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Affinity of a vancomycin polymer with bacterial surface models

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Abstract—The affinity between a vancomycin polymer (**3**) and cell wall intermediate mimics of vancomycin resistant bacteria (VRE) was determined by use of surface plasmon resonance (SPR). The increased affinity of **3** over monomeric vancomycin derivatives **1** and **2** suggests the importance of tighter binding to VRE surfaces in the enhanced antibacterial activities of **3**.

The evolution of multi-resistant bacteria is a serious worldwide problem. The vancomycin-class glycopeptide antibiotics are the last resort against MRSA (methicilin resistant *Staphylococcus aureus*). However, VRE (vancomycin resistant *enterococci*) has emerged, and the possibility of this resistance being transferred to *S. aureus* is a cause of major concern.[1] The binding of vancomycin is believed to be important in interference with bacterial peptideglycan biosynthesis.

In our efforts toward the design of new semisynthetic antibiotics, we recently reported that a vancomycin polymer **3** displayed potency against VRE up to 60 times greater than vancomycin itself (Fig. 1).[2]

The mechanism of this enhancement in antibacterial activity remains elusive, but might derive from tighter binding of the polymer to the resistant bacteria surface.

It has also been demonstrated that the array of sugars on the polymers could enhance its binding to the receptors due to the 'multi-valent or cluster effect',[3] which suggests that a vancomycin polymer could have similar types of interactions (Fig. 2).

To probe the basis for the enhanced activity of vancomycin polymer **3**, we examined the binding of **3** with bacterial cell wall model peptides by surface plasmon resonance (SPR).

SPR is a powerful method for examination of the affinity between surface presenting receptor molecules and ligand in solution.[4]

The utility of the SPR methods has been demonstrated in a variety of applications in the past decade, including a recent determination of the binding constant of van-

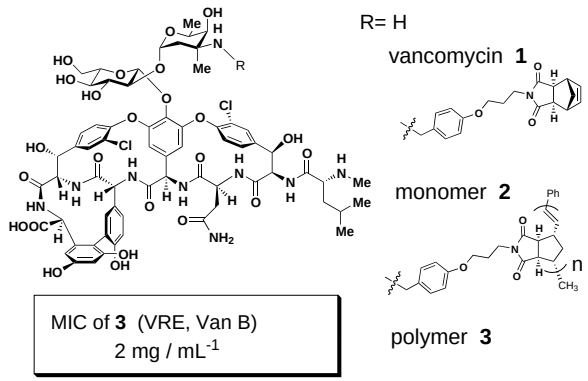

Figure 1. Structure and antibacterial activity of a vancomycin polymer **3**.

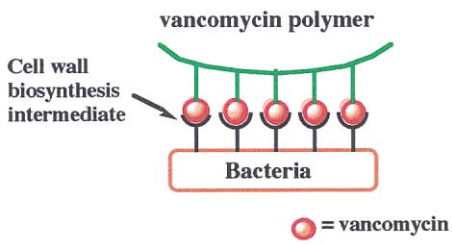

Figure 2. Schematic presentation of the possible interaction of a vancomycin polymer with a bacterial cell wall biosynthesis intermediate.

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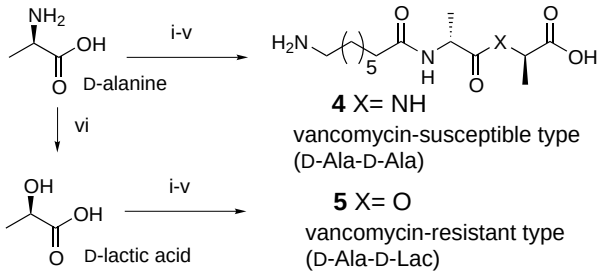

Scheme 1. Synthesis of the cell wall models. *Reagents and conditions*: (i) benzyl alcohol, H^+; (ii) *N*-Boc-alanine, EDCI, HOBt, DMAP, CH_2Cl_2; (iii) TFA, rt; (iv) *N*-Cbz-8-amino-caprylic acid, EDCI, HOBt, CH_2Cl_2; (v) Pd–C, H_2, methanol; (vi) $NaNO_2$, aq. CH_3COOH.

