Communication

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Using Surface Plasmon Resonance to Study the Binding of Vancomycin and Its Dimer to Self-Assembled Monolayers Presenting D-Ala-D-Ala

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The binding of vancomycin (Van) to the C-terminal D-Ala-D-Ala (DADA) group of Gram-positive bacterial cell wall precursors inhibits the cross-linking of the cell walls and is responsible for the biological activity of Van.[1,2] Williams et al. demonstrated that Van spontaneously forms a moderately stable, noncovalent dimer ($K_{dim} \approx 700\ M^{-1}$),[3] and proposed that divalency—the simultaneous interaction of two associated Van moieties with two DADA groups—is important in the action of Van.[4−6] Griffin demonstrated that a synthetic divalent variant of Van is more active against Van-resistant organisms than is Van itself.[7] The interaction of Van and DADA has been extensively studied in solution,[1,8−10] and the interaction at cellular and other surfaces is now a subject of research.[11] This paper compares the binding of Van and a synthetic divalent version of Van (Van−R_d−Van, R_d = $NHCH_2$-$C_6H_4CH_2NH$)[12] to N^{α}-Ac-L-Lys-D-Ala-D-Ala (N^{α}-AcKDADA) groups presented on self-assembled monolayers (SAMs). This comparison provides an estimate of the influence of divalency on the binding in this structurally well-defined model system, illustrates the value of surface plasmon resonance (SPR) as an analytical technique[13] in examining oligovalent binding at surfaces, and demonstrates the synergy between SAMs and SPR in studying this type of binding.

We generated SAMs that presented N^{α}-AcKDADA* groups (**L***, the asterisk * indicates an N^{α}-AcKDADA group attached to the surface of the SAM) by reaction of an ϵ-amino group of this tripeptide with a SAM composed of the interchain carboxylic anhydride derived from 16-mercaptohexadecanoic acid.[14] The reaction yielded a mixed SAM presenting roughly equal numbers of **L*** and carboxylic acid groups: that is, $\chi_{L*} \approx 0.50$, where χ_{L*} is the mole fraction of surface groups terminating in **L***.[15] X-ray photoelectron spectroscopy of the resulting substrate showed an N(1s) peak (at 400 eV) and confirmed the coupling of **L** to the SAM.

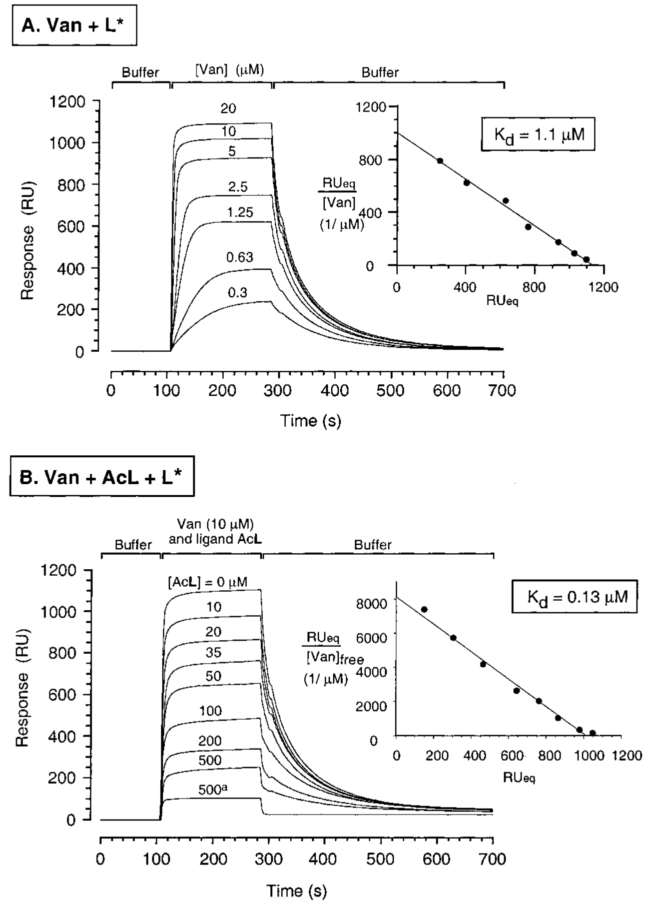

Figure 1. (A) The binding of Van to a SAM presenting L* at $\chi_{L*} \approx 0.50$; the concentration of Van in the buffer is indicated by [Van]. The inset is a Scatchard plot of the data; RUeq is the SPR response (RU = resonance units) when the binding reaches equilibrium on the surface. (B) The binding of Van (10 μM) to a mixed SAM ($\chi_{L*} \approx 0.50$) was inhibited by the addition of the soluble ligand Ac**L** to Van-containing solution; the concentrations of the soluble ligand ([Ac**L**]) are indicated in the plot. [a]There was no Van in this control experiment.

