Pure & Appl. Chem., Vol. 61, No. 3, pp. 585–588, 1989.
Printed in Great Britain.
© 1989 IUPAC

Molecular basis of the activity of antibiotics of the vancomycin group

Dudley H. Williams and Jonathan P. Waltho

University Chemical Laboratory, Lensfield Rd., Cambridge, U.K.

Abstract

The formation of a carboxylate anion binding pocket in the antibiotics of the vancomycin group is made energetically more favorable by the S stereochemistry of residue 3 relative to the R stereochemistries of residues 1, 2, and 4. Pocket formation is further favoured by the covalent cross-linking of the sidechains of residues 2 and 4, and in some cases, also of residues 1 and 3. In the absence of the latter cross-link in vancomycin itself, the pocket is only partially formed in the free antibiotic, and is in equilibrium with a second conformation in which the peptide backbone alternates as in a β-pleated sheet.

The sugar vancosamine plays a role in the thermodynamics of binding of vancomycin to cell wall analogues. This role is based upon a hydrophobic interaction between the 6-methyl group of the sugar and the methyl group of the C-terminal alanine of the cell-wall analogue. The hydrophobic interaction is however only significant in the presence of the nearby charged amino group of the sugar. It is believed that the charged group, by local ordering of the water structure, strengthens the hydrophobic interaction.

The sugar mannose in ristocetin A is able, by partially shielding the non-C-terminal alanine methyl group of the dipeptide cell wall analogue from water, to increase the binding of ristocetin A to this analogue. However, in the binding of the tripeptide cell-wall analogue, this same methyl group is shielded from water by the sidechain of lysine, and mannose loses a significant role in the thermodynamics of binding. This observation accounts for the much larger increase in binding, on passing from di- to tri-peptide, observed for vancomycin (which lacks mannose) relative to ristocetin A (which possesses mannose).

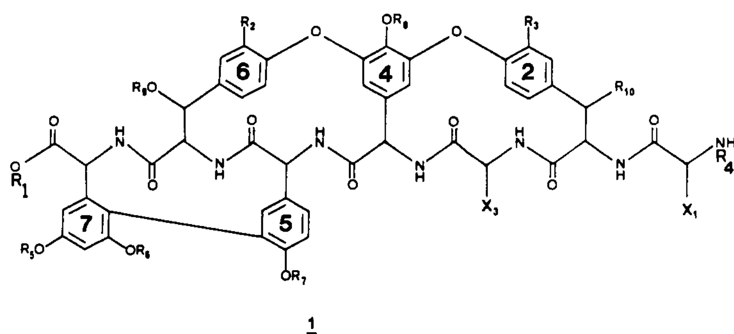
INTRODUCTION

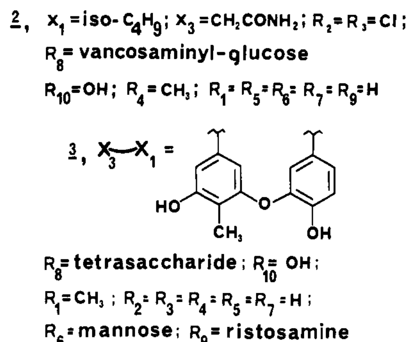
Antibiotics of the vancomycin group have assumed increasing clinical importance during the last fifteen years, in part because of the increasing prevalence of Staph. aureus bacteria which are resistant to methicillin (ref. 1). In addition, vancomycin itself has found extensive use in the treatment of post-operative diarrhoea, caused by Clostridium difficile in the gut. The antibiotic is then given orally, and has been found to be very efficient in curing a dangerous condition. The vancomycin group consists of a large number of structures, all of which are heptapeptides. Most of the variants can be encompassed by the general structure 1 (see, for example, ref. 2).

Our work to elucidate the molecular basis for the mode of action of these antibiotics has been built on a finding (ref. 3) that vancomycin itself (2) and another member of the group, ristocetin A (3), bind to cell wall mucopeptide precursors terminating in the peptide -D-Ala-D-Ala. Given this knowledge, we were able to show (ref. 4) by proton NMR that the two components interact as shown in fig. 1 (hydrogen bonds shown by dotted lines). The molecular basis of action of the antibiotics is therefore well-founded, and additionally one can measure the binding constant between the two components by the use of UV-difference spectroscopy (ref. 5). These measurements have not only been carried out for numerous antibiotics of the vancomycin group with N-acetyl-D-Ala-D-Ala, but also to the extended cell wall analogue, di-N-diacetyl-L-Lys-D-Ala-D-Ala. Thus, we have been investigating recently how these systems can be used to further an understanding of molecular recognition.