comycin dimer to a bacterial cell surface membrane model.[5]

Our studies employed a commercially available automated SPR system (BIAcore 2000, PE biosystems). The tripeptides **4** and **5** were designed as immobilized ligands on optical biosensors, which mimic the C-terminal segments of bacterial peptideglycan precursors, D-alanine-D-alanine (vancomycin susceptible) and D-alanine-D-lactate (vancomycin resistant), respectively (Scheme 1). The synthesis of **4** and **5** was achieved with traditional techniques, as depicted in Scheme 1.

These peptides were immobilized to an optical biosensor ('sensor chip' CM5) with four independent flow cells, where thin gold surfaces are coated with carboxylated dextran.

First, the carboxylate terminus of carboxylated dextran were activated by a mixture of *N*-ethyl-*N*′-(diethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). Then, ligands **4** and **5** were injected to independent flow cells to form amide linkages with the activated dextran layer. The remaining activated carboxylate groups were capped by injection of ethanolamine. The control flow cell was prepared by immobilizing only ethanolamine without the addition of **4** and **5**.

To the resultant SPR sensors, vancomycin solution (in 10 mM HEPES buffer, containing NaCl 75 mM, surfactant P20 0.5 ppm, 1% DMSO, pH 7.4) at concentrations ranging from 22 to 0.2 μM, were injected. The equilibrium dissociation constants K_d were estimated by an analysis (BIAevaluation 3.0 program) of the sensorgrams (Fig. 3). Under these conditions, nonspecific binding to the control surface was not observed (data are not shown).

The K_d values obtained were 3.25×10^{-5} M for D-Ala-D-Ala and $>1\times10^{-4}$ M (above measurable limit) for D-Ala-D-Lac, respectively. These values are in accordance with the trends observed in previous solution phase studies (K_d of vancomycin with *N*-Ac_2-L-Lys-D-Ala-D-Ala 10^{-6} M and with *N*-Ac_2-L-Lys-D-Ala-D-Lac 10^{-3}–10^{-4} M),[6] which proved the validity of the SPR surface.

The affinities of the monomer **2** and polymer **3** to the optical sensors presenting peptide **4** (vancomycin susceptible type) and **5** (vancomycin resistant type) were determined in a similar fashion (Figs. 4 and 5). Since polymer **3** is a mixture of 2- to ca. 15-mers,[7] rigorous analysis of the data was not trivial. Thus, apparent dissociation constants K_d were estimated on a per residue basis. These results are summarized in Table 1.

The sensorgram clearly indicates an enhanced affinity of polymer **3** for each SPR surface presenting **4** and **5** relative to monomer **2**. Most strikingly, the apparent K_d value of **3** with resistant bacteria mimic **5** was enhanced to the same range ($\sim10^{-5}$ M) as that of vancomycin **1**

