Binding of a vancomycin group antibiotic to a cell wall analogue from vancomycin-resistant bacteria

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Glycopeptide antibiotics bind to bacterial cell wall peptide analogues terminating in –L-Lys–D-Ala–D-Lac in a similar manner to that of cell-wall analogues terminating in -L-Lys–D-Ala–D-Ala.

In the fight against antibiotic resistant bacteria, the vancomycin group of antibiotics represent a last line of defence against the serious clinical pathogen MRSA (methicillin resistant *Staphylococcus aureus*).[1,2] The vancomycin group antibiotics interfere with cell-wall biosynthesis by binding to the peptide sequence –L-lysyl-D-alanyl-D-alanine (–L-Lys-D-Ala-D-Ala)[3–6] which is present in growing bacterial cell walls, resulting in cell death.[7]

Unfortunately, the increased use of vancomycin in hospitals has lead to the emergence of vancomycin resistance in some bacteria, particularly enterococci.[8–11] This resistance is conferred by a change in the cell wall structure from –L-Lys-D-Ala-D-Ala to –L-lysyl-D-alanyl-D-lactate (–L-Lys-D-Ala-D-Lac). The net effect of this change is to replace an NH, which normally forms a hydrogen bond to a carbonyl group in the antibiotic, with an oxygen, giving rise to a repulsive interaction (Fig. 1).[12,13] This results in a large decrease in affinity for the ligand (approximately 1000-fold),[14] though a precise figure for the binding constant is not known.

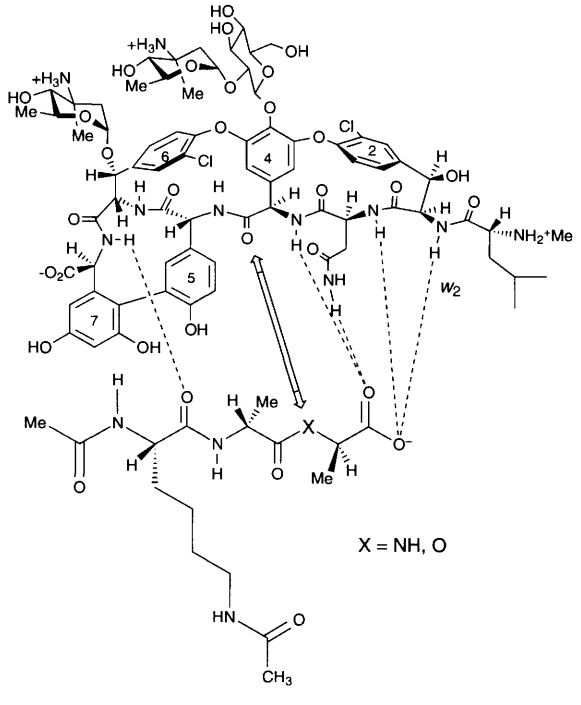

Fig. 1 Exploded view of the complex formed between chloroeremomycin and either di-*N*-acetyl-L-Lys-D-Ala-D-Lac (X = O) or di-*N*-acetyl-L-Lys-D-Ala-D-Ala (X = NH). The double-headed arrow indicates either a hydrogen bond in the case of –D-Ala or a repulsive interaction in the case of –D-Lac.

However, a number of antibiotics belonging to the vancomycin family have been reported which show activity *in vivo* against vancomycin-resistant bacteria.[15,16] This raises the question as to whether or not these new antibiotics have the same mode of action as vancomycin. Here we report evidence showing that chloroeremomycin,† a representative vancomycin group antibiotic, binds to peptides terminating in –D-Lac in a very similar manner to that of peptides terminating in –D-Ala, and also report the first measured binding constant of such an interaction.

UV spectrophotometry was used to determine the binding constant of chloroeremomycin and di-*N*-acetyl-L-Lys-D-Ala-D-Lac (synthesised in our laboratory), which was found to be 245 ± 10 $dm^3\ mol^{-1}$,‡ dramatically lower than that of di-*N*-acetyl-L-Lys-D-Ala-D-Ala, which is 1.3 x $10^6\ dm^3\ mol^{-1}$.[17] The structure of the complex in aqueous solution was studied by NMR spectroscopy. Because of the low binding constant, a high ligand concentration was used to ensure a high population of bound antibiotic. The first indication of ligand binding was provided by the chemical shift of the amide proton of residue 2 (w_2). In complexes with ligands terminating in –D-Ala, w_2 forms a hydrogen bond to the ligand carboxylate, which leads to a large downfield shift for the proton.[18] In this investigation, the ligand terminating in –D-Lac induces a similar downfield shift in w_2, from 8.48 ppm in the free antibiotic to 10.90 ppm in the complex, indicating that the carboxylate is bound in the 'binding pocket'. NOESY experiments§ provided evidence of a number of key close contacts which define the structure further. Some of the key crosspeaks are shown in Fig. 2, and the contacts they indicate are shown diagrammatically in Fig. 3.¶ They locate the methyl group of the –D-Lac residue over the face of ring 4, the methyl group of the –D-Ala residue over ring 7 and adjacent to ring 5, the sidechain of the –L-Lys residue over ring 7, and the methyl group of the *N*-α-acetyl group adjacent to the equatorial methyl group of the residue 6 *epi*-vancosamine (V_{13}). In combination, these demonstrate that the gross conformation

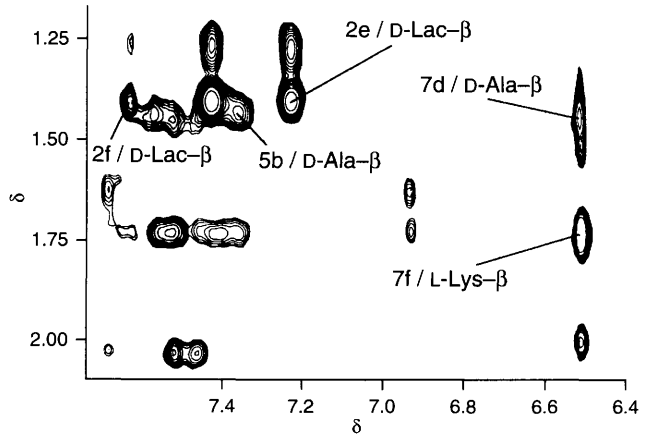

Fig. 2 A portion of the NOESY spectrum of the complex formed between chloroeremomycin and di-*N*-acetyl-L-Lys-D-Ala-D-Lac, indicating some of the key crosspeaks

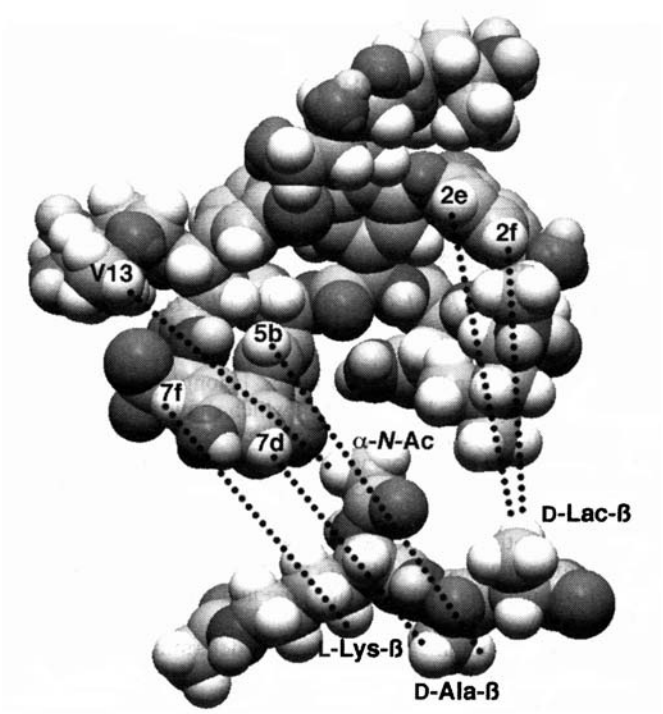

Fig. 3 Exploded diagram illustrating spatial constraints derived from NOESY crosspeaks observed between chloroeremomycin and the depsipeptide ligand

of the ligand–antibiotic complex is essentially identical to that previously reported for complexes of glycopeptide antibiotics with cell-wall analogues terminating in –D-Ala.[19–23]

In conclusion, we have demonstrated that chloroeremomycin binds di-*N*-acetyl-L-Lys-D-Ala-D-Lac in the same fashion as di-*N*-acetyl-L-Lys-D-Ala-D-Ala, albeit with a substantially reduced binding constant. This suggests that vancomycin group antibiotics have essentially the same mode of action against both vancomycin-resistant and vancomycin-susceptible bacteria.

We thank the Wellcome Trust (R. J. D.), Xenova (A. C. T.) and the EPSRC (G. J. S.) for financial support, and Eli Lilly for the gift of chloroeremomycin. We also thank the NIMR, Mill Hill, London for access to NMR spectrometers.

Footnotes

† Our nomenclature; also known as chloroorienticin and LY264826B.

‡ 0.1 mol dm^{-3} phosphate buffer, pH 4.5, 298 K. The method used was the same as previously described.[17]

§ The spectrum was obtained from a sample containing chloroeremomycin (10 mmol dm^{-3}) and di-*N*-acetyl-L-Lys-D-Ala-D-Lac (100 mmol dm^{-3}) in 10% D_2O/H_2O at pH 4.5 and 298 K, conditions which ensure a high population of bound antibiotic, and give sharp amide NH resonances. The NOESY spectrum was recorded on a Bruker AM 500 spectrometer in phase-sensitive mode using time proportional phase incrementation to give quadrature detection in f_1. 2048 complex data points were recorded in f_2 and 512 real points in f_1, using a mixing time of 100 ms. Zero filling was used once on f_2 and twice on f_1 to give a final transformed matrix of 2048 x 2048 real points. All crosspeaks reported here are positive peaks.

¶ It is possible that the apparent contact between D-Lac-β and 2f arises *via* spin diffusion from 2e.

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Received, 1st April 1996; Com. 6/02234K