Binding of a dimeric derivative of vancomycin to L-Lys–D-Ala–D-lactate in solution and at a surface

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Background: The emergence of bacteria that are resistant to vancomycin (V), a glycopeptide antibiotic, results from the replacement of the carboxy-terminal D-Ala–D-Ala of bacterial cell wall precursors by D-Ala–D-lactate. Recently, it has been demonstrated that covalent dimeric variants of V are active against vancomycin-resistant enterococci (VRE). To study the contribution of divalency to the activities of these variants, we modeled the interactions of V and a dimeric V with L-Lys–D-Ala–D-lactate, an analog of the cell-wall precursors of the vancomycin-resistant bacteria.

Results: A dimeric derivative of V (V–R_d–V) was found to be much more effective than V in inhibiting the growth of VRE. The interactions of V and V–R_d–V with a monomeric lactate ligand — diacetyl-L-Lys–D-Ala–D-lactate (Ac_2KDADLac) — and a dimeric derivative of L-Lys–D-Ala–D-lactate (Lac–R'_d–Lac) in solution have been examined using isothermal titration calorimetry and UV spectroscopy titrations; the results reveal that V–R_d–V binds Lac–R'_d–Lac approximately 40 times more tightly than V binds Ac_2KDADLac. Binding of V and of V–R_d–V to N^{α}-Ac-L-Lys–D-Ala–D-lactate presented on the surface of mixed self-assembled monolayers (SAMs) of alkanethiolates on gold indicates that the apparent off-rate for dissociation of V–R_d–V from the surface is much slower than that of V from the same surface.

Conclusions: The results are compatible with the hypothesis that divalency is responsible for tight binding, which correlates with small values of minimum inhibitory concentrations of V and V–R_d–V.

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Key words: dimeric vancomycin, divalency, isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), vancomycin-resistant enterococci (VRE)

Received: **14 January 1999**
Revisions requested: **17 February 1999**
Revisions received: **10 March 1999**
Accepted: **11 March 1999**

Published: **18 May 1999**

Chemistry & Biology June 1999, 6:353–359
http://biomednet.com/elecref/1074552100600353

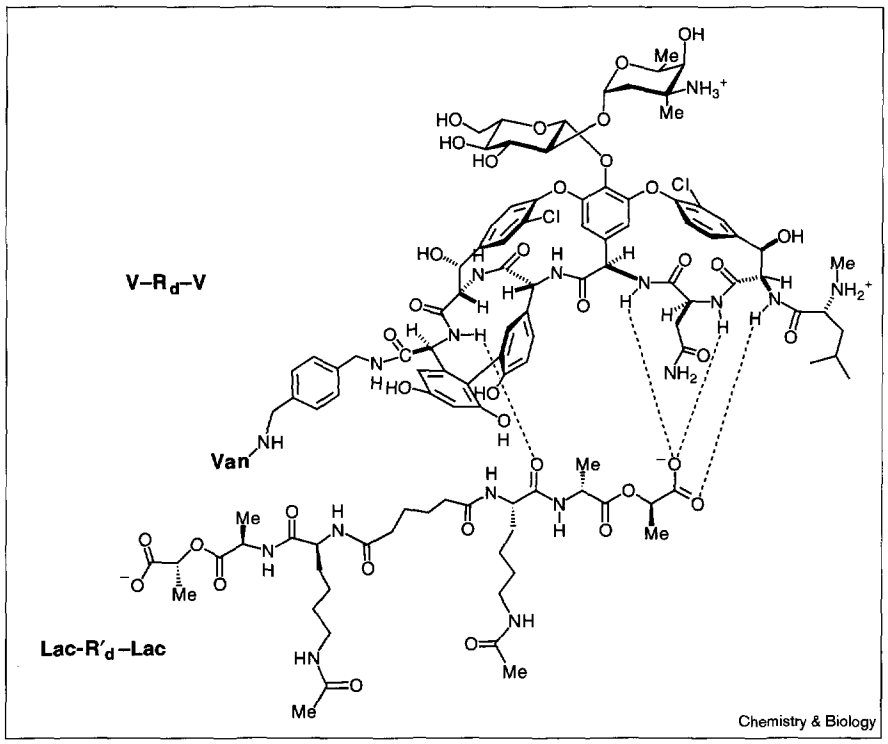
 ISSN 1074-5521

Introduction

The emergence of bacteria that are resistant to vancomycin (V) — an important member of the group of glycopeptide antibiotics that are active against gram-positive bacteria — is a growing problem in clinical practice [1,2]. Vancomycin acts, at least in part, by binding to the carboxy-terminal D-Ala–D-Ala (DADA) of the bacterial cell wall mucopeptide precursors, and creates a weak point in the cell walls; this weak point is believed to render the bacterial cells susceptible to lysis [3–7]. One mechanism for resistance to V is the replacement of the carboxy-terminal DADA by D-Ala–D-lactate (DADLac); the change of the amide NH group to an oxygen atom greatly decreases the affinity of V to DADLac relative to that for DADA [1,2,7,8].

