPES, pH 7.5, 10 mM $CaCl_2$) to a final volume of 10 μL. The reactions were initiated by adding enzyme (90 nM) and were quenched with 10 μL of ice-cold 10 mM Tris (pH 8.0) containing 0.2% Trition X-100. The reaction mixture was immediately spotted on cellulose chromatography paper (3MM Whatmann). Products and starting material were separated using chromatography (isobutyric acid/1 N NH_4OH = 5:3) and quantitated by scintillation counting.
(15) Segel, I. H. *Enzyme Kinetics*; Wiley & Sons: New York, 1975.
(16) Booth, P. M.; Williams, D. H. *J. Chem. Soc., Perkin Trans. 1* **1989**, 2335−2339.
(17) Malabarba, A.; Ciabatti, R.; Kettenring, J.; Ferrari, P.; Vekey, K.; Bellasio, E.; Denaro, M. *J. Org. Chem.* **1996**, *61*, 2137−2150.
(18) Compound **4b** was not subjected to kinetic characterization, as it would be impossible to achieve concentrations of lipid II above the IC_{50}.
(19) Pucci, M. J.; Dougherty, T. J. *J. Bacteriol.* **2002**, *184*, 588−591.
(20) Pinho, M. G.; de Lencastre, H.; Tomasz, A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10886−10891.
(21) Severin, A.; Wu, W. S.; Keiko, T.; Tomasz, A. *Antimicrob. Agents Chemother.* **2004**, *48*, 4566−4573.
(22) Reaction conditions: 4 μM ^{14}C-GlcNAC-labeled lipid II analogue and various amounts of glycopeptide in DMSO were mixed with 1000 units/mL of penicillin G and buffer (50 mM HEPES, pH 7.5, 10 mM $CaCl_2$) to a final volume of 10 μL. The reactions were initiated and quenched as described in ref 14. The data were fit using Prism, as described in ref 10.
(23) Williams, D. H.; Bardsley, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1173−1193.
(24) Allen, N. E.; Nicas, T. I. *FEMS Microbiol. Rev.* **2003**, *26*, 511−532.
(25) For a description of conditions used in the dimerization studies, see ref 11. For details using ACE to measure dimerization of glycopeptides, see: LeTourneau, D. L.; Allen, N. E. *Anal. Biochem.* **1997**, *246*, 62−66.
(26) Allen, N. E.; LeTourneau, D. L.; Hobbs, J. N.; Thompson, R. C. *Antimicrob. Agents Chemother.* **2002**, *46*, 2344−2348.
(27) Jain, R. K.; Trias, J.; Ellman, J. A. *J. Am. Chem. Soc.* **2003**, *125*, 8740−8741.

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