We measured the binding of Van (at concentrations ranging from 20 to 0.3 μM) to this mixed SAM (Figure 1A). Van in solution reaches equilibrium rapidly with Van bound to **L***. Scatchard analysis of the amount of Van bound at the surface as a function of the concentration of Van in the buffer gave a value for the equilibrium dissociation constant of $K_d \approx 1.1\ \mu M$;[16] the corresponding value in solution is $\sim 1\ \mu M$.[1,10,17] The similarity of these values indicates that the binding of Van to **L*** at the surface is thermodynamically comparable to that in solution.

The binding of Van (10 μM) to **L*** was inhibited by the addition of the substrate analogue, $N^{\alpha,\epsilon}$-diacetyl-L-Lys-D-Ala-D-Ala (Ac**L**), to the Van-containing solution (Figure 1B). The observation that the amount of Van bound to the SAM decreased with increasing concentrations of Ac**L** in solution confirms that the interaction between Van and **L*** is biospecific. Since the

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(14) Samples of SAMs of the interchain carboxylic anhydride on gold were prepared as described previously (Yan, L.; Marzolin, C.; Terfort, A.; Whitesides, G. M. *Langmuir* **1997**, *13*, 6704). The tripeptide was introduced by subsequent treatment of the anhydride substrate with a 10 mM solution of N^{α}-AcKDADA (pH = 10).

(15) We did not directly measure the surface density of **L*** group. We assumed that the interchain anhydride at the surface completely reacted with the **L** groups under our experimental conditions.

(16) For all of the Scatchard analyses in this paper, the bulk effects were subtracted from the data used.

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binding constant of Ac**L** to Van in solution is known (~1 μM), it is possible to calculate the concentration of free Van in the mixture. Scatchard analysis of the amount of Van bound at the surface as a function of the concentration of free Van in the solution would, therefore, afford an estimate of the equilibrium dissociation constant of K_d in the presence of the soluble ligand Ac**L**. The inset in Figure 1B indicates a value of K_d ~0.13 μM; this value is, surprisingly, about 9 times smaller than that in the absence of Ac**L**. Why did the presence of a soluble ligand of Van *increase* the binding constant of Van to **L*** at the surface? Williams recently reported that Van complexed with Ac**L** dimerizes more strongly than free Van in solution: $\Delta G°$ is 1.3 kcal/mol more favorable for 2Ac**L**·Van $\rightleftharpoons$ Ac**L**·Van·Van·Ac**L** than for 2Van $\rightleftharpoons$ Van·Van.[18] This ligand-promoted dimerization could affect the binding through at least two possible mechanisms: (i) the reaction of the dimeric species Ac**L**·Van·Van·Ac**L** in solution with **L*** on the surface of the SAM is entropically favorable as a consequence of the release of two molecules of Ac**L** (Ac**L**·Van·Van·Ac**L** + 2**L*** $\rightleftharpoons$ **L***·Van·Van·**L*** + 2Ac**L**), and (ii) Ac**L** could also promote formation of mixed dimeric species at the surface (2Van + Ac**L** + **L*** $\rightleftharpoons$ **L***·Van·Van·Ac**L**); these reactions might be enthalpically favorable.

We then examined the binding of Van−R_d−Van to **L*** (Figure 2A). The apparent rate of dissociation of this surface-associated complex was clearly much slower than that of the monomeric Van at the surface; however, the SPR sensorgrams for both association and dissociation of Van−R_d−Van to **L*** were biphasic, and their kinetic analysis was rendered intractably complicated by mass transport and by the presence of at least two binding modes at the surface: as a monovalent complex and as one or more divalent complexes. We thus set out to estimate the affinity of the binding of Van−R_d−Van to **L*** through an inhibition experiment.[19] We measured the binding of a solution of Van−R_d−Van containing various concentrations of Ac**L** over a SAM with $\chi_{L*} \approx 0.05$ (Figure 2B).[20] The decrease of the amount of Van−R_d−Van bound to the SAM with increasing concentrations of Ac**L** in solution confirms that the interaction between Van−R_d−Van and **L*** is also biospecific. We were not able to inhibit the binding of Van−R_d−Van completely under our experiment conditions, because the binding of Van−R_d−Van to **L*** in these experiments is too tight. The concentrations of free Van−R_d−Van were calculated, assuming that the two sites in Van−R_d−Van are identical and independent. Scatchard analysis of the data yielded $K_d \approx 0.5$ nM; this value is comparable to the dissociation constant of the complex of this Van dimer and a dimeric derivative of N^{α}-AcKDADA in solution ($K_d \approx 1.1$ nM).[12]