Polyvalency has been demonstrated to increase the strength of binding in weak-binding systems successfully [9,10]. We are developing the system of V and DADA as a model with which to study the physical-organic chemistry of polyvalency [11–13]. Recent work — by Williams and coworkers, with noncovalent dimers of V, and by Griffin and coworkers, with covalent dimers — has suggested that these dimers have different activities than monomeric V. Williams and coworkers [14,15] have proposed that noncovalent dimerization of V and some of its analogs is important for their antibacterial activities, and recent X-ray crystal structures of V support this model [16,17]. Griffin and colleagues [18] also demonstrated that dimers were more active against vancomycin-resistant bacteria by a factor of about 100 than was V itself. One possible explanation for this enhanced activity is divalent binding to DADLac groups in the cell walls. Here we describe a study of the binding of monomeric V and a dimeric analog (V–R_d–V) to the DADLac moiety, in solution (as both monomer and dimer) and at the surface of a self-assembled monolayer (SAM). The results of these studies are compatible with the hypothesis that divalency can contribute to tight binding and demonstrate that tighter binding correlates with better microbiological inhibition (therefore lowering the minimum inhibitory concentrations) by V–R_d–V. Also the results support the hypothesis that divalency contributes to the biological activity of these species.

Figure 1



The structures of the dimeric vancomycin (V–R$_d$–V) and dimeric lactate ligand (Lac–R'$_d$–Lac).

Results and discussion

Synthesis of N^{α}-Ac-L-Lys-D-Ala-D-lactate and dimeric derivative of L-Lys-D-Ala-D-lactate (Lac-R'$_d$-Lac)

We synthesized V–R$_d$–V by covalently linking the carboxyl terminus of V with a rigid *p*-xylylenediamine (Figure 1), as reported previously [12]. A corresponding dimeric derivative of L-Lys–D-Ala–D-Lac (Lac–R'$_d$–Lac) was designed and synthesized by forming the amide links to the two α-amino groups of lysine with adipic acid (Figure 1). Ligand N^{α}–Ac–L-Lys–D-Ala–D-lactate (AcKDADLac) was synthesized for surface plasmon resonance studies of V–R$_d$–V and V at the surface of a SAM.

Microbial assays of activities of V and V-R$_d$-V

We tested the antibacterial activities of the monomeric and dimeric derivatives of V using broth microdilution assays (Table 1). V–R$_d$–V showed activity against vancomycin-susceptible gram-positive bacterial strains similar to that of V, but showed substantially higher activity against vancomycin-resistant enterococci (VRE; Table 1). The minimum inhibitory concentration (MIC) value of V–R$_d$–V against vancomycin-resistant *Enterococcus faecium* (Van B phenotype) is ~5.6 μg/ml (1.8 μM), and ~16.7 μg/ml (5.5 μM) against vancomycin-resistant *E. faecalis* (Van A phenotype). The reported average MIC value

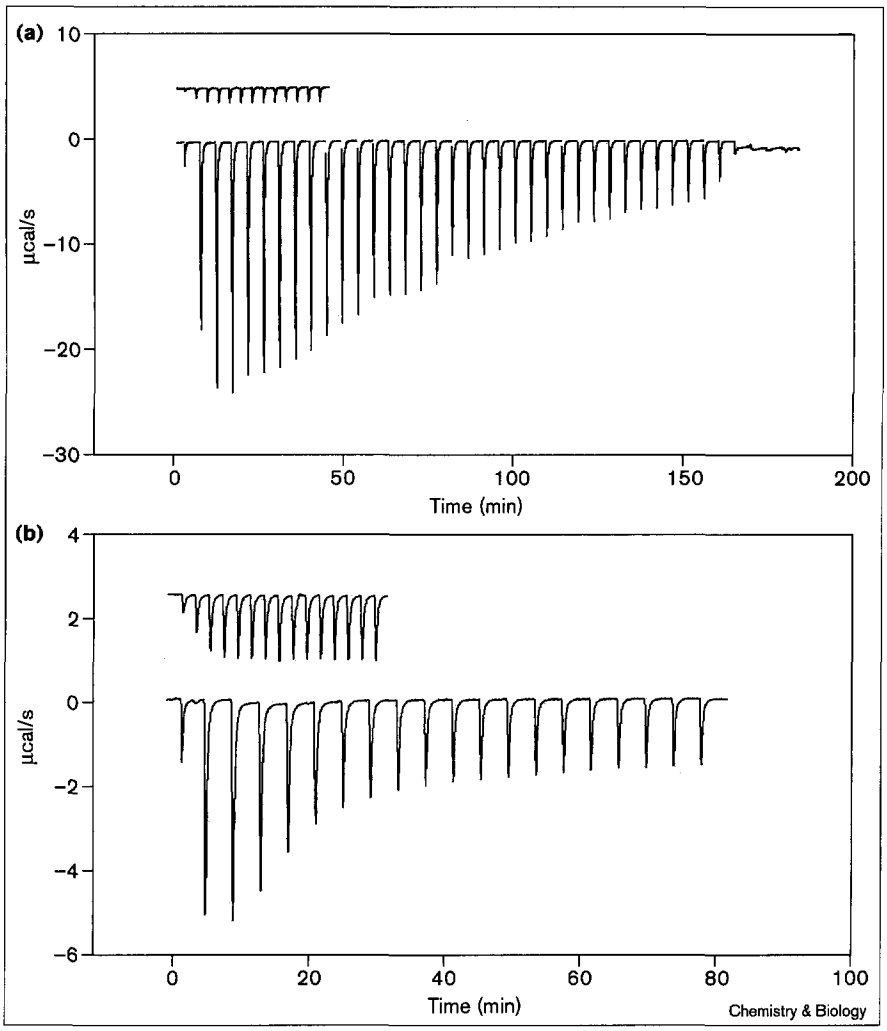
Table 1

Affinity of vancomycin and dimer V-R$_d$-V for lactate ligands in solution and at the surface, and their *in vitro* antibacterial activities.

