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 (dAlaDAla) 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. Williams et al. demonstrated that Van spontaneously forms a moderately stable, noncovalent dimer (K_{dimer} ≈ 700 M^{-1}), and proposed that divalency—the simultaneous interaction of two associated Van moieties with two dAlaDAla groups—is important in the action of Van. Griffin demonstrated that a synthetic divalent variant of Van is more active against Van-resistant organisms than is Van itself. The interaction of Van and dAlaDAla has been extensively studied in solution, and the interaction at cellular and other surfaces is now a subject of research. This paper compares the binding of Van and a synthetic divalent version of Van (Van-R-dimer-Van, R_d = NHCH2C6H4CH2NH) to N\textsubscript{3}-Ac-t-Lys-d-Ala-d-Ala (N\textsubscript{3}-AcKdAdaD) groups presented on self-assembled monolayers (SAMs). This comparison provides an estimate of the influence of divality on the binding in this structurally well-defined model system, illustrates the value of surface plasmon resonance (SPR) as an analytical technique 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\textsubscript{3}-AcKdAdaD* groups (L*), the asterisk * indicates an N\textsubscript{3}-AcKdAdaD group attached to the surface of the SAM) by reaction of an \epsilon-amino group of this tripeptide with a SAM composed of the interchain carboxylic anhydride derived from 16-mercaptobenzoic acid. The reaction yielded a mixed SAM presenting roughly equal numbers of L* and carboxylic acid groups: that is, \chi_{L*} ≈ 0.50, where \chi_{L*} is the mole fraction of surface groups terminating in L*. 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.

We measured the binding of Van (at concentrations ranging from 20 to 0.3 \mu M) 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} ≈ 1.1 \mu M; 16 the corresponding value in solution is \sim 1 \mu M. 10,13 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 \mu M) to L* was inhibited by the addition of the soluble ligand AcL to Van-containing solution; the concentrations of the solubie ligand ([AcL]) are indicated in the plot. There was no Van in this control experiment.

Figure 1. (A) The binding of Van to a SAM presenting L* at \chi_{L*} ≈ 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 \mu M) to a mixed SAM (\chi_{L*} ≈ 0.50) was inhibited by the addition of the soluble ligand AcL to Van-containing solution; the concentrations of the solubie ligand ([AcL]) are indicated in the plot. 

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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\textsubscript{3}-AcKdAdaD (pH = 10).

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binding constant of AcL 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 AcL. 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 AcL. 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 AcL dimerizes more strongly than free Van in solution: $\Delta G^\circ$ is 1.3 kcal/mol more favorable for 2AcL-Van $\leftrightarrow$ AcL-Van$^*$/Van$^*$/AcL than for 2Van $\leftrightarrow$ Van$^*$/Van.18 This ligand-promoted dimerization could affect the binding through at least two possible mechanisms: (i) the reaction of the dimeric species AcL-Van$^*$/Van$^*$ in solution with L* on the surface of the SAM is entropically favorable as a consequence of the release of two molecules of AcL (AcL-Van$^*$/Van$^*$ + 2L* $\leftrightarrow$ L*$_1$/Van$^*$/Van$^*$ + 2AcL), and (ii) AcL could also promote formation of mixed dimeric species at the surface (2Van + AcL + L* $\leftrightarrow$ L$_1$/Van$^*$/Van$^*$/AcL); these reactions might be enthalpically favorable.

We then examined the binding of 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$^*$ 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$^*$ to L* through an inhibition experiment.19 We measured the binding of a solution of Van$^*$-AcK D A D A in solution ($\chi_L \approx 0.05$) was inhibited by the addition of the soluble ligand AcL to the containing solution; the concentrations of AcL were 0, 0.15, 1, 2, 3, 5.8 mM, from the top to bottom, respectively. The bulk effects of AcL in the solution have been examined in separate control runs and subtracted from the sensorgrams.

(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 sensogram indicated by $\chi_L \approx 0$ 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 AcL to Van-containing solution; the concentrations of AcL were 0, 0.15, 1, 2, 3, 5.8 mM, from the top to bottom, respectively. The bulk effects of AcL in the solution have been examined in separate control runs and subtracted from the sensorgrams.

![Figure 2](image-url)

**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 sensogram indicated by $\chi_L \approx 0$ 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 AcL to Van-containing solution; the concentrations of AcL were 0, 0.15, 1, 2, 3, 5.8 mM, from the top to bottom, respectively. The bulk effects of AcL in the solution have been examined in separate control runs and subtracted from the sensorgrams.

strength of binding of the divalent derivative of vancomycin to two N$_2$-AcK D A D A 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.

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

(20) To generate a surface presenting L*$_1$ group at a density of $\chi_L = 0.05$, 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$_2$-AcK D A D A (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.