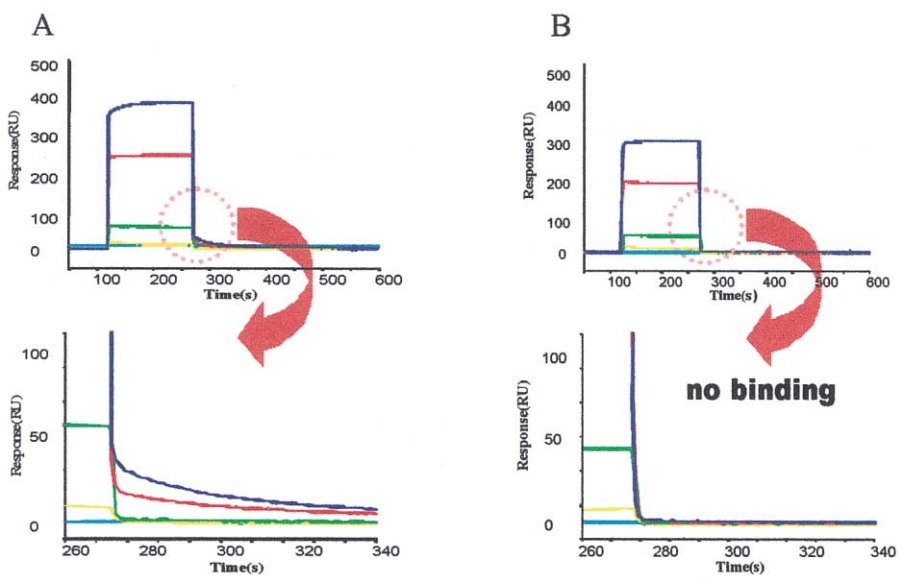

Figure 3. The binding of vancomycin to a SPR surface presenting **4** (A) and **5** (B): concentration of vancomycin. Blue 22.9 μM, red 11.5 μM, green 2.3 μM, yellow 0.23 μM, light blue 0 μM.

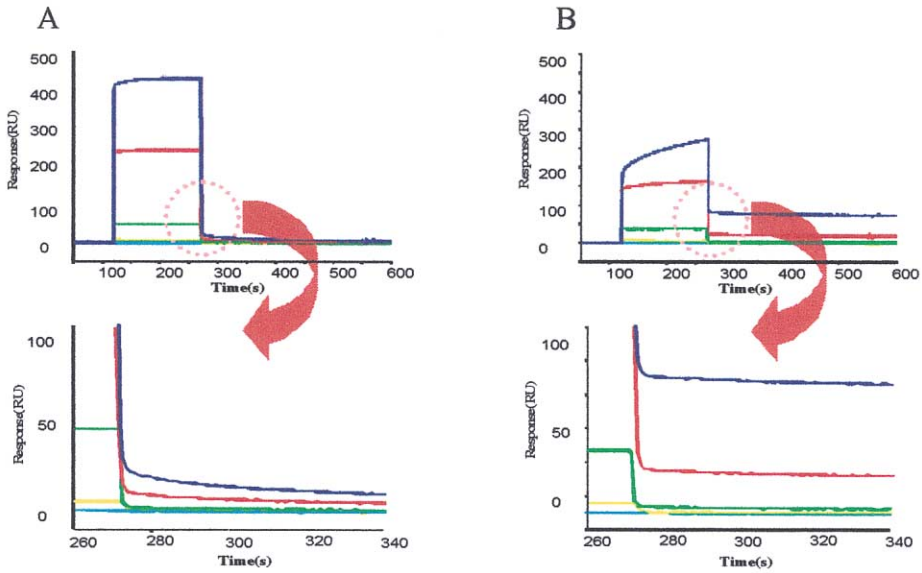

Figure 4. The binding of vancomycin monomer **2** (A) and polymer **3** (B) to a SPR surface presenting vancomycin susceptible Gram positive bacteria model **4**: concentration of vancomycin, blue 22.9 μM, red 11.5 μM, green 2.3 μM, yellow 0.23 μM, light blue 0 μM.

