BIOCHEMICAL LETTERS

Association constants for the binding of vancomycin and teicoplanin to *N*-acetyl-D-alanyl-D-alanine and *N*-acetyl-D-alanyl-D-serine

Glycopeptides such as vancomycin and teicoplanin form a complex with the C-terminal dipeptide moiety of nascent bacterial peptidoglycan and its precursors [1,2]. This leads to the inhibition of peptidoglycan synthesis, by impeding the action of the transglycosylases and transpeptidases [3]. It was recently shown [4] in *Enterococcus gallinarum* that, in the presence of vancomycin, an additional and unusual peptidoglycan precursor, UDP-MurNAc-pentapeptide (Mur, muramic acid), ending in D-alanyl-D-serine was synthesized, whereas a marked decrease in the pool of the normal UDP-MurNAc-pentapeptide precursor ending in D-alanyl-D-alanine was observed. This was associated with a moderate resistance to vancomycin [minimum inhibitory concentration (MIC) 16 $\mu\text{g/ml}$], but not to teicoplanin (MIC 1 $\mu\text{g/ml}$). We have therefore determined the association constants for the binding between these glycopeptides and two analogues of the C-terminal dipeptide of the UDP-MurNAc-pentapeptide precursors, *N*-acetyl-D-alanyl-D-alanine and *N*-acetyl-D-alanyl-D-serine.

Table 1 Association constants, K_A (M^{-1}), for the combination of vancomycin or teicoplanin with *N*-acetyl-dipeptides

	Ac-D-Ala-D-Ala	Ac-D-Ala-D-Ser
Vancomycin	3.6×10^4	0.52×10^4
Teicoplanin	17×10^4	6×10^4

N-Acetyl-D-alanyl-D-alanine was synthesized by acetylation (acetic anhydride/pyridine) of D-Ala-D-Ala-OBn. $\text{CF}_3\text{CO}_2\text{H}$ [5], followed by hydrogenolysis of the benzyl (Bn) group. *N*-Acetyl-D-alanyl-D-serine was synthesized by coupling Z-D-Ala with D-Ser(Bu')-OBu^t using dicyclohexylcarbodi-imide. The benzyl-oxycarbonyl (Z) group was removed by catalytic hydrogenolysis. After acetylation of the amino function (acetic anhydride/pyridine), the desired product was obtained by acidolysis (trifluoroacetic acid/anisole) of the two t-butyl groups. Both *N*-acetyl-dipeptides were homogeneous by t.l.c. in several solvent systems.

Absorbance measurements of the complexes between vancomycin or teicoplanin and Ac-D-Ala-D-Ala or Ac-D-Ala-D-Ser were carried out in 1 cm-optical-path cells [1,2] with a Cary 118 double-beam spectrophotometer, and the difference between the spectra at 250 and 282 nm was determined. All experiments were carried out at room temperature in 20 mM citrate buffer, pH 5.1. Results of the spectrophotometric titration are shown in Figure 1. Association constants (Table 1) were calculated from these curves by the Wilkinson method [6]. The K_A for vancomycin and Ac-D-Ala-D-Ala was very similar to that found by Nieto and Perkins [5]; however, the minor discrepancy between our K_A value for vancomycin and that given by Nieto and Perkins ($2.0 \times 10^4 \text{ M}^{-1}$) could be due to the fact that the commercial vancomycin we used was not purified as described by those authors. The K_A for vancomycin and Ac-D-Ala-D-Ser was 6-fold lower than that observed with Ac-D-Ala-D-Ala. This could somehow reflect the affinity of vancomycin for the unusual precursor present in *E. gallinarum* and therefore explain the higher MIC compared with that found for the other species of Enterococci which produce the normal precursor [7,8]. It is noteworthy that, after inactivation of the *vanC* gene of *E. gallinarum*, which presumably encodes a ligase catalysing the synthesis of D-Ala-D-Ser and therefore is involved in the synthesis of the D-serine precursor [9], an 8-fold decrease in the vancomycin MIC (16 to 2 $\mu\text{g/ml}$) was observed [10]. This ratio corresponds to that determined for the K_A values for vancomycin and the two *N*-acetyl-dipeptides. In contrast, the K_A for teicoplanin and Ac-D-Ala-D-Ser, which is of the same order of magnitude as that observed for vancomycin and Ac-D-Ala-D-Ala, could account for the lower MIC of teicoplanin for *E. gallinarum*.

Figure 1 Spectrophotometric titration of vancomycin and teicoplanin with *N*-acetyl-dipeptides

Curves were obtained by the Wilkinson [6] method from the differential-absorption data of vancomycin or teicoplanin during addition of *N*-acetyl-dipeptides: (a) vancomycin/Ac-D-Ala-D-Ala; (b) vancomycin/Ac-D-Ala-D-Ser; (c) teicoplanin/Ac-D-Ala-D-Ala; (d) teicoplanin/Ac-D-Ala-D-Ser. Both the reference and sample cells contained 40 μM vancomycin or teicoplanin. The concentration of *N*-acetyl-dipeptide in the sample cell was increased in a stepwise manner by adding, before each measurement, the appropriate amount from a concentrated stock solution. In all cases an equilibrium value was reached in less than 15 min.

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