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INVESTIGATIONS INTO SELF-ASSOCIATION OF VANCOMYCIN COVALENT DIMERS USING SURFACE PLASMON RESONANCE TECHNOLOGY

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Abstract: Covalent dimers of vancomycin linked through the vancosamine sugar moieties of the glycopeptide antibiotic have been synthesized in one step in 67–69% yield. The propensity for self-association of these and related vancomycin covalent dimers is evaluated using surface plasmon resonance technology.

The glycopeptide family of antibiotics exemplified by vancomycin (**1**), eremomycin, orienticin, teicoplanin, and ristocetin is an important class of naturally occurring antibacterial agents. Specifically, vancomycin has been widely used for the treatment of Gram-positive infections caused by methicillin resistant *Staphylococcus aureus* and for the treatment of bacterial infections in patients allergic to β-lactam antibiotics.[1,2] Glycopeptide antibiotics manifest their activity by binding carboxy terminal Lys-D-Ala-D-Ala peptide intermediates involved in the biosynthesis of the bacterial cell wall. Binding of the antibiotic inhibits crosslinking of the growing peptidoglycan making the cell susceptible to lysis through osmotic shock.[3,4]

With the exception of teicoplanin, a recognition factor recently identified as critical for enhancing glycopeptide binding to bacterial cell walls is the self-association of two antibiotic molecules into homodimers.[5,6] Self-association of glycopeptide antibiotics has been shown to be cooperative with the binding of peptide ligands and highly favorable based on entropic considerations.[7–9] Such observations indicate that self-association may play an important role in the mode of action of these antibiotics.

In efforts to further exploit the self-association phenomenon and potentially enhance antibiotic activity several groups have prepared covalent dimers of vancomycin.[10–12] Dimers linked head-to-head through the vancomycin carboxy termini (HH-cov-dimers) have been prepared by Sundram et al. and by Rao and Whitesides.[10,11] Staroske and Williams have prepared dimers linked from the N-terminus to the C-terminus of vancomycin (HT-cov-dimers).[12] Interestingly, the HH-cov-dimers retained comparable activity against susceptible Gram-positive organisms relative to vancomycin while exhibiting up to a 60-fold increase in antibiotic activity against vancomycin-resistant enterococci. Biological activity data for the HT-cov-dimers has not been reported.

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As previously noted by Staroske and Williams, depending on the linker arm utilized and the orientation of the individual glycopeptide antibiotic monomers in the covalent dimer species, covalent dimers may form dimer-dimer complexes, dimer complexes where only two of four glycopeptide moieties interact, polymeric species, or no complexes at all (Figure 1).[12] In fact, studies to evaluate such dimer-dimer interactions have not been reported, and which complex is formed may play an important role in antibiotic potency. We describe here the synthesis of alternative vancomycin dimers covalently linked through the vancosamine sugar moieties (VV-cov-dimers, **2** and **3**) and an evaluation of potential vancomycin covalent dimer-dimer binding interactions using surface plasmon resonance (SPR) technology.

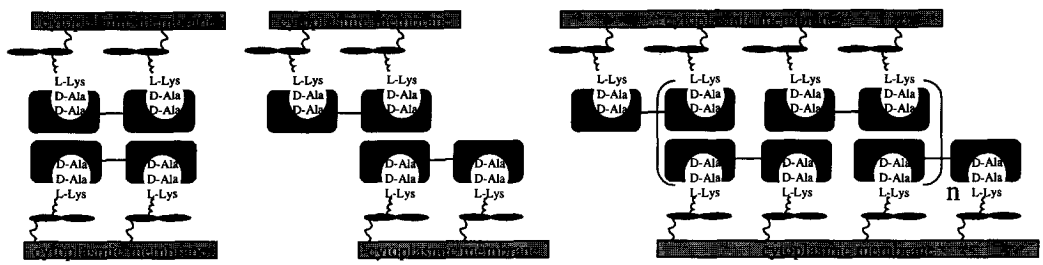
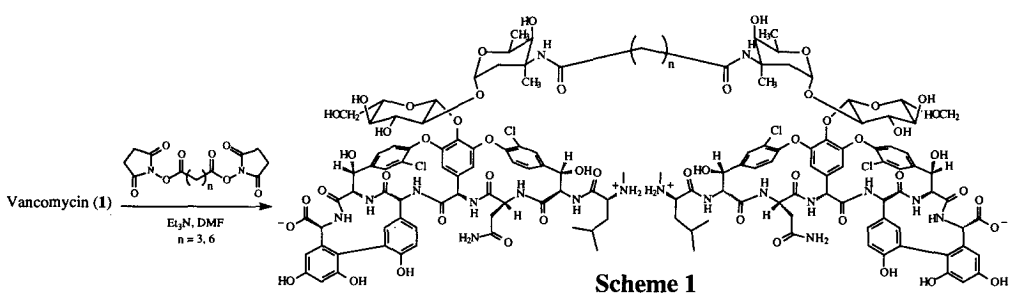


Figure 1. Schematic representation of potential covalent glycopeptide dimer-dimer binding interactions in a bacterial cell wall

VV-cov-dimers **2** and **3** were synthesized by reacting 2.5 equivalents of vancomycin (**1**) in DMF with the bis-*N*-hydroxysuccinimidyl active esters of glutaric or suberic acid in the presence of excess triethylamine (25 equiv.) (Scheme 1).[13] Purification of the reaction mixtures by reversed-phase HPLC provided **2** and **3** cleanly in 69% and 67% yield, respectively, with less than 2% of coupling products originating from the *N*-methylleucyl residue of vancomycin being observed. Structures of all covalent dimers isolated were assigned by ESI mass spectral fragmentation patterns and selected ^{1}H NMR chemical shifts.[12,13] Derivatives of vancomycin acylated at the vancosaminyl amine with β-alanine or 6-aminohexanoic acid linkers have been previously described.[12] However, the coupling conditions utilized (PyBOP/DMF or DMSO) provided mixtures of all possible acylated vancomycin derivatives resulting in significantly lowered yields. Furthermore, coupling of a second vancomycin moiety to generate VV-cov-dimers as presented here was not described.