	K$_d$ in solution (μM)		K$_d$ at surface (μM)*		MIC (μg/ml)	
	Ac_2KDADLac	Lac-R'$_d$–Lac	AcKDADLac	AcKDADA [13]	Sensitive	VRE
V	660†; 1.7 × 10^3‡	1.8 × 10^3‡	330	1.1	0.3§; 0.6#; 1.9¶; 2.1¥	>50.0**
V–R$_d$–V	NB††	17.5†; 42.6‡	<3.3	0.005	0.2§; 2.5#; 1.9¶; 8.3¥	5.6¥; 16.7‡‡

*Determined using SPR spectroscopy over AcKDADLac- or AcKDADA-presenting SAMs; †determined using UV difference spectroscopy titration in 10 mM phosphate buffer at pH 7.2; ‡determined using isothermal titration microcalorimetry in 200 mM phosphate buffer at pH 7.0 (for V) and 50 mM phosphate buffer at pH 7.0 (for V–R$_d$–V); §against *S. pneumoniae*; #against alpha hemolytic *Streptococcus*; ¶against vancomycin-sensitive *E. faecalis*; ¥against Coagulase-negative *Staphylococcus*;**against *E. faecium* (Van B phenotype) and *E. faecalis* (Van A phenotype); literature value is 1024 μg/ml [18]; ††no binding (NB) was observed when titrated V–R$_d$–V (81 μM) with Ac2KDADLac (26.81 mM) in 50 mM phosphate buffer using isothermal titration microcalorimetry; ‡‡against *E. faecium* (Van B phenotype); §§against *E. faecalis* (Van A phenotype).

Figure 2



Isothermal titrations of V and V–R_d–V with lactate ligands at 25°C. **(a)** Titration of V (2.06 mM) with Ac_2KDADLac in 200 mM phosphate buffer at pH 7.0 (lower trace), and background titration of Ac_2KDADLac in the absence of V otherwise under the same condition (upper trace). **(b)** Titration of V–R_d–V (64.5 µM) with the dimeric lactate ligand (Lac–R'_d–Lac, 7.97 mM) in 50 mM phosphate buffer at pH 7.0 (lower trace), and background titration of Lac-R'_d–Lac (upper trace). See the Materials and methods section for experimental details.

of V is ~1024 µg/ml (690 µM) against VRE [18]; V–R_d–V is therefore approximately a hundredfold more effective than V in inhibiting growth of VRE.

Interactions of V–R_d–V and V with Lac–R'_d–Lac and diacetyl-L-Lys-D-Ala-D-lactate in solution

To examine the contribution of divalency to the antibacterial activity of V–R_d–V, we compared the affinity of Lac–R'_d–Lac for V–R_d–V with that of diacetyl-L-Lys-D-Ala–D-lactate (Ac_2KDADLac) for V. Isothermal titration calorimetry (ITC) [19] and UV difference spectrophotometry [6] were used to characterize the binding of Lac–R'_d–Lac to V–R_d–V. Representative data from ITC titrations are shown in Figure 2. Figure 2a shows a titration of 2.06 mM of V with a solution of 53.3 mM of Ac_2KDADLac in 200 mM phosphate buffer (pH 7.0) at 25°C, and Figure 2b shows a titration of 64 µM of V–R_d–V with a solution of 8 mM of Lac–R'_d–Lac in 50 mM phosphate buffer (pH 7.0) at 25°C. The raw titration data were integrated and fitted to the single-set-of-sites model to yield values of $\Delta H°$ and K_d: $\Delta H° \approx -3.7$ kcal/mol and $K_d \approx 1745$ µM for binding of Ac_2KDADLac to V, and $\Delta H° \approx -6.5$ kcal/mol and $K_d \approx 43$ µM for binding of Lac-R'_d–Lac to V–R_d–V. The binding affinity of V–R_d–V to Lac–R'_d–Lac is therefore approximately 40 times tighter than that of V to Ac_2KDADLac. UV difference spectrophotometry titrations have been performed in 20 mM phosphate buffer: K_d of Ac_2KDADLac for V ≈ 660 µM, and K_d of Lac–R'_d–Lac for V–R_d–V ≈ 17.5 µM. It was consistent with ITC results that the binding of V–R_d–V to Lac-R'_d–Lac is also approximately 40 times stronger than that of V to Ac_2KDADLac. The reason why the values of K_d determined using ITC were about threefold larger than those determined using UV titrations was not clear; part of this difference might be due to a difference in the ionic strength of the two buffer solutions.