THE GENERATION OF THE CARBOXYLATE ANION BINDING POCKET

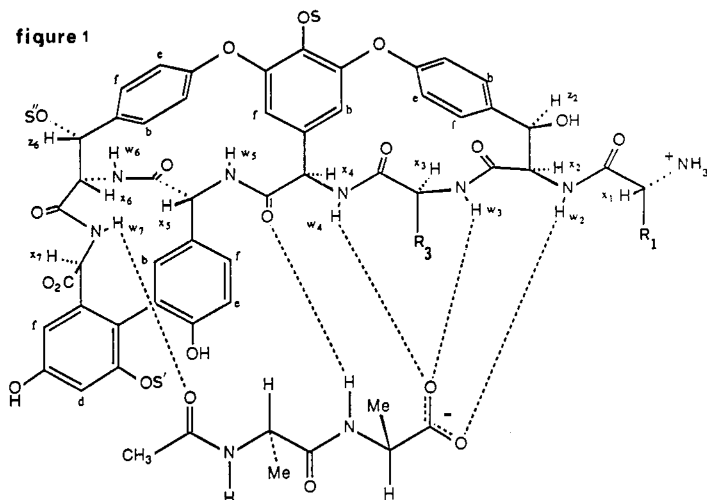
All members of the vancomycin group of antibiotics so far discovered, exhibit in their bound state, a carboxylate binding pocket whose most general feature is three inward pointing NHs, derived from three successive amides of a peptide backbone (the amides of amino acids 2, 3 and 4 from the N-terminus; see 1). The nature of this pocket, and the three hydrogen bonds formed between it and the carboxylate anion of the cell wall analogue is seen in fig. 1. A key question is "how are these three NHs made to point in the same direction, when normally in a poly-R or poly-S-peptide in its β-pleated sheet form they would alternate

