Structural Features that affect the Binding of Teicoplanin, Ristocetin A, and their Derivatives to the Bacterial Cell-wall Model *N*-Acetyl-D-alanyl-D-alanine

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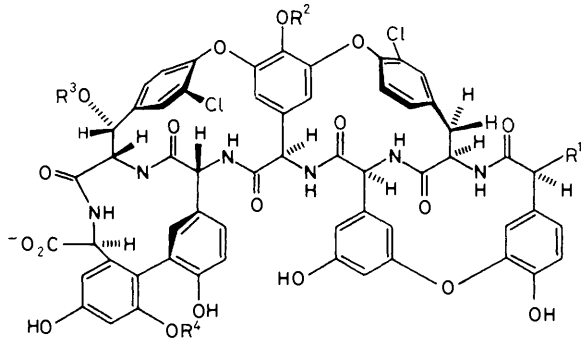
Studies with the title antibiotics have confirmed that the N-terminal amino groups of such glycopeptides play a major rôle in binding to *N*-acetyl-D-alanyl-D-alanine; one sugar is found to affect free energies of binding but the other sugars and fatty acid groups attached to the aglycones have more uncertain rôles.

We have recently elucidated the structures of the major factor of the antibiotic teicoplanin (**1**), and of its degradation products lacking one (**2**), two (**3**), and three (**4**) of the attached sugar moieties.[1] Teicoplanin is a member of the vancomycin class of antibiotics[2] and has close structural resemblances to ristocetin A (**5**). The availability of the derivatives (**2**)—(**4**) has allowed us to study the rôle of the sugars in the binding of the antibiotics to the bacterial cell-wall model *N*-acetyl-D-alanyl-D-alanine (**9**) (Ac-D-Ala-D-Ala). In addition, the *N*-acetylated derivatives (**7**) and (**8**) have given further data on the rôle of the N-terminal amino groups.



(**1**) $R^1 = NH_3^+$; R^2 = *N*-(8-methylnonanoyl)-2-amino-2-deoxy-β-D-glucopyranosyl; R^3 = 2-acetamido-2-deoxy-β-D-glucopyranosyl; R^4 = α-D-mannopyranosyl

(**2**) $R^1 = NH_3^+$; R^2 = H; R^3 = 2-acetamido-2-deoxy-β-D-glucopyranosyl; R^4 = α-D-mannopyranosyl

(**3**) $R^1 = NH_3^+$; $R^2 = R^4$ = H; R^3 = 2-acetamido-2-deoxy-β-D-glucopyranosyl

(**4**) $R^1 = NH_3^+$; $R^2 = R^3 = R^4$ = H

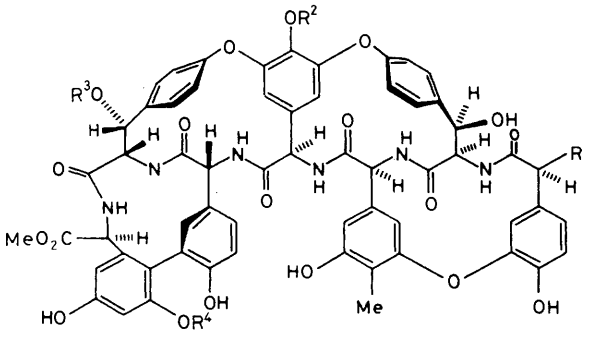
(**8**) R^1 = NHAc; $R^2 = R^4$ = H; R^3 = 2-acetamido-2-deoxy-β-D-glucopyranosyl

Measurements of binding energies (Table 1) and ^{1}H n.m.r. experiments using teicoplanin (**1**) and (**3**) in combination with (**9**), including measurements of intermolecular nuclear Overhauser effects (n.O.e.s),[1] indicate that teicoplanin forms a complex with Ac-D-Ala-D-Ala that has a very similar structure to that formed by ristocetin A. Strikingly, the chemical shifts of the two alanyl methyl groups in the bound state are very

Table 1. Free energies of binding (association) for antibiotics and Ac-D-Ala-D-Ala at 28 °C.[a]

Structure	$-\Delta G$/kJ mol^{-1}	Structure	$-\Delta G$/kJ mol^{-1}
(**1**)	35.0 ± 1	(**5**), pH 5	31.2 ± 0.4[b]
(**2**)	35.2 ± 1	(**5**), pH 10	18.4 ± 2[b]
(**3**)	28.8 ± 1	(**6**)	26.0 ± 1[c]
(**4**)	32.3 ± 2	(**7**)	15.2 ± 2[d]
(**8**)	23.0 ± 1		

[a] Measured by u.v. difference spectroscopy[7] in 0.02 M citrate at pH 5 unless otherwise stated. [b] Taken from ref. 5. [c] Taken from ref. 8. [d] Measured by ^{1}H n.m.r. in D_2O containing NaCl.



(**5**) $R^1 = NH_3^+$; R^2 = tetrasaccharide residue; R^3 = ristosamine residue; R^4 = α-D-mannopyranosyl

(**6**) $R^1 = NH_3^+$; $R^2 = R^4 = H$; R^3 = ristosamine residue

(**7**) R^1 = NHAc; R^2 = tetrasaccharide residue; R^3 = *N*-acetylristosamine residue; R^4 = α-D-mannopyranosyl

Table 2. Kinetic parameters for ristocetin binding to Ac-D-Ala-D-Ala at 26 °C.[a]

Structure	Dissociation rate/s^{-1}	Association rate/ $l\,mol^{-1}\,s^{-1}$
(**5**), pH 5	42 ± 20	1.2 ± 0.6 × 10^7
(**5**), pH 10	113 ± 55	2.0 ± 1.4 × 10^5
(**7**), pH 5	41 ± 20	3.2 ± 2.8 × 10^4

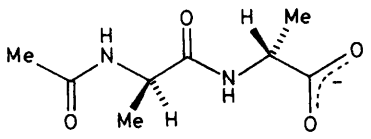
[a] Data for (**5**) from ref. 5; data for (**7**) estimated using ^{1}H n.m.r. at the coalescence temperature.

similar for teicoplanin and ristocetin A. Thus for (**1**) they resonate at δ (^{1}H) 0.44 and 0.87 (compare δ 0.38 and 0.87 for ristocetin A[3]).

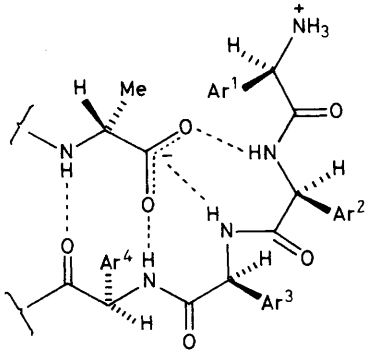
The structure of the complex between ristocetin A (**5**) and Ac-D-Ala-D-Ala has been deduced previously. Several hydrogen bonds and, presumably, hydrophobic interactions between the peptide and the antibiotic aglycone result in strong binding. In the case of the analogous tripeptide model, Ac_2-L-Lys-D-Ala-D-Ala, interactions between the peptide and D-mannose (R^4) and D-glucose have been noted.[4]

Some data have been presented[5] indicating that the charged N-terminus of ristocetin A (**5**) is important in stabilizing an initial weak Coulombic complex, which undergoes a slow conformational change to the fully bound state. The present work with *N*-acetylated compounds supports this conclusion.

It can be seen from Table 1 that both deprotonation, (**5**) (pH 10), and *N*-acetylation, (**7**), lower the free energy of binding (ΔG) for ristocetin A by similar amounts. This is consistent with the model (**10**) in which there is no direct interaction between the amino group of ristocetin A and the carboxylate group of the peptide in the bound state, since otherwise the different size of the acetylated amino group in (**7**) would lower ΔG more than does deprotonation. Moreover, the very similar dissociation rates for all three cases given in Table 2 suggest that there is no direct interaction between the amino group and the carboxylate group in the transition state for binding. The changes that occur in going from the bound state to the transition state would not, therefore, involve attractions between the amino group and carboxylate group. The rôle played by the amino group of ristocetin A is indicated by the association rates given in Table 2, which show that deprotonation of the amino group, whether



(**9**)



(**10**)

by change in pH or by acetylation, changes the association rates by similar amounts. This absence of a steric effect is what one would expect for the model proposed.[5]

We now consider the part played by the sugars. In teicoplanin (**1**) it is possible to remove the sugars in a stepwise manner.[1] Binding energies (ΔG) for these derivatives have been measured and are given in Table 1, from which it is clear that only the removal of the D-mannose residue (R^4) has any significant effect on ΔG. Data for ristocetin A show the same effect [(**5**) and (**6**) in Table 1] and we assume these results reflect the rôle played by the D-mannose residue in forming part of the binding pocket for Ac-D-Ala-D-Ala. Sugars at positions R^2 and R^3 in teicoplanin have little effect on ΔG. By implication, the tetrasaccharide at R^2 in ristocetin A also has little effect.

Table 1 shows that *N*-acetylation, compound (**8**), of a teicoplanin derivative reduces ΔG, but to a lesser extent than does *N*-acetylation of ristocetin A. This may be because, in *N,N'*-diacetyl-ristocetin A, the ristosamine is also *N*-acetylated. This would imply a previously undescribed rôle for the charge on the amino sugars in the binding of such antibiotics. A similar effect may be operating in the structurally similar antibiotic vancomycin,[2] where removal of an amino-sugar from position R^2 causes a significant drop in the strength of binding to Ac-D-Ala-D-Ala,[6] in contrast to the lack of effect of removing uncharged sugars from the analogous positions in teicoplanin and ristocetin A.

The rôles played by the sugars, apart from the D-mannose residue, are poorly understood. The same may be said of the acyl groups in teicoplanin. However, with the exception of the D-mannose and possibly the charged amino-sugars, these groups do not appear to affect the energies of binding to Ac-D-Ala-D-Ala. Most importantly, there is little correlation between the binding energies observed with models and the *in vitro* activities observed with Gram-positive bacteria. Thus, perhaps, the sugars and fatty acid groups are important for transport and/or cell-surface recognition.

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