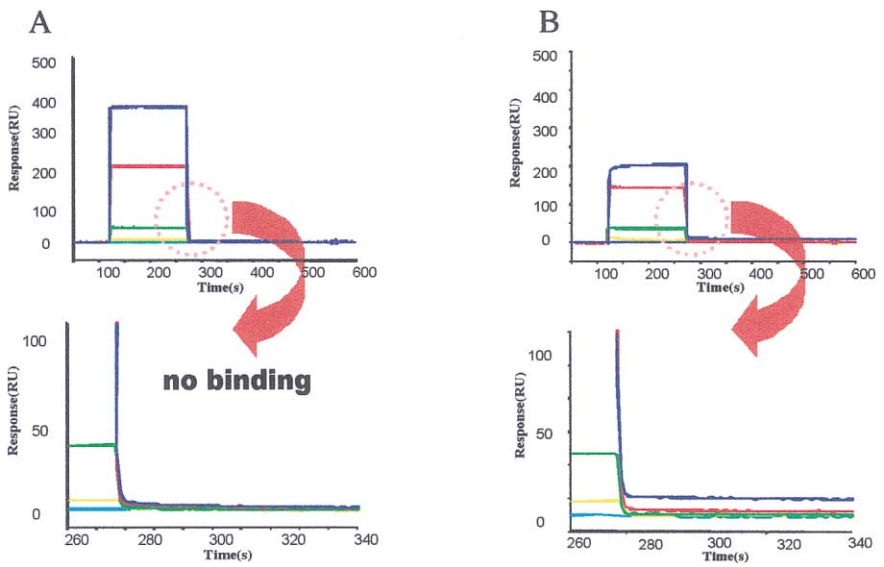

Figure 5. The binding of vancomycin monomer **2** (A) and polymer **3** (B) to a SPR surface presenting VRE model peptide **5**: concentration of vancomycin, blue 22.9 μM, red 11.5 μM, green 2.3 μM, yellow 0.23 μM, light blue 0 μM.

to sensitive bacteria mimic **4**. This might be important to an increase of the antibacterial activity of polymeric vancomycin **3** against VRE (Table 2).[2]

The results also suggest that the observed increases in affinity for **4** and **5** do not quantitatively account for the biological activities. For example, polymer **3** also binds with increased affinity to peptide **4**, but it was not reflected in the potency against vancomycin *susceptible* bacteria. Other additional factors could also contribute to the antibacterial nature of these compounds.

We have described in this letter, the enhanced affinities of the polymer **3** for resistant bacterial cell wall models, which was measured by surface plasmon resonance (SPR). The SPR analyses by use of commercially available carboxylated dextran optical sensors would be a convenient and powerful tool in the search for more potent vancomycin polymers. A screening of polymeric vancomycin derivatives is currently under way using SPR and will be reported in due course.

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Table 1. Ligand-binding affinities of vancomycin **1**, monomer **2**, and polymer **3**

	K_d(M)		
	vancomycin **1**	monomer **2**	polymer **3**
Vancomycin susceptible D-Ala-D-Ala model	3.25×10^{-5}	4.65×10^{-5}	2.83×10^{-6}
Vancomycin resistant D-Ala-D-Lac model	no binding	no binding	2.00×10^{-5}

Table 2. In vitro antibacterial activities of vancomycin **1**, monomer **2**, and polymer **3**[2]

Antibacterial Activity	MIC(μg/mL)		
	vancomycin **1**	monomer **2**	polymer **3**
S. aureus [a]	0.2	0.2	2
VRE (Van B) [b]	125	125	2

a) means of 6 strains of *S. aureus*, which include clinicaly isolated MRSA.

b) RV1

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References

1. For recent reviews, see: Niccolai, D.; Tarsi, L.; Thomas, R. J. *Chem. Commun.* **1997**, 2333. See also: Williams, D. H.; Bardsley, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1173.
2. Arimoto, H.; Nishimura, K.; Kinumi, T.; Hayakawa, I.; Uemura, D. *Chem. Commun.* **1999**, 1361.
3. (a) Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71; (b) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754.
4. For a short review of SPR principles and applications, Weimer, T. *Angew. Chem., Int. Ed.* **2000**, *39*, 1219.
5. Rao, J.; Yan, L.; Xu, B.; Whitesides, G. M. *J. Am. Chem. Soc.* **1999**, *121*, 2629.
6. For some recent examples: (a) Xu, R.; Greiveldinger, G.; Marenus, L. E.; Cooper, A.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 4898. (b) Sundram, U. N.; Griffin, J. H.; Nicas, T. I. *J. Am. Chem. Soc.* **1996**, *118*, 13107.
7. Polymer **3** prepared in our preliminary studies[2] was used in this study. The degree of polymerization was judged from SDS-PAGE analysis, see Figure 2 in Ref. 2.