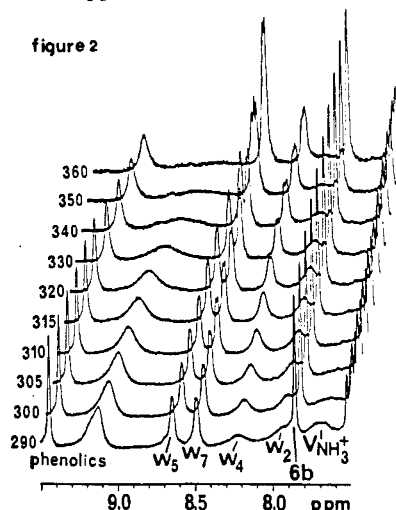


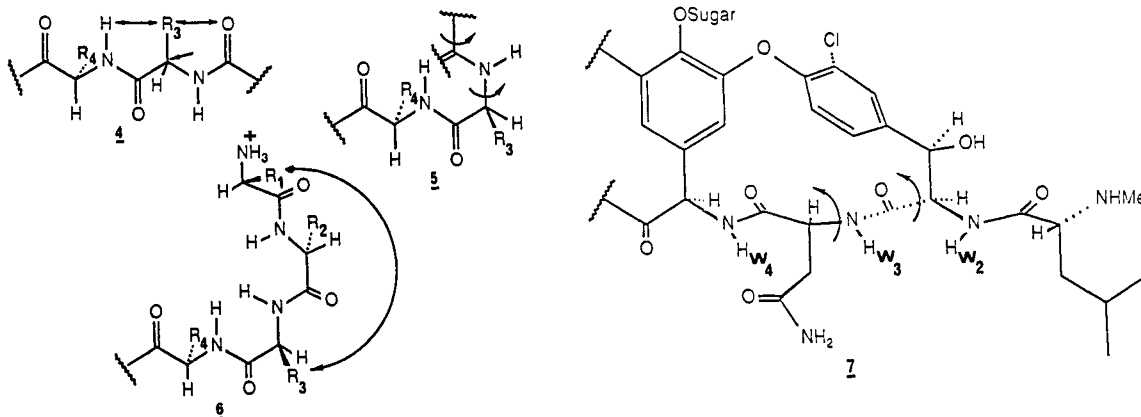


up/down/up ?". We have concluded that the low energy requirement for pocket formation in vancomycin mainly derives from the fact that the amino acids have the R, R, S, and R configurations at positions 1, 2, 3, and 4 from the N-terminus, i.e. residue 3 has the opposite absolute configuration to residues 1, 2, and 4 (see 1). As a consequence, a β-pleated sheet form would contain the two repulsive 1,3-interactions indicated by double headed arrows in 4. These interactions can be relieved by rotating the backbone to produce 5, but the carbonyl oxygen atom which is omitted in 5 now has an unfavourable steric interaction with the NH of amino acid 4. This interaction can in turn be relieved by rotating the amide bond which is bounded by arrows in 5 through @ 180°. The conformation 6 results, and this is essentially the shape found in the carboxylate anion binding pockets of the antibiotics. Note that in 6, the three inwardly pointing NH groups of amino acids 2, 3, and 4 can be constrained together if the sidechains of residues 1 and 3 are held approximately coplanar on the top side of 6 as presented, and the sidechains of residues 2 and 4 are similarly held approximately coplanar on the bottom side of 6. In fact, in all known antibiotics of the vancomycin group residues 2 and 4 are indeed constrained near the same plane by covalent cross-linking of their sidechains (see, for example, 2 and 3). In ristocetin A (3), the sidechains of residues 1 and 3 are also covalently cross-linked. The result of the two sets of cross-links makes it appear, from CPK models, that the geometry of the desired binding pocket is constrained by a kinetic barrier. In contrast, in vancomycin (2), the sidechains of residues 1 and 3 are not covalently cross-linked. Molecular models suggest that in this case, the binding pocket might be more flexible along the amide backbone, and this possibility has accordingly been investigated by proton NMR spectroscopy.

In free vancomycin monohydrochloride in d_6-DMSO solution, nOes are observed between the amide NH (coded W_3) of the asparagine residue (residue 3) and the α-CH (coded X_2) of residue 2, the benzylic C<u>H</u>-OH (coded Z_2) of residue 2, and the aromatic ring proton (coded 2b) adjacent to chlorine in residue 2. These nOes establish that the amide proton of residue 3 spends some of its time on the "non-binding" face of vancomycin. However, W_3 also shows nOes to the amide NHs of residues 2 and 4 (coded W_2 and W_4), and to the aromatic ring proton (coded 2f) on the front face of residue 2 .These nOes establish that a second conformation is populated, in which W_3 is on the front (i.e., binding) face of the structure, and the carboxylate anion binding pocket is indeed formed to an appreciable extent







prior to binding the cell wall analogue. The interconvertion of the two conformers requires the rotation, through approximatety 180°, of the amide bond formed between the carbonyl of residue 2 and the NH of residue 3.

The above process is slow on the NMR timescale (ref. 6). At room temperature, the resonances of the amide NH protons W_2, W_3, and W_4, associated with residues 2, 3, and 4, are very broad compared with those (W_5, W_6, and W_7) of the other amide NHs (fig. 2). On heating the solution, the resonances of W_2 (initially at least), W_3, and W_4 sharpen, with those of W_3 and W_4 attaining approximately the same linewidth as those of W_5 and W_7 at @ 350°K (fig. 2). Above 310°K, the exchange of W_2 with traces of water in the solvent becomes the primary determinant of the linewidth of its resonance; this process is incidental to our present considerations, but is catalysed by the basic NH_2 group at the N-terminus of the antibiotic (ref. 6). Similar, though less dramatic sharpening is observed for the resonances of X_2, X_3, and Z_2 but not for other α-CH and β-CH protons. These selective sharpenings of the specified lines indicate a process which is passing from an intermediate exchange rate to the fast exchange limit as the temperature is increased.

In the light of the above nOe evidence, and the fact that the selectively sharpened resonances are all due to protons in the vicinity of the amide bond connecting residues 2 and 3, we conclude that this amide bond (bounded by arrows in 7, which depicts the NH's of residues 2, 3, and 4 on the front face of the antibiotic, as required for binding) is undergoing slow rotation. Estimates of the chemical shift differences which might be observed in the slow exchange limit lead us to conclude that the amide bond is rotating at a frequency in the range 200-1000 Hz (ref. 6). Thus the barrier to the rotation must lie near to 12-14 kcal mol^{-1}.

The above conclusions may be of utility in the design of a carboxylate anion binding pocket. They indicate that an appropriate pocket might be found within a tetrapeptide unit if the residues (counted from the N-terminus) had R, R, S, and R (or S, S, R, and S) configurations, and an appropriate connection of the sidechains of residues 2 and 4.

THE ROLE OF THE ANTIBIOTIC SUGARS IN BINDING

From the early work, it is evident that the major basis for the antibiotic-cell wall analogue interaction lies in the aglycone portion of the antibiotics. However, the antibiotics are found to carry 1 to 7 sugars. For example, the sugars glucose and vancosamine are found in vancomycin (2), and mannose, ristosamine, and a tetrasaccharide are found in ristocetin A (3). While it always appeared likely that these sugars would promote *in vivo* activity of the antibiotics by promoting their aqueous solubility, it is only now becoming evident that at least some of them aid binding in subtle and interesting ways.

(i) The role of vancosamine in vancomycin

When vancosamine is selectively removed from vancomycin, the binding constant to the cell wall analogue di-N-acetyl-L-Lys-D-Ala-D-Ala drops by a factor of @ 3 (ref. 7). Inspection of an X-ray structure of a degradation product of vancomycin, CDP-1, immediately suggests a reason for this drop. In the X-ray structure, the 6-methyl group of vancosamine lies immediately next to the benzene ring of the amino acid residue 4; it is this benzene ring that undergoes a hydrophobic interaction with the methyl group of the C-terminal alanine residue of the cell wall analogue. Thus, the 6-methyl group of vancosamine can block the access of water to the methyl group of the C-terminal alanine residue, and so improve the overall hydrophobic interactions of the latter.

Further experiments show that although this idea is basically correct, the significance of the interaction is subtly modified by the presence of the NH_3^+ group of the amino-sugar vancosamine. Thus, acetylation of the amino group of vancosamine also reduces the binding constant to the same cell wall analogue by a factor of @ 3 (ref. 7). Determination of nOes when the cell wall analogue is bound to vancomycin, and when bound to vancomycin N-acetylated in the sugar residue, establishes that the 6-methyl group of vancosamine is adjacent to the methyl group of the C-terminal alanine residue in both complexes. Hence, we are forced to the conclusion that the associated hydrophobic interaction is strengthened (and indeed only contributes significantly to binding) in the presence of the charged amino group.

The physical basis for such an effect is clear. Water molecules in the vicinity of the NH_3^+ group will be relatively strongly bound to each other, and relatively highly ordered. As a consequence, the penalty in free energy to be paid for disrupting this local water structure by a hydrocarbon surface will be larger than in the absence of the charge. Thus, the hydrophobic effect is strengthened by the proximate charge. A macroscopic expression of this kind of effect is found in the familiar "salting-out" phenomenon used in working up some organic reactions; a substance of limited hydrophobicity can be induced to pass from an aqueous to a non-polar phase by increasing the ionic strength in the aqueous phase. Since the effect in the antibiotic is intramolecular, the local "salting out" is highly effective.

(ii) The role of mannose in ristocetin A

The removal of mannose from residue 7 (see (1)) of ristocetin A (3) reduces the binding to N-acetyl-D-Ala-D-Ala by slightly in excess of 1kcal mol^{-1} (@ a factor of 10 reduction in the binding constant) (ref. 8). The molecular basis of this effect seems clear from earlier proton nOe experiments (ref. 9). The anomeric proton of the mannose residue shows an nOe to the methyl sidechain of the central alanine residue of the tripeptide cell wall analogue. Hence, it is concluded that a portion of this methyl group can be shielded from water by a relatively hydrophobic portion of the sugar residue.

However, the binding constant of ristocetin A to tripeptide is not changed significantly upon removal of mannose (and simultaneously of the tetrasaccharide) (ref. 10). Thus, it must be concluded that although the mannose residue is important in shielding the methyl group of the non-C-terminal alanine residue from water in dipeptide, it has no such significant role when the tripeptide binds. This apparent anomaly is resolved by the observation that when the tripeptide binds to ristocetin A in aqueous solution, the lysine sidechain folds over the aromatic ring of residue 7. Evidently, the lysine sidechain protects the methyl group of the non-C-terminal alanine from solvent to an extent that makes the corresponding role of mannose insignificant. It may additionally be that in the complex with tripeptide, the lysine sidechain and the mannose show an unfavourable steric interaction that prevents them from being able to shield the methyl group simultaneously and optimally from water. In this connection, it is noteworthy that vancomycin, which lacks the mannose attached to amino acid 5, binds much more strongly to tripeptide (1.5×10^6) than to dipeptide (6.4×10^4 mol^{-1}).

REFERENCES

1. R. Wise and D. Reeves, (Eds.)(1984) Symposium on vancomycin therapy. J. Antimicrob. Chemother. 14, suppl. D.
2. J. C. J. Barna and D. H. Williams, Ann. Rev. Microbiol., 38, 339-357 (1984).
3. H. R. Perkins, Biochem. J., 111, 195-205 (1969).
4. J. R. Kalman and D. H. Williams, J. Amer. Chem. Soc., 102, 897-905 and 906-912 (1980).
5. M. Nieto and H. R. Perkins, Biochem. J., 123, 773-787 (1971).
6. J. P. Waltho, D. H. Williams, D. J. M. Stone, and N. J. Skelton, submitted for publication.
7. K. Rajamoorthi, C. M. Harris, T. M. Harris, J. P. Waltho, N. J. Skelton and D. H. Williams, J. Amer. Chem. Soc., in press.
8. J. C. J. Barna, D. H. Williams, and M. P. Williamson, J. Chem. Soc., Chem. Commun., 254-256 (1985).
9. D. H. Williams, M. P. Williamson, D. W. Butcher, and S. J. Hammond, J. Amer. Chem. Soc., 105, 1332-1339 (1983).
10. T. R. Herrin, A. M. Thomas, T. J. Perun, J. C. Mao, and S. W. Fesik, J. Med. Chem., 28, 1371-1375 (1985).