This work examines the binding of vancomycin and a divalent derivative of vancomycin with the surface of a SAM presenting N^{α}-AcKDADA groups and provides an estimate of the influence of divalency—noncovalent and covalent—in determining the strength of binding of the divalent derivative of vancomycin to two N^{α}-AcKDADA groups on this surface. The values of binding constants support the hypothesis that divalency contributes to the observed antibacterial activity of a divalent variant of vancomycin against vancomycin-resistant bacteria.[7] It also establishes that the surface of a SAM is capable of organizing ligands for divalent binding in a way that can be analogous to divalent interactions in solution, and it demonstrates the synergy of SPR spectroscopy and SAMs in investigating multivalency interactions at surfaces.

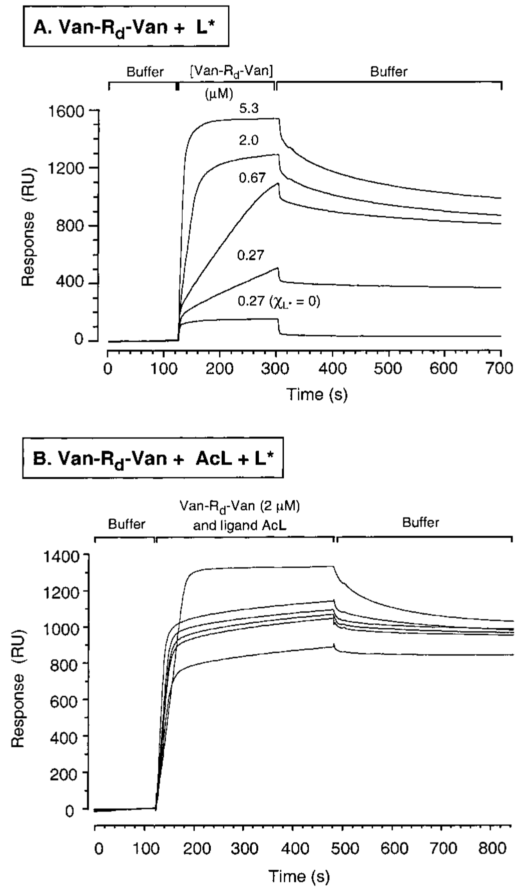

Figure 2. (A) The binding of a Van dimer (Van−R_d−Van) at different concentrations ([Van−R_d−Van]) to a mixed SAM ($\chi_{L*} \approx 0.50$). The sensorgram indicated by $\chi_{L*} = 0$ had only carboxylic acid groups on the surface. (B) The binding of Van-R_d-Van (2 μM) to a mixed SAM ($\chi_{L*} \approx 0.05$) was inhibited by the addition of the soluble ligand Ac**L** to Van-containing solution; the concentrations of Ac**L** were 0, 0.15, 1, 2, 3, 5.8 mM, from the top to bottom, respectively. The bulk effects of Ac**L** in the solution have been examined in separate control runs and subtracted from the sensorgrams.

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(19) The observation of a smaller value of RU and slower dissociation rate with the addition of 150 μM of Ac**L** reflected the blocking of the monovalent binding by the large excess of Ac**L** in the solution.

(20) To generate a surface presenting **L*** group at a density of $\chi_{L*} \approx 0.05$, a different method was used: the gold substrate was soaked for 8−12 h in the ethanol solution of 11-tri(ethylene glycol)-undecyl-1-thiol and 11-[19-carboxymethyl-hexa(ethylene glycol)]-undecyl-1-thiol (the molar ratio was 19:1) and then allowed to react with an aqueous solution of *N*-hydroxysuccinimide (0.10 M) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.4 M) for 30 min and subsequently, N^{α}-AcKDADA (2 mg/mL) in 25 mM phosphate buffer at pH 8.1 for 30 min. Lahiri, J.; Isaacs, L.; Tien, J.; Whitesides, G. M. *Anal. Chem.* **1999**, *71*, 777.