In comparing the thermodynamic data describing the binding of V to diacetyl-L-Lys–D-Ala–D-Ala with those of

Table 2

Thermodynamic parameters of binding of vancomycin derivitives to depsipeptide/peptide ligands at 298K.

Receptor	Ligand	ΔG° (kcal/mol)	ΔH° (kcal/mol)	−TΔS° (kcal/mol)
V	Ac_2KDADLac	−3.8	−3.7	−0.1
V–R_d–V	Lac–R′$_d$–Lac	−6.0	−6.5	0.5
V*	Ac_2KDADA	−7.9	−12.8	4.9

Values of ΔH° were determined by duplicated ITC titrations at pH 7.0 in phosphate buffer at 298K; values for the free energy ΔG° were calculated from K_d, measured in the same solution; values of −TΔS° were calculated from ΔG° = ΔH°−TΔS°. *Measured in 20 mM phosphate buffer at 298K; the results agree well with the literature values [27].

V to Ac_2KDADLac (Table 2), it is interesting to note that the replacement of the amide in diacetyl-L-Lys-D-Ala-D-Ala by an ester bond in Ac_2KDADLac results in a much smaller binding enthalpy but also a smaller entropic loss. This type of enthalpy/entropy compensation has been discussed in other contexts by Searle and Williams [20]. The loss of one hydrogen bond seems to loosen greatly the binding interface between V and Ac_2KDADLac.

The reported dimerization constant of V is about 700 M^{-1} [21]; at the concentrations of V we used in the experiments, V could partly exist as the noncovalent dimer. The ITC data, however, did not show any of the cooperativity observed by Williams *et al.* [22] in their study of the binding of V with diacetyl-L-Lys-D-Ala-D-Ala. A possible reason could be that the binding of V to DADLac is so weak that the ligand-induced cooperative binding by DADLac is too small to be observed.

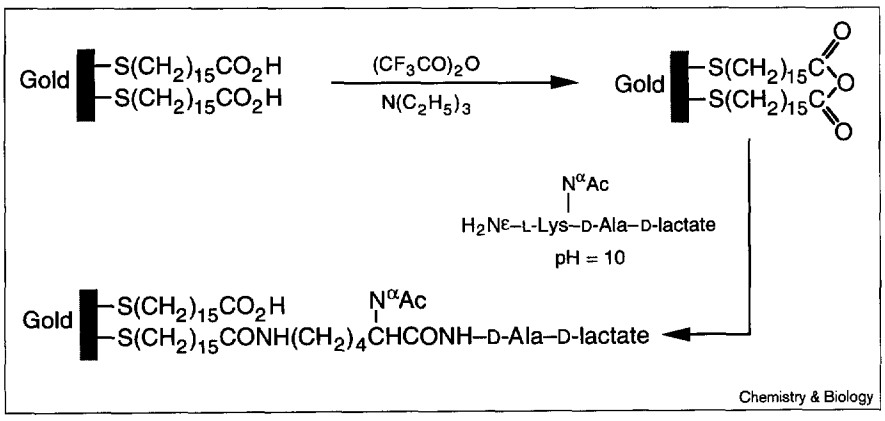
We also examined the interaction between V and Lac-R′$_d$–Lac by ITC. We treated the two sites of Lac–R′$_d$–Lac identically in analyzing the data to obtain affinities; the average dissociation constant ($K_d \approx 1835$ μM; Table 1) was similar to that between V and Ac_2KDADLac. Moreover, under conditions similar to those used in the titration of V–R_d–V (64 μM) with Lac–R′$_d$–Lac, ITC titration showed no detectable binding of V–R_d–V (81 μM) with Ac_2KDADLac. These results established that binding is enhanced only between the dimeric V and the dimeric ligand. Comparison of the thermodynamic parameters for the monovalent binding of V to Ac_2KDADLac (Table 2) with those of the binding of V–R_d–V to Lac–R′$_d$–Lac clarified the origin of the enhanced affinity of the divalent binding — $\Delta\Delta H^\circ \approx -2.8$ kcal/mol, and $T\Delta\Delta S^\circ \approx -0.6$ kcal/mol. The observation that enthalpy increased without a large compensating loss in entropy is compatible with the hypothesis that divalency is the basis for the enhanced affinity of Lac–R′$_d$–Lac for V–R_d–V.

Surface plasmon resonance spectroscopy study of V and V–R_d–V at the surface of a SAM presenting N^α-Ac-L-Lys-D-Ala-D-lactate

The interactions that determine the antibacterial activities of V and its derivatives occur at the surface of the cell membrane of the bacterium (i.e. the interface between the cell membrane and the peptidoglycan layer of the cell wall). To model this interaction, we have examined the interactions of V–R_d–V and V with DADLac at the surface of a SAM. The combination of surface plasmon resonance (SPR) spectroscopy and SAMs of alkanethiolates on gold provides an easily manipulated and highly sensitive analytical system with which to study molecular recognition at surfaces [13,23].

