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Nuclear Magnetic Resonance Studies on the Interaction of Avoparcin with Model Receptors of Bacterial Cell Walls

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SUMMARY

On the basis of nuclear Overhauser enhancement and ^1H chemical shift data obtained in aqueous solution, a model is proposed for the interaction of β-avoparcin and epi-β-avoparcin with acetyl-D-alanyl-D-alanine (Ac-D-Ala-D-Ala) and diacetyl-L-lysyl-D-alanyl-D-alanine (Ac_2-L-Lys-D-Ala-D-Ala). For the β-avoparcin: Ac_2-L-Lys-D-Ala-Ala complex, the COOH-terminal end of the tripeptide is located near the NH_2 terminus of the antibiotic with the tripeptide extending across the peptide backbone of β-avoparcin toward its COOH-terminal end. In our proposed structure, the three amino acid residues of the peptide span the entire length of the antibiotic, and the aliphatic side chain of the lysine residue extends over the D-ring of β-avoparcin. The structure of the epi-β-avoparcin:Ac_2-L-Lys-D-Ala-D-Ala complex was found to be similar to the β-avoparcin complex at the binding site for the lysine residue at the COOH-terminal end of the antibiotic, but differed in the interactions at the NH_2 terminus. These results are consistent with the similarities in the COOH-terminal conformations and the differences in conformations at the NH_2 terminus found for β-avoparcin and epi-β-avoparcin which were described in the preceding paper [Fesik, S. W., I. M. Armitage, G. A. Ellestad, and W. J. McGahren, *Mol. Pharmacol.* **25**:275–280 (1984)]. The association constants (measured by UV methods) for both β-avoparcin:peptide complexes were greater than those measured for epi-β-avoparcin and correlated with their differences in antibacterial activity. Epi-β-avoparcin exhibited no measurable binding to the dipeptide; however, a significant affinity was measured for the tripeptide, indicating that the interactions with the NH_2 terminus of the antibiotics provide binding energy for the antibiotic peptide complex but that the COOH-terminal end of the antibiotics also plays an important role in the binding interaction. These results are interesting in light of the similarities in the structural and conformational features at the COOH terminus for all of the glycopeptide antibiotics.

INTRODUCTION

In the preceding paper (1), we investigated the conformational properties of β-avoparcin and a series of structurally related analogues. On the basis of an analysis of the ^1H chemical shifts, ^1H-^1H NOE[3] data, and pH titration experiments, it was concluded that the COOH-terminal conformations of all of the compounds examined were similar and resembled that previously found for other glycopeptide antibiotics of this class (vancomycin and ristocetin). However, for β-avoparcin and epi-β-avoparcin (Fig. 1), a diastereomer with 10- to 100-fold less antibacterial activity (2), conformational differences were observed at the NH_2-terminal end of the molecules. In order to correlate these conformational properties with their different antibacterial activities, more information is needed on the structural details of their interaction with the receptor sites on the bacterial cell wall.

As with the other glycopeptide antibiotics of this class, avoparcin inhibits the growth of bacterial cell walls by preventing the formation of the peptidoglycan through its interaction with cell wall mucopeptide precursors ending in D-Ala-D-Ala (3). For the glycopeptide antibiotics, vancomycin and ristocetin, attempts have been made to characterize their interaction with peptide models of bacterial mucopeptides. In a study of the specificity of vancomycin:peptide complexes, Nieto and Perkins (4) measured the association constants for the binding of vancomycin to several synthetic peptides by UV difference spectroscopy. Peptides ending in D-Ala-D-Ala with

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[3] The abbreviations used are: NOE, nuclear Overhauser enhancement; Me_2SO, dimethyl sulfoxide; Ac-D-Ala-D-Ala, acetyl-D-alanyl-D-alanine; Ac_2-L-Lys-D-Ala-D-Ala, diacetyl-L-lysyl-D-alanyl-D-alanine.

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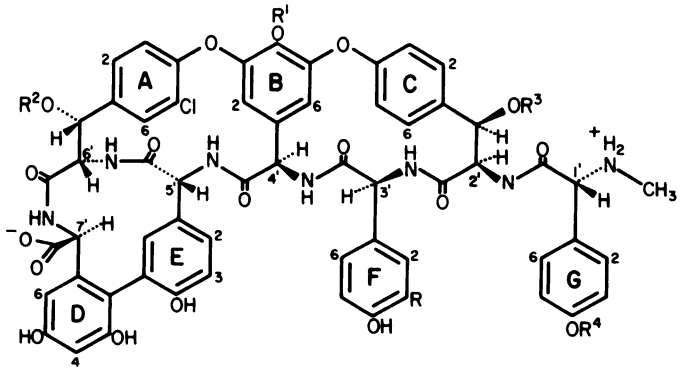

