Common factors in the mode of action of vancomycin group antibiotics active against resistant bacteria

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It is shown that a semisynthetic glycopeptide, with activity against vancomycin resistant bacteria, possesses (as do other glycopeptides active in this way) features which promote its dimerisation and membrane anchoring.

Vancomycin resistance is a growing problem in the treatment of bacterial infection.[1–5] It arises as a result of a modification of the bacterial cell wall in which the C-terminal d-alanine of cell-wall peptides, to which the antibiotic normally binds, is replaced by d-lactate.[6–8] This results in a repulsive interaction between ligand and antibiotic, and consequently a large decrease in affinity for cell-wall peptides. However, a number of semi-synthetic glycopeptides have recently been developed which show remarkable activity against vancomycin-resistant bacteria.[9] In recent work,[10] we have provided evidence that one such antibiotic, biphenylchloroeremomycin (BCE) or LY307599, developed by researchers at Eli Lilly & Co. in Indianapolis, owes its activity against vancomycin-resistant bacteria to its ability to dimerise and anchor to bacterial cell membranes. We have demonstrated that these two locating devices, which we have previously identified as being important in the mode of action of glycopeptides,[11,12] act cooperatively together to greatly increase the low, intrinsic free solution binding of ligands terminating in d-lactate. We now show that a second semisynthetic glycopeptide (MDL 63246) with activity against vancomycin-resistant bacteria, developed independently by researchers at Lepetit, also appears to share the same locating devices.

MDL 63246 (Fig. 1) is prepared semisynthetically from the antibiotic A40926,[13,14] a relative of the clinically important teicoplanin.[15] Teicoplanin (Fig. 1) shows no evidence of

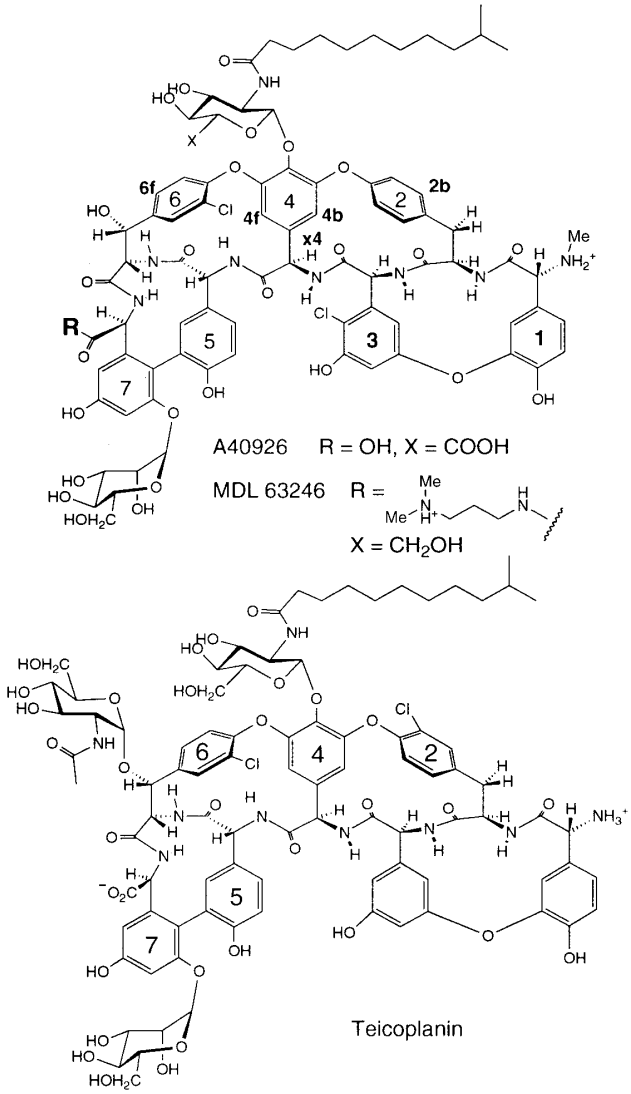

Fig. 1 Structure of the glycopeptide antibiotics teicoplanin, A40926 and MDL 63246

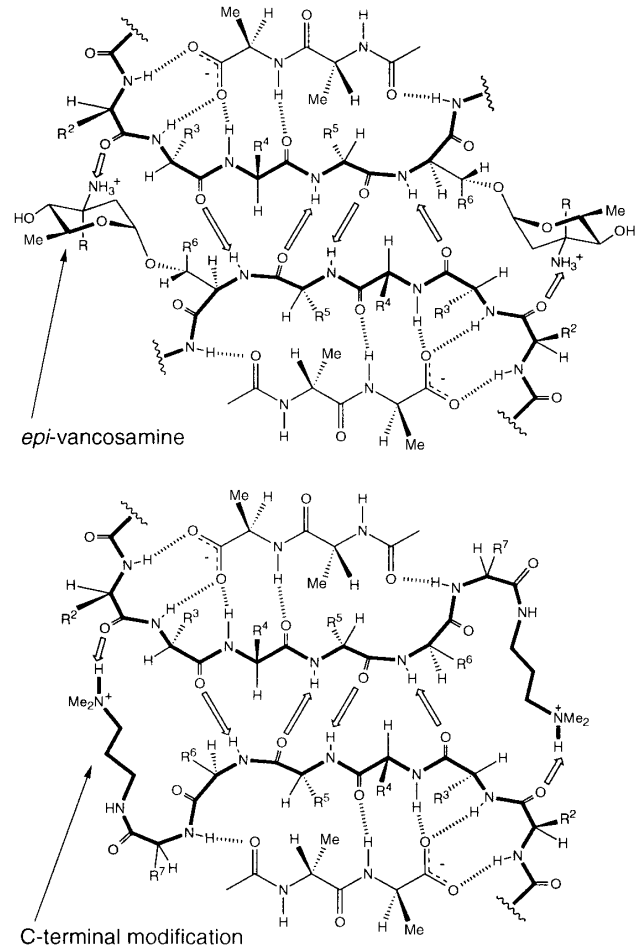

Fig. 2 Structure of the dimer interface of MDL 63246 and eremomycin, showing how the C-terminal diamine of the former can fulfil a similar role to the *epi*-vancosamine sugar of the latter

dimerisation, but it does possess a membrane anchoring C_{11} chain attached *via* the ring 4 sugar,[16] a feature shared by A40926. However, there are a number of structural differences between A40926 and teicoplanin. A40926 has a ring 3 chlorine, lacks a ring 2 chlorine, and perhaps most importantly in the present context, lacks the acetylated ring 6 sugar found in teicoplanin. The acetylated ring 6 sugar is thought to be the main factor which prevents teicoplanin from dimerising, as it would lead to an unfavourable steric interaction in the dimer structure. It was thus hypothesised that A40926 might dimerise to a limited extent. The structures of the dimers formed by several members of the vancomycin group have been determined by X-ray crystallography[17,18] and two dimensional NMR spectroscopy.[19,20] The use of a CPK model revealed that the diamine present on the C-terminus of MDL 63246 could stabilise such a dimer structure in a similar manner to the *epi*-vancosamine sugar of the antibiotic eremomycin, which increases the dimerisation constant by a factor of *ca*. 100.[21] This increase is due, at least in part, to the formation of a hydrogen bond between the ammonium ion of the *epi*-vancosamine with an amide carbonyl on the other side of the dimer. It further promotes dimerisation in complexes with cell-wall analogues due to an extended salt bridge, mediated through this amide between ammonium ion on one side of the dimer and carboxylate of the cell-wall peptide in the binding pocket of the other side of the dimer (Fig. 2).[19] Thus it was hypothesised that MDL 63246 might dimerise, and that its activity could be explained on the basis of the combined effect of dimerisation and membrane anchoring, exactly as for BCE.

To test this hypothesis, evidence was sought that MDL 63246 did indeed dimerise. When glycopeptide antibiotics dimerise, many ^{1}H NMR resonances change their chemical shift in a characteristic way and these shifts are thus indicative of dimerisation. The α-proton of residue 4 (x4) is particularly useful in this respect, as it shifts from *ca*. δ 5.5 in monomer to *ca*. δ 6.5 in dimer. Hence, where the process of dimerisation is in fast exchange, the chemical shift of x4 increases as the antibiotic concentration and proportion of dimer increase.[21] As x4 moves through a region of the spectrum which is relatively uncluttered by other peaks, it serves as an excellent probe for dimerisation. A 3 mm sample of the parent compound A40926 was thus prepared with the ligand di-*N*-Ac-Lys-d-Ala-d-Ala. The ^{1}H NMR spectrum was assigned from DQF-COSY, NOESY and TOCSY spectra. The chemical shift of x4 was δ 5.61. On dilution to 0.5 mm, x4 did shift upfield, but only very slightly (< 0.05 ppm), suggesting very weak dimerisation. However, for MDL 63246, a 3 mm solution gave an x4 shift of δ 6.12, changing to δ 5.84 at 0.5 mm. This concentration dependent x4 chemical shift strongly suggested that dimerisation was taking place. A plot of x4 chemical shift versus antibiotic concentration was therefore used to calculate the dimerisation constant by fitting the curve to the expression for dimerisation using a least squares algorithm. Such a plot yielded a dimerisation constant, K_{dim}, of 600 dm^3 mol^{-1} for MDL 63246 (Fig. 3).

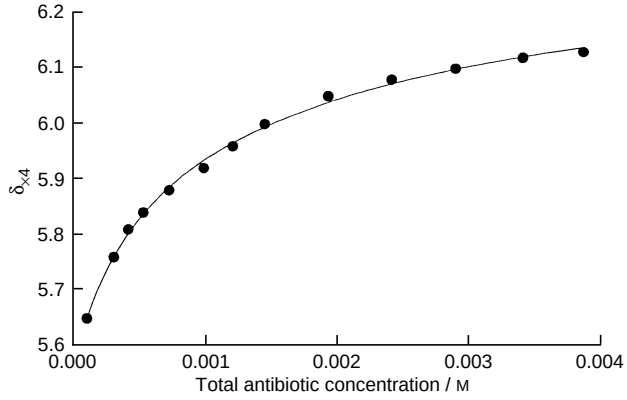

Fig. 3 Plot of x4 chemical shift against total MDL 63246 concentration. This curve yields a dimerisation constant of 600 ± 100 dm^3 mol^{-1}.

The dimer structure was investigated further by two dimensional NMR spectroscopy. DQF-COSY, TOCSY and NOESY experiments were used to assign the structure and study its conformation. Several NOE enhancements were observed which were inconsistent with the covalent structure of the antibiotic, but which were indicative of a dimer. These included NOEs between x4 and 6f, an aromatic proton on residue 6 of the antibiotic, and between 6f and 4b (Fig. 1). An NOE enhancement was also observed between 2b and the *N*-methyl groups of the C-terminal diamine, suggesting the diamine was in a position where it could hydrogen bond to the amide between residues 2 and 3, and participate in an extended salt bridge in the presence of cell wall peptides (Fig. 2).

The second example of an antibiotic which dimerises and possesses a membrane anchor, and which exhibits enhanced activity, lends further support to the hypothesis that these two locating devices act cooperatively to enhance binding at the surface of the bacteria. In the case of MDL 63246, it may be possible to improve activity further by modifications which improve dimerisation. This could be achieved in a number of ways, such as optimisation of the C-terminal diamine, through changes in length or removal of rotors. Use of a different base antibiotic, such as teicoplanin with the ring 6 sugar removed, could also improve dimerisation; this antibiotic possesses a ring 2 chlorine, a feature known to enhance dimerisation.[21]

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