Cooperativity in ligand binding expressed at a model cell membrane by the vancomycin group antibiotics

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Dimerisation or the use of a membrane anchor enhances the binding of the glycopeptide antibiotics at the surface of a model cell membrane.

Studies of the clinically important vancomycin group glycopeptide antibiotics have shown that the antibiotics specifically bind to the peptide sequence -L-Lys-D-Ala-D-Ala which is present in the immature cell-wall of Gram-positive bacteria (Fig. 1).[1–3] More recently, it has been shown that this group of antibiotics form dimers and that the process of dimerisation is cooperative with ligand binding.[4,5] That is, ligand binds to antibiotic dimer more strongly than to monomer, and the dimerisation constant is greater in the presence of ligand. Ristocetin A is an exception to this generalisation (see below).[4]

Of the vancomycin group antibiotics that have been characterised, teicoplanin is unique in that it shows no evidence of dimerisation and it has a C_{11} acyl chain attached to the residue 4 sugar substituent.[6] It has been shown that this hydrophobic chain can act as a membrane anchor,[7] such that binding to the cell-wall precursor at a cell surface is potentially intramolecular as both the antibiotic and its target are attached to the same template.[4] It has been postulated that this intramolecular binding would enhance the activity,[8] and indeed it has been shown that when the residue 4 sugar and the membrane anchor are removed the activity against staphylococci is reduced by a factor of 2–15.[9] Additionally, in accord with the hypotheses that dimerisation and membrane anchors promote antibiotic activity, it has been demonstrated that these features make the antagonism of antibiotic action by externally added di-*N*-acetyl-Lys-D-Ala-D-Ala much more difficult.[8]

We have recently observed a relationship between the overall free energy of ligand binding and the limiting chemical shift of the amide proton $w_2(\delta_{w2}^{limit})$ in aqueous solution which occurs at lower field for a stronger complex (w_2 is labelled in Fig. 1).[10–12] The limiting chemical shift is attained when the antibiotic is >95% bound. Using δ_{w2}^{limit} as a measure of the relative strength of ligand binding, sodium dodecylsulphate (SDS) micelles were used as model cell membranes to study the interaction of teicoplanin with *N*-α-decanoyl-*N*-ε-acetyl-Lys-D-Ala-D-Ala (*N*-dec-*N*-ac-KDADA, synthesised in this laboratory) which is also anchored into the micelles and di-*N*-acetyl-Lys-D-Ala-D-Ala (di-*N*-ac-KDADA, available from Sigma), which is free in solution as it lacks the hydrophobic membrane anchor. Using 500 MHz ^{1}H NMR, δ_{w2}^{limit} of the complexes with each of the ligands with teicoplanin were determined in the presence of micelles and compared to that when free (7.78 ppm). For the complex of teicoplanin with *N*-dec-*N*-ac-KDADA on the surface of a micelle δ_{w2}^{limit} = 11.77, further downfield than the signal in the di-*N*-ac-KDADA complex in the presence of micelles (11.36 ppm). Thus, when the ligand binding is essentially intramolecular on the surface of a micelle, the overall free energy of binding is more favourable (Fig. 2). UV spectrophotometry was used to determine the binding constants of di-*N*-ac-KDADA and *N*-dec-*N*-ac-KDADA with teicoplanin in the presence of SDS which were found to be 3.2×10^5 and 1.7×10^6 dm^3 mol^{-1} respectively. These results are in agreement with the qualitative trend observed from the NMR spectra. On the basis of the UV data, there is a benefit from the intramolecular binding on the surface of a micelle which is worth 4.1 kJ mol^{-1} in free energy.

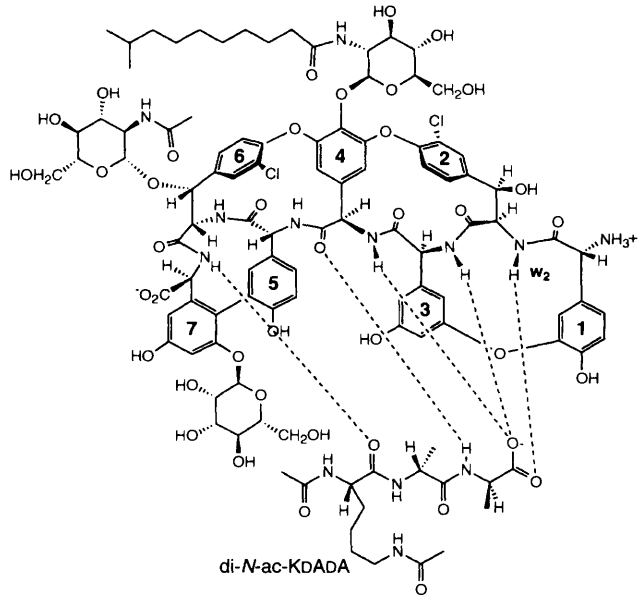

Fig. 1 Exploded view of the complex between teicoplanin and the cell-wall analogue di-*N*-ac-KDADA. The dotted lines represent intermolecular hydrogen bonds. Note the acyl chain attached to the glucosamine of residue 4.