FIG. 1. *Structure of β-avoparcin*

R = H; R^1 = 2-O-(α-L-ristosaminyl)-O-β-D-glucose; R^2 = O-α-L-ristosamine; R^3 = O-α-D-mannose; R^4 = O-α-L-rhamnose. Epi-β-avoparcin is the 1′ epimer.

a third residue in the L-configuration displayed the strongest association for vancomycin. Subsequently, using NMR methods, more detailed information was obtained on the structure of the antibiotic-peptide complexes. Based on the N-H temperature coefficients, ^{1}H chemical shifts, and the observed intermolecular NOE obtained in Me_2SO (5), a model was postulated for the interaction of ristocetin with Ac-D-Ala-D-Ala. In the proposed model, the complex is stabilized by several hydrogen bonds, including three between the COOH terminus of the dipeptide and the "pocket" of NH protons at the NH_2-terminal end of ristocetin A. In NMR studies (6, 7) of the vancomycin:peptide complexes, a similar model was proposed involving hydrogen bonding at the NH_2 terminus of vancomycin. To accommodate the experimental evidence for an electrostatic interaction between the NH_2 terminus of the antibiotic and the carboxyl group of the peptide, a conformational change in the *N*-methyl leucine moiety upon binding was postulated.

If the NH_2 terminus of avoparcin plays an important role in binding bacterial mucopeptides, as with other glycopeptide antibiotics, the different NH_2-terminal conformations that we have found for β-avoparcin and epi-β-avoparcin could explain their 10- to 100-fold differences in antibacterial activities. In an effort to characterize the functional groups and conformational features of the avoparcin antibiotics that are necessary for a favorable interaction with bacterial mucopeptides, we have studied the interaction of β-avoparcin and epi-β-avoparcin with Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala by NMR. In addition, we have compared the binding affinities of β-avoparcin and epi-β-avoparcin for these peptides as measured by UV methods. From these experiments, we have been able to characterize the interaction of β-avoparcin with these peptide models for the bacterial cell wall mucopeptides and provide a structural rationale for the different antibacterial activity displayed by β-avoparcin and epi-β-avoparcin.

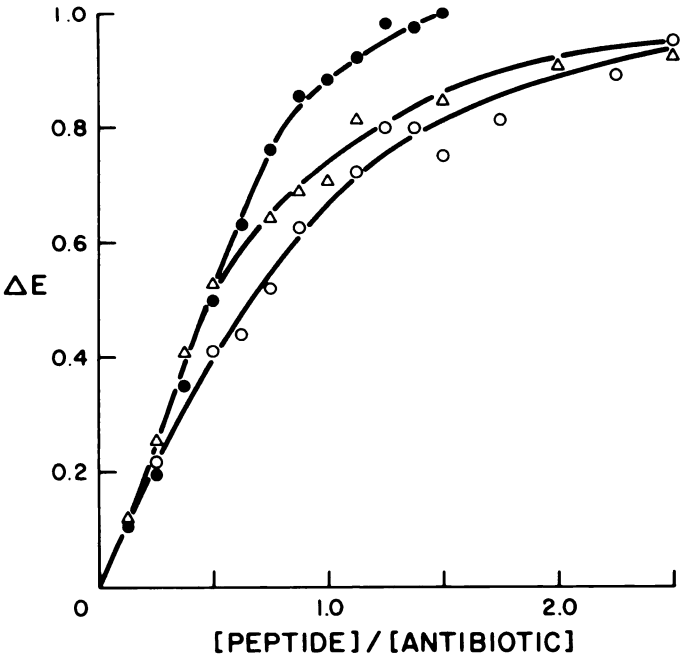

FIG. 2. *Difference in UV absorption (ΔE) of a 0.1 mg/ml solution of antibiotic (pH = 5.5) at 283 nm with the addition of peptide*

●, β-Avoparcin and Ac_2-L-Lys-D-Ala-D-Ala; ○, epi-β-avoparcin and Ac_2-L-Lys-D-Ala-D-Ala; △, β-avoparcin and Ac-D-Ala-D-Ala.

EXPERIMENTAL PROCEDURES

UV. The binding of β-avoparcin and epi-β-avoparcin to Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala was studied using UV methods as previously described (4). To a dilute solution (0.05–0.1 mM) of glycopeptide antibiotic (pH 5.5), increasing amounts of peptide were added, and the absorbance changes at 283 nm were monitored in the UV difference spectrum. UV spectra were recorded on a Cary 118 UV-visible spectrophotometer at room temperature. Association constants (K_a) were calculated from the data using the equation K_a = [complex]/[antibiotic][peptide].

NMR. NMR samples were prepared by mixing small amounts (1–3 mg) of antibiotic and peptide in ^2H_2O. The pH of the samples was adjusted with NaOD and DCL and is reported as the uