

# Surface plasmon resonance analysis of glycopeptide antibiotic activity at a model membrane surface

Matthew A. Cooper, Dudley H. Williams\* and Younghoon R. Cho

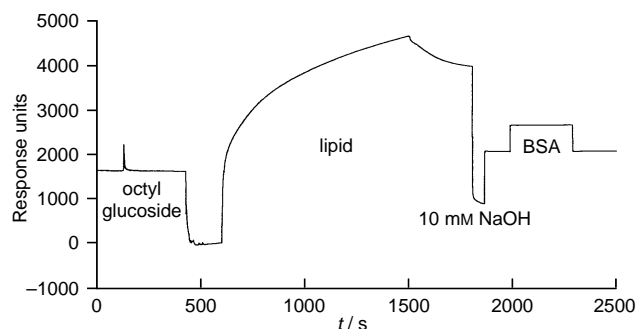
Cambridge Centre for Molecular Recognition, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, UK CB2 1EW

## Affinity constants of glycopeptide antibiotics for peptides anchored in a lipid monolayer measured by surface plasmon resonance show a correlation with *in vitro* antimicrobial activity.

The vancomycin group of glycopeptide antibiotics is active against a wide range of Gram-positive bacteria, particularly many *staphylococci* and *enterococci* responsible for post-surgical infections. Unfortunately resistance to vancomycin is now widespread,<sup>1</sup> and the accompanying increase in the number of deaths from bacterial infections has given new urgency to the search for novel antibiotics. The activity of the glycopeptide antibiotics arises from their ability to bind mucopeptide precursors terminating in the sequence –Lys-D-Ala-D-Ala (–KAA).<sup>2,3</sup> We have shown previously that with the exception of the teicoplanins, glycopeptide antibiotics dimerise in aqueous solution<sup>4</sup> and that dimerisation plays an important role in their biological activity.<sup>5,6</sup> We have employed sodium dodecyl sulfate micelles as model membranes to study the binding interactions between antibiotic and peptide ligands anchored to the lipid surface.<sup>7,8</sup> The small size of the micelles allows NMR analysis of antibiotic–ligand–micelle aggregates, but their high radius of curvature and surfactant properties make them a poor membrane model.

Biacore AB have recently commercialised a hydrophobic association chip for use in their surface plasmon resonance (SPR) instruments, which consists of a self assembled monolayer of alkanethiol on a gold film. Vesicles spontaneously absorb onto the alkane surface to form a supported lipid monolayer<sup>9</sup> which chemically and physically resembles the surface of a cell membrane. The phenomenon of surface plasmon resonance can then be exploited to study interactions with lipophilic ligands inserted into the monolayer. Changes in the measured refractive index at the interface, given in response units, are proportional to the amount of material in the immediate vicinity of the sensor surface.<sup>10</sup> Buffered solutions of an analyte are passed over the surface and the affinity of the binding event can be calculated from analysis of the resultant binding curve.

A phosphate buffered solution of phosphatidylcholine vesicles (0.5 mM) formed by extrusion through a 50 nm polycarbonate filter<sup>11</sup> was loaded on the chip at low flow rate (2  $\mu\text{l min}^{-1}$ ) immediately after an injection of octyl glucoside, a non-ionic detergent used to clean the alkanethiol surface (Fig. 1). Sodium hydroxide (10 mM) was then injected at high flow rate (to remove any multilamellar structures) which resulted in a stable baseline. Complete coverage of the chip surface with lipid was confirmed by the lack of non-specific binding of bovine serum albumin, which binds strongly to the hydrophobic self assembled monolayer alone. *N*- $\alpha$ -Docosanoyl- $\epsilon$ -acetyl-Lys-D-Ala-D-Ala (Doc-KAA) was then inserted into the lipid monolayer by direct injection across the surface as a dilute solution (data not shown). Antibiotics possessing some variation in measured binding affinities to di-*N*-acetyl-Lys-D-Ala-D-Ala (Ac-KAA) in free solution, but varying more in their propensity to dimerise (Table 1), were then exposed to the ligand–lipid monolayer (Fig. 2). Affinity constants were

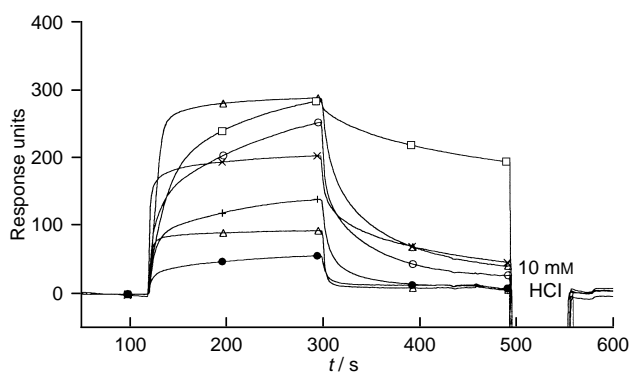


**Fig. 1** Loading of the chip with phosphatidylcholine vesicles formed by extrusion. (Note that dramatic changes in the measured response at the point of injection are due to bulk refractive index shifts between buffer and the injected solution.)

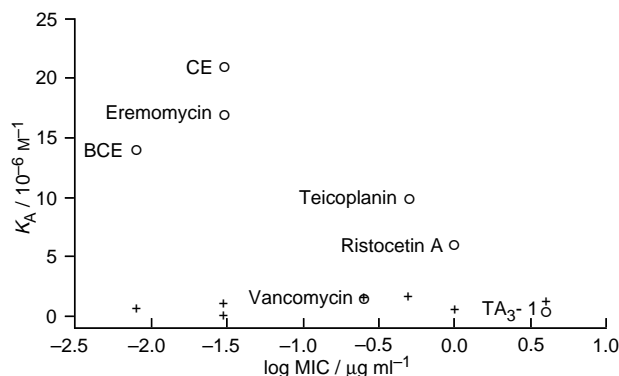
**Table 1** Minimum inhibitory concentrations, affinity constants ( $K_A$ ) for Ac-KAA and Doc-KAA and dimerisation constants ( $K_{dim}$ ) for various antibiotics

Antibiotic	MIC <sup>a</sup> for <i>B. Subtilis</i> / $\mu\text{g ml}^{-1}$	$K_{dim}$ / $\text{M}^{-1b}$	$K_A/\text{M}^{-1c}$	
			Ac-KAA	Doc-KAA
TA <sub>3</sub> -1	4	—	$1.2 \times 10^6$	$4.9 \times 10^5$
Vancomycin	0.25	700	$2.4 \times 10^6$	$1.4 \times 10^6$
Ristocetin A	1	300	$5.9 \times 10^5$	$6.3 \times 10^6$
Eremomycin	0.03	$4.0 \times 10^5$	$3.0 \times 10^4$	$1.7 \times 10^7$
CE	0.03	$1.6 \times 10^4$	$1.0 \times 10^6$	$2.1 \times 10^7$
BCE	0.008	$2.0 \times 10^5$	$5.9 \times 10^5$	$1.4 \times 10^7$
Teicoplanin	0.5	—	$1.6 \times 10^6$	$1.0 \times 10^7$

<sup>a</sup> Minimum inhibitory concentration. <sup>b</sup> Ref. 12. <sup>c</sup> Refs. 4 and 17. <sup>d</sup> Refs. 13–16.



**Fig. 2** Binding of glycopeptide antibiotics at 2  $\mu\text{M}$  to a Doc-KAA–lipid monolayer and removal of bound antibiotic with 10 mM HCl; ( $\Delta$ ) ristocetin A, ( $\square$ ) BCE, ( $\circ$ ) CE, ( $\times$ ) teicoplanin, (+) eremomycin, ( $\Delta$ ) TA<sub>3</sub>-1 and ( $\bullet$ ) vancomycin



**Fig. 3** Correlation between surface affinity constants (○) of glycopeptide antibiotics to mucopeptide analogues and MIC values against *B. Subtilis*. Solution affinity constants (+) do not correlate with MIC values.

determined by Scatchard analysis of the equilibrium response levels at varying concentrations (kinetic analysis will be discussed in a full paper). Data from a flow cell containing the ligand–lipid monolayer were subtracted from data from a control flow cell containing lipid only. This control negates changes in the SPR signal due to unbound antibiotic in solution.

Affinity constants of antibiotics for Doc-KAA at the membrane-like surface determined by SPR showed a much better correlation with antimicrobial activity<sup>12</sup> than those measured to Ac-KAA<sup>13–16</sup> in solution by UV difference spectroscopy (Table 1, Fig. 3). Eremomycin, for example, has a low affinity constant to Ac-KAA in solution, but nevertheless has a low minimum inhibitory concentration (MIC) value (Table 1). This is because eremomycin dimerises strongly and is able to bind cooperatively<sup>7</sup> to a pair of ligands anchored to the surface. The initial binding of ligand to one half of the dimer is intermolecular, whereas binding to the other half of the dimer at the surface is effectively intramolecular and results in an affinity constant over 500-fold greater than that in solution (Table 1). In contrast the antibiotic TA<sub>3</sub>-1, which has a high affinity for peptide in solution but is not known to dimerise,<sup>16</sup> has a relatively high MIC value. In this case there is no cooperative benefit to be gained from templated binding at a surface (Table 1) as the antibiotic can only bind as monomer. In general, strongly dimerising antibiotics<sup>17</sup> such as eremomycin and chloroeremomycin (CE, or LY264826)<sup>18</sup> have enhanced affinities for Doc-KAA at the surface, and weakly dimerising antibiotics such as ristocetin A<sup>4</sup> have correspondingly weaker enhancements. Teicoplanin is exceptional as it does not dimerise, yet has a higher affinity constant at the surface than in free solution. This is because teicoplanin possesses a C<sub>11</sub> acyl chain which functions as a membrane anchor to localise the antibiotic at the surface.<sup>7</sup> Binding to peptide in this case is cooperative as the antibiotic and peptide are attached to the same template and this event is effectively intramolecular as is the case with the dimerising antibiotics. Biphenylchloroeremomycin (BCE, or LY307599),<sup>19</sup> a semisynthetic derivative of CE that dimerises strongly and possesses a biphenyl substituent which can function as a membrane anchor,<sup>8</sup> also

binds tightly to Doc-KAA at the surface. In accord with the hypothesis that dimerisation and membrane anchoring promote antibiotic activity, it has been shown that these features make the antagonism of antibiotic action by externally added Ac-KAA *in vitro* much more difficult.<sup>6</sup>

We have shown that SPR is well suited for the analysis of binding interactions at a model membrane surface and that the calculated binding affinities at the surface are more relevant to *in vitro* activity than corresponding values determined in free solution. We have shown that cooperativity in the binding of glycopeptides to bacterial cell wall analogues is promoted by dimerisation and membrane anchoring of the antibiotic. The joint operation of these two locating devices is crucial for the activity of semisynthetic antibiotics against vancomycin-resistant bacteria.<sup>8,20</sup>

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### Footnote and References

\* E-mail: dhwl@cam.ac.uk

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