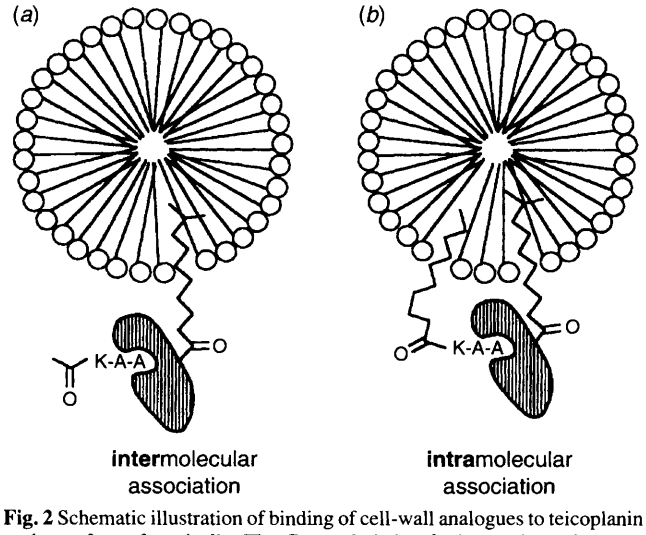

Fig. 2 Schematic illustration of binding of cell-wall analogues to teicoplanin at the surface of a micelle. The C_{11} acyl chain of teicoplanin anchors the antibiotic to the micelle. (*a*) The complex with di-*N*-ac-KDADA is intermolecular but (*b*) the complex with *N*-dec-*N*-ac-KDADA is essentially intramolecular, conferring extra stability.

The dimerisation of another member of the group, ristocetin A, has been observed to be anticooperative with the binding of ligand (*e.g.* di-*N*-ac-KDADA). That is, dimerisation is reduced when the ligand is fully bound.[4,13] Despite this anticooperative effect, ristocetin A is an effective antibiotic.[14] Thus, it has been postulated that on the surface of a cell (or micelle with anchored ligands) both dimerisation and ligand binding should be enhanced such that the anticooperative effect is overcome.[4]

In order to test this hypothesis, δ_{w2}^{limit} was measured for the complexes of ristocetin A with *N*-decanoyl-D-Ala-D-Ala (*N*-dec-DADA, synthesised in this laboratory) and *N*-acetyl-D-Ala-D-Ala (*N*-ac-DADA, synthesised in this laboratory) in the presence of micelles (Fig. 3).

The intermolecular complex between ristocetin A and *N*-ac-DADA in the presence of micelles gave $\delta_{w2}^{limit} = 11.43$. In the postulated intramolecular complex at the surface of a micelle between ristocetin A and *N*-dec-DADA, δ_{w2}^{limit} was observed to be further downfield at 11.65 ppm. UV spectrophotometry was again used to determine the binding constants of *N*-ac-DADA and *N*-dec-DADA with ristocetin A in the presence of SDS which where found to be 4.1×10^4 and 1.6×10^7 dm^{-3} mol^{-1} respectively. Thus, the intramolecularity gives an overall favourable free energy contribution to the whole system of 14.8 kJ mol^{-1} despite the unfavourable contribution from the anticooperativity that is inherent in the ristocetin A system.

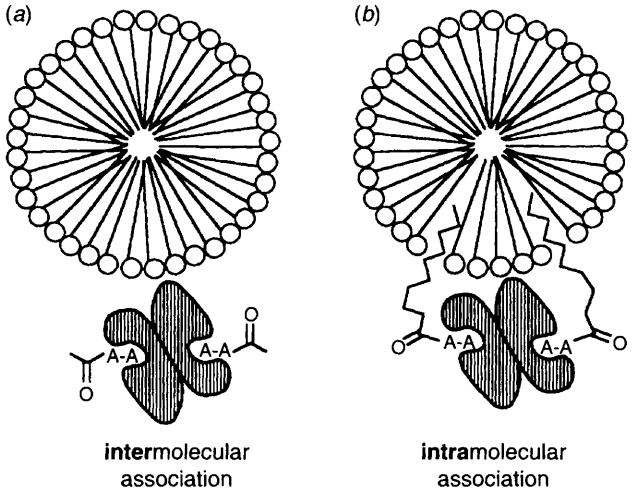

Fig. 3 Schematic illustration of binding of cell-wall analogues to a ristocetin dimer at the surface of a micelle. (*a*) The complex with *N*-ac-DADA is intermolecular but (*b*) the complex with *N*-dec-*N*-DADA is essentially intramolecular, conferring extra stability.

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