Scheme 1

Covalent dimer-dimer interactions were evaluated by the equilibrium SPR response (R_{eq}) obtained upon binding of vancomycin dimers to an (N^{ε}-acetyl)KDADA tripeptide immobilized on the carboxymethyl dextran surface of a CM-5 sensor chip (BIAcore, Inc.) through an aminocaproate linker.[14] The binding of vancomycin to the (N^{ε}-acetyl)KDADA tripeptide surface was also evaluated and served as a reference. Thus, several concentrations of vancomycin or vancomycin analog (0.01–10 μM) were injected over the sensor surface at 10 μL/min for 4 min. The binding response at equilibrium (R_{eq}) for each concentration tested was then determined by averaging the response observed between 210–230 s postinjection (Figure 2a). A plot of R_{eq} versus concentration of vancomycin analog injected provided the curves depicted in Figure 2b.

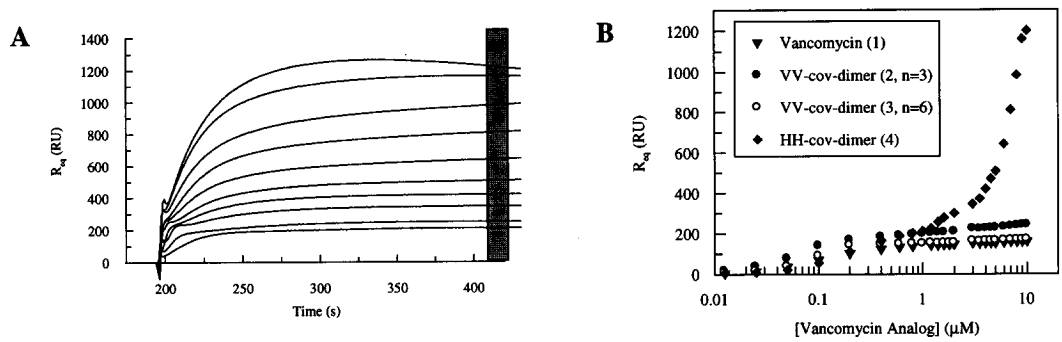


Figure 2. (**A**) Representative overlay plot of sensorgrams generated for covalent vancomycin dimers binding to (N^{ε}-acetyl)KDADA tripeptide surface (data shown is for HH-cov-dimer (**4**)). Response at equilibrium (R_{eq}) was determined from the shaded region of the curves. (**B**) Plots of R_{eq} versus vancomycin analog concentrations.

The (N^{ε}-acetyl)KDADA tripeptide surface had a maximum binding capacity for vancomycin of ~160 RUs for the concentration range examined. At the concentrations examined essentially all vancomycin is bound as the monomer (K_{dim} = 700 M^{-1}).[15] Similarly, binding of covalent dimers **2** and **3** to the peptide surface qualitatively approximate the response obtained for vancomycin. The data obtained indicate that covalent dimers **2** and **3** bind the peptide surface bivalently and that covalent dimers linked through the vancosamine moiety have no propensity for self-association at the concentrations tested. This may reflect a low dimerization constant for **2** and **3** or an inability of the VV-cov-dimers to adopt conformations amenable to self-association. In contrast, an HH-cov-dimer (**4**) containing a 1,6-diaminohexane linker synthesized by the method of Sundram et al.[10] readily assembles into higher order complexes (dimer-dimer and polymeric species) at concentrations > 1 μM still capable of binding to the tripeptide surface. This propensity of the HH-cov-dimer for formation of higer order complexes at significantly lower concentrations than vancomycin itself is consistent with the hypothesis that self-association may be contributing to the enhanced antibacterial potency previously observed for this compound.[10] Thus, preliminary results clearly show surface plasmon resonance technology can be a valuable tool for rapid investigations into the propensity for self-association of vancomycin covalent dimers.[16]

References and Notes

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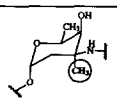
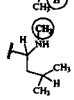
13. Analytical data: Compound **2**: Analytical HPLC (linear gradient of 5 to 50% CH_3CN in 25 mM aqueous ammonium formate over 15 min, 2 mL/min): retention time, 12.8 min. ESI-MS *m/z*: 1497 (MH_2^{2+}), 1690 (vancomycin-linker-vancosamine + H^+), 1305 (MH^+ - vancomycin-linker-vancosamine). Compound **3**: Analytical HPLC (linear gradient of 5 to 50% CH_3CN in 0.1% aqueous TFA over 20 min, 2 mL/min): retention time, 9.0 min. ESI-MS *m/z*: 1519 (MH_2^{2+}), 1731 (vancomycin-linker-vancosamine + H^+), 1305 (MH^+ - vancomycin-linker-vancosamine). Selected ^1H NMR (500 MHz, DMSO-d_6) chemical shifts δ:

	Vancomycin	VV-cov-dimer (**2**)	VV-cov-dimer (**3**)
	1.33 (s, 3H)	1.43 (s, 3H)	1.43 (s, 3H)
	1.68–1.72 (m 1H)	1.67–1.71 (m, 1H)	1.67–1.71 (m, 1H)
	2.39 (s, 3H)	2.39 (s, 3H)	2.38 (s, 3H)
	4.08–4.10 (m, 1H)	4.16–4.18 (m, 1H)	4.16–4.20 (m, 1H)

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