We treated SAMs of 16-mercaptohexadecanoic acid on gold with trifluoroacetic anhydride and triethylamine in anhydrous *N*, *N*-dimethylformamide to generate SAMs of the interchain carboxylic anhydride (Figure 3) [24]. The AcKDADLac was immobilized at the surface of SAMs by subsequent treatment of the anhydride substrate with a 10 mM solution of AcKDADLac (pH ≈ 10). The resulting mixed SAMs presented AcKDADLac and COOH groups at the surface; the mole fraction of AcKDADLac groups in the

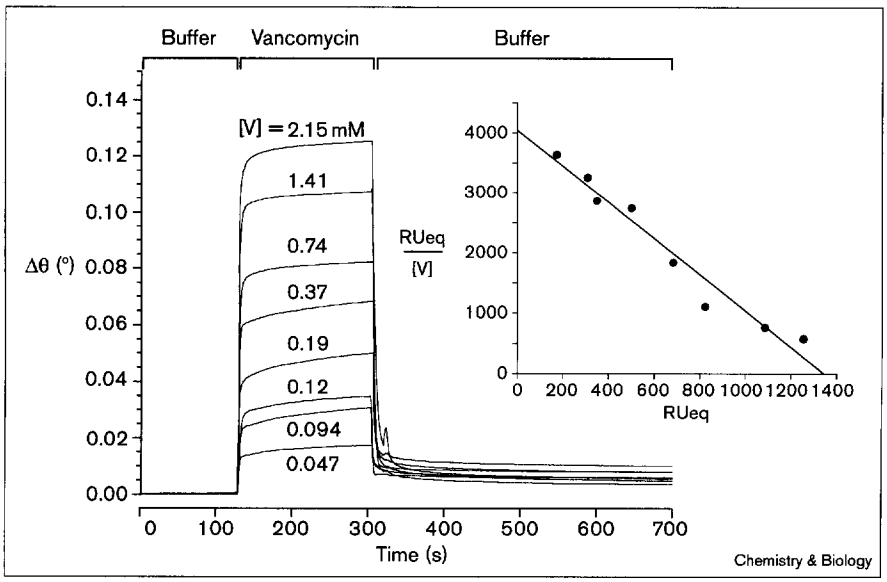
Figure 3



Preparation of mixed SAMs on gold presenting N^α-Ac-L-Lys-D-Ala-D-lactate (AcKDADLac).

Figure 4

The binding of V to mixed SAM presenting AcKDADLac groups. The inset is a Scatchard plot of these data; RUeq is the equilibrium response (1° = 10,000 RU). RU, resonance units.



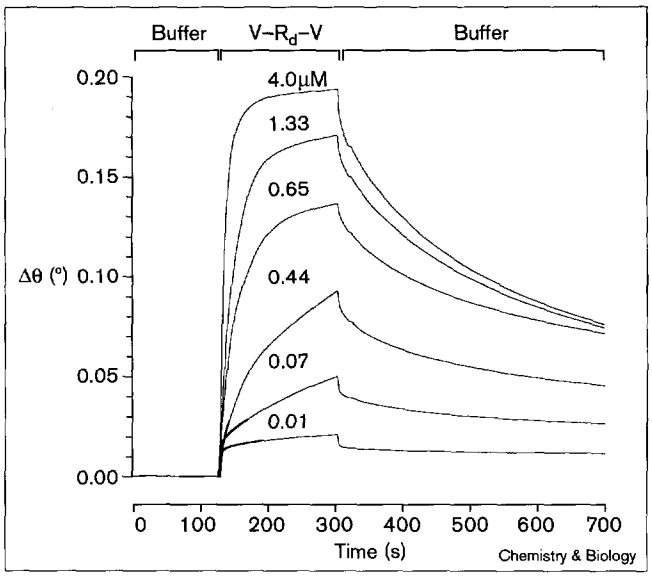
mixed SAMs was $\chi_{(AcKDADLac)}$ ~0.50 and that of COOH was $\chi_{(COOH)}$ ~0.50) [13]. We then examined the interaction of vancomycin (at concentrations ranging from 2.15 mM to 47 μM) with these SAMs. The increase in the amplitude of the SPR signal on adding V suggested that V was binding to AcKDADLac at the SAM; the rate of dissociation of V from the surface (k_{off}) was, however, too fast (> 0.5 s^{-1}) to be estimated accurately (Figure 4). We therefore estimated the affinity of V with AcKDADLac at the SAMs using Scatchard analysis. The values of k_{off} and k_{on} are sufficiently high that V in solution reaches equilibrium with V bound to AcKDADLac at the surface. Scatchard analysis of the equilibrium amount of V bound to AcKDADLac at the surface as a function of the concentration of V in the buffer solutions yielded a value of $K_{d,V}^{Sur}$ ~0.33 mM. Comparison of the binding at the surface with that in solution indicates that the value at the surface is twofold or sixfold smaller than that in solution (depending on the value chosen for comparison; Table 1). The sensorgrams of binding indicate that less than 10% of V remains at the surface after the binding. The nonspecific interaction between V and the surface presenting AcKDADLac is, at least in part, responsible for the tighter binding at the surface measured in our system than that in solution.

The affinity of V for AcKDADLac on SAMs (K_d ~0.33 mM) is 300-fold less than that for N^{α}–Ac–L-Lys–D-Ala–D-Ala on SAMs (K_d ~1.1 μM) [13]. The MIC values of V for VRE are approximately a thousandfold larger than those for vancomycin-susceptible strains. Those observations — a weak binding and fast dissociation of V observed in this model system — suggest that the weak interaction between V and AcKDADLac on SAMs correlates (and perhaps models) the weak antibacterial activity of V against vancomycin-resistant bacteria using DADLac as the cell wall precursors.

We then examined the interaction of V–R_d–V (at concentrations from 4.0 μM to 10 nM) with AcKDADLac on the SAMs under the same experimental conditions (Figure 5). The interaction was substantially different from that between V and AcKDADLac: the dissociation of V–R_d–V was much slower than that of V. Analysis of the dissociation regions gave a value of the apparent off-rate of about 5×10^{-3} s^{-1}; this value was at least 100 times slower than that of V from the same surface (> 0.5 s^{-1}). Due to the complexity introduced into the analysis of the data by mass transport in the binding of V–R_d–V at the surface, we were not able to estimate the on-rate. By assuming that the on-rate of V–R_d–V is similar to that of V, we could estimate the upper limit of the binding affinity of V–R_d–V at the surface as $<K_{d,V}^{Sur}/100$ ~3.3 μM.

The maximum response in the binding of V–R_d–V observed by SPR is higher than that in the binding of V by about 600 resonance units (Figure 5). We suspect that this larger value of resonance units might result from at least one other binding mode — monovalent binding — and nonspecific adsorption of V–R_d–V at high concentrations of V–R_d–V. In principle, V–R_d–V can also bind monovalently with AcKDADLac and therefore give a larger SPR response than bivalent binding. The higher the concentration of V–R_d–V, the more important the monovalent binding mode, and the more serious the nonspecific adsorption of V–R_d–V by the SAM surface.

Figure 5



The binding of V–R_d–V to a mixed SAM presenting AcKDADLac groups.

The SPR studies indicate that V–R_d–V binds the lactate ligand AcKDADLac, when present at a high surface density ($\chi \approx 0.50$), more than 100 times more tightly than does V. This result correlates well with the observation in the microbiological study that the MIC values of V–R_d–V against VRE are approximately 100 times smaller than that of V, and is consistent with the hypothesis that divalency contributes to the antibacterial activity.

Significance

This study proposes a model system with which to examine one possible contribution to the enhanced activity of a dimeric derivative of vancomycin (V) against vancomycin-resistant bacteria. Understanding the origin of this enhanced activity might suggest leads to the design of other antibiotics effective against vancomycin-resistant bacteria.

The data from our study of this model system have demonstrated a correlation between the antibacterial activity of V and a dimeric derivative of V (V–R_d–V) against vancomycin-resistant bacteria and their binding affinities with their lactate ligands in solution and at the surface. V–R_d–V binds more tightly to a dimeric lactate ligand Lac–R'_d–Lac in solution than does V, binds more tightly to a monomeric lactate ligand AcKDADLac at the surface than does V, and is more effective in inhibiting the growth of vancomycin-resistant enterococci than is V. This correlation between divalency, tight binding and antibacterial activity cannot, however, be applied to the D-Ala–D-Ala ligands and vancomycin-susceptible bacteria: although V–R_d–V binds more tightly with a dimeric D-Ala–D-Ala ligand in solution [12] and AcKDADA at the surface than does V [13], it is not more effective than V against vancomycin-susceptible bacteria. The reasons why this correlation exists for vancomycin-resistant bacteria but not for vancomycin-susceptible bacteria are not currently clear to us, and suggest that there be important aspects of the antibiotic activity of vancomycin that are not adequately described by current mechanisms of this activity [3–7].

Materials and methods

Vancomycin hydrochloride was purchased from Sigma and used without further purification. V–R_d–V was available from previous studies [12,13]. Amino acids including Ac_2KDADLac were purchased from Sigma except D-Ala–O–tBu from BACHEM Bioscience, and the peptide-coupling reagent 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Applied Biosystem. Part of AcKDADLac used in our study was synthesized according to reported procedures [25], and part was purchased from BACHEM Bioscience.

Synthesis of Lac–R′$_d$–Lac

To 300 mg (0.576 mmol) of N^{α}–*t*-boc–N^{α}–Ac–L-Lys–D-Ala–D-Lac–O–Benzyl ester (synthesized as literature reported procedures [25]) and 39 mg of adipic acid (0.267 mmol) in 10 ml of acetonitrile solution was added 231 mg of HBTU (0.609 mmol) and 157 mg of diisopropyltheylamine (1.217 mmol). The reaction was stirred at room temperature overnight; a white, cloudy precipitate formed. The precipitate was collected after filtration and washed with saturated NaCl and ethyl acetate. The hexadepsi-peptide ester (230 mg) was obtained as white solid (0.242 mmol, 84%). The crude ester was dissolved in absolute ethanol and hydrogenated over 5% palladium on activated carbon overnight. The reaction mixture was then filtered through a bed of Celite, and the filtrate was evaporated and purified using HPLC to afford the final depsipeptide. ^{1}H-NMR (400 MHz, DMSO-d_6) 8.34 (d, 2H), 7.83 (d, 2H), 7.78 (t, 2H), 4.92 (q, 2H), 4.27 (m, 4H), 2.96 (q, 4H), 2.10 (m, 4H), 1.78 (s, 6H), 1.63–1.53 (m, 2H), 1.52–1.41 (m, 6H), 1.39 (d, 6H), 1.37-1.31 (m, 4H), 1.27 (d, 6H), 1.25-1.15 (m, 4H); FAB-MS, for $C_{32}H_{54}N_8O_{12}Na$ ($M + Na^+$), calc'd m/z 795.3752, found m/z 795.3735.

Isothermal titration calorimetry

Titrations were carried out using a MicroCal MCS titration calorimeter with Observer software for instrument control, data acquisition and analysis. Instrumentation, software and data analysis have been described elsewhere [19]. Titration buffers were 200 or 50 mM phosphate buffer at pH 7.0 as indicated. Titrations typically consisted of 3 μl or 5 μl injections in a schedule of 20–30 injections spaced at intervals of 4–5 min and a syringe speed of 500 rpm. The concentrations of V–R_d–V were determined using UV–Vis absorption spectrometry assuming that the value of ε_{280} of V–R_d–V was double of that of V (for V, $E^{1\%}_{1\,cm}$ at 280 nm = 45 [6]).

Antibiotic broth microdilution assays

MIC values of antibiotics were determined for each organism exactly as described by the National Committee for Clinical Laboratory Standards [26]. The bacteria studied were clinical strains obtained from Massachusetts General Hospital Microbiology Laboratory. Bacteria, adjusted to appropriate bacterial densities in Mueller–Hinton broth supplemented with divalent cations (and in some cases lysed horse blood), were incubated with dilutions of the vancomycin compounds for 16–20 h at 37°C. MIC value was determined as the lowest concentration of antibiotic allowing no visible growth.

Preparation of mixed SAMs

The gold substrates were prepared by electron beam evaporation of 1.5 nm of titanium and 38 nm of gold onto glass cover slips as

previously described [24]. The gold-coated slips were cut into approximately 1 cm × 2 cm pieces and washed with absolute ethanol before adsorption of alkanethiol. SAMs were prepared by overnight exposure of the freshly prepared substrates to a 2 mM ethanolic solution of 16-mercaptohexadecanoic acid at room temperature in a 20 ml scintillation vial. The resulted SAMs were immersed in a 10 ml solution of 0.1 M trifluoroacetic anhydride and 0.2 M triethylamine in anhydrous *N, N*-dimethylformamide for 20 min at room temperature, removed and rinsed thoroughly with dichloromethane, and dried in a stream of nitrogen. The substrates of the interchain anhydride were treated with a 10 mM solution of AcKDAbLac (pH = 10) and afforded mixed SAMs with a ligand density of approximately 0.50.

Surface plasmon resonance measurements

SPR measurements were made with a BIACore instrument (Pharmacia Biosensor), and were conducted with a constant 5 µl/min flow of solution over the surfaces. The binding of vancomycin or the dimeric vancomycin was carried out by sequential injection of 10 µl of PBS solution. The surface was washed with PBS after each injection. The dissociation of vancomycin or the dimeric vancomycin resulted in a shift in the resonance angle that was reported in resonance units (RU; 10,000 RU = 1.0°). The data were analyzed using the program provided by BIACore.

Acknowledgements

This work was funded in part by the Defense Advanced Research Projects Agency (DARPA/SPAWAR; DARPA/ONR), the National Science Foundation (NSF ECS-9729405), the National Institutes of Health (GM 30367, GM 53210 to R.M.W. and R01-AI39617 to H.S.W) and Shriners Burns Institute grant number 8704 (to H.S.W.). We thank Emily Zanzot for carrying out the *in vitro* activity assays. J.R. thanks Eli Lilly (1996-97), Hoffman-La Roche (1997-98) and Glaxo Wellcome (1998-99) for predoctoral fellowships.

References

1. Walsh, C.T. (1993). Vancomycin resistance – decoding the molecular logic. *Science* **261**, 308-309.
2. Walsh, C.T., Fisher, S.L., Park, I.-S., Prahalad, M. & Wu, Z. (1996). Bacterial resistance to vancomycin: five genes and one missing hydrogen bond tell the story. *Chem. Biol.* **3**, 21-28.
3. Barna, J.C.J. & Williams, D.H. (1984). The structure and mode of action of glycopeptide antibiotics of the vancomycin group. *Ann. Rev. Microbiol.* **38**, 339-357.
4. Nagarajan, R. (1991). Antibacterial activities and modes of action of vancomycin and related glycopeptides. *Antimicrob. Agents Chemother.* **35**, 605-609.
5. Reynolds, P.E. (1989). Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.* **8**, 943-950.
6. Nieto, M. & Perkins, H.R. (1971). Physicochemical properties of vancomycin and iodovancomycin and their complexes with diacetyl-L-lysyl-D-alanyl-D-alanine. *Biochem. J.* **123**, 773-787.
7. Wright, G.D. & Walsh, C.T. (1992). D-Alanyl–D-alanine ligases and the molecular mechanism of vancomycin resistance. *Accounts. Chem. Res.* **25**, 468-473.
8. Bugg, T.D., Wright, G.D., Dutka-Malen, S., Arthur, M., Courvalin, P. & Walsh, C.T. (1991). Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. *Biochemistry* **30**, 10408-10415.
9. Sauter, N.K., *et al.*, & Wiley, D.C. (1989). Hemagglutinins from two influenza virus variants bind to sialic acid derivatives with millimolar dissociation constants: a 500 MHz proton nuclear magnetic resonance study. *Biochemistry* **28**, 8388-8396.
10. Sigal, G.B., Mammen, M., Dahmann, G. & Whitesides, G.M. (1996). Polyacrylamides bearing pendant α-sialoside groups strongly inhibit agglutination of erythrocytes by influenza virus: the strong inhibition reflects enhanced binding through cooperative polyvalent interactions. *J. Am. Chem. Soc.* **118**, 3789-3800.
11. Rao, J., Lahiri, J., Isaacs, L., Weis, M.R. & Whitesides, G.M. (1998). A trivalent system from vancomycin•D-Ala–D-Ala with higher affinity than avidin•biotin. *Science* **280**, 708-711.
12. Rao, J. & Whitesides, G.M. (1997). Tight binding of a dimeric derivative of vancomycin with a dimeric L-Lys–D-Ala–D-Ala. *J. Am. Chem. Soc.* **119**, 10286-10290.
13. Rao, J., Yan, L., Xu, B. & Whitesides, G.M. (1999). Using SPR to study the binding of vancomycin and its dimer to self-assembled monolayers presenting D-Ala-D-Ala. *J. Am. Chem. Soc.*, **121**, 2629-2630.
14. Mackay, J.P., Gerhard, U., Beauregard, D.A., Maplestone, R.A. & Williams, D.H. (1994). Dissection of the contributions toward dimerization of glycopeptide antibiotics. *J. Am. Chem. Soc.* **116**, 4573-4580.
15. Mackay, J.P., Gerhard, U., Beauregard, D.A., Westwell, M.S., Searle, M.S. & Williams, D.H. (1994). Glycopeptide antibiotic activity and the possible role of dimerization: a model for biological signaling. *J. Am. Chem. Soc.* **116**, 4581-4590.
16. Loll, P.J., Bevivino, A.E., Korty, B.D. & Axelsen, P.H. (1997). Simultaneous recognition of a carboxylate-containing ligand and an intramolecular surrogate ligand in the crystal structure of an asymmetric vancomycin dimer. *J. Am. Chem. Soc.* **119**, 1516-1522.
17. Schafer, M., Schneider, T.R. & Scheldrick, G.M. (1996). Crystal structure of vancomycin. *Structure* **4**, 1509-1515.
18. Sundram, U.N., Griffin, J.H. & Nicas, T.I. (1996). Novel vancomycin dimers with activity against vancomycin-resistant enterococci. *J. Am. Chem. Soc.* **118**, 13107-13108.
19. Wiseman, T., Williston, S., Brandt, J.F. & Lin, L.-N. (1989). Rapid measurement of binding constants and heats of binding using a new titration calorimetry. *Anal. Biochem.* **179**, 131-137.
20. Searle, M.S. & Williams, D.H. (1992). The cost of conformational order: entropy changes in molecular associations. *J. Am. Chem. Soc.* **114**, 10690-10697.
21. Gerhard, U., Mackay, J.P., Maplestone, R.A. & Williams, D.H. (1993). The role of the sugar and chlorine substitutents in the dimerization of vancomycin antibiotics. *J. Am. Chem. Soc.* **115**, 232-237.
22. Williams, D.H., Maguire, A.J., Tsuzuki, W. & Westwell, M.S. (1998). An analysis of the origins of a cooperative binding energy of dimerization. *Science*, **280**, 711-714.
23. Mrksich, M., Grunwell, J.R. & Whitesides, G.M. (1995). Biospecific adsorption of carbonic anhydrase to self-assembled monolayers of alkanethiolates that present benzenesulfonamide groups on gold. *J. Am. Chem. Soc.* **117**, 12009-12010.
24. Yan, L., Marzolin, C., Terfort, A., & Whitesides, G.M. (1997). Formation and reaction of interchain carboxylic anhydride groups on self-assembled monolayers on gold. *Langmuir* **13**, 6704-6712.
25. Sharman, G.J. et al., & Williams, D.H. (1997) The roles of dimerization and membrane anchoring in activity of glycopeptide antibiotics aganist vancomycin-resistant bacteria. *J. Am. Chem. Soc.* **119**, 12041-12047.
26. National Committee for Clinical Laboratory Standards (1993). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*; Approved Standard, (3rd edn), NCLLS document M7-A3, Villanova, PA.
27. Cooper, A. & McAuley-Hecht, K.E. (1993) Microcalorimetry and the molecular recognition of peptides and proteins. *Phil. Trans. R. Soc. Lond. A* **345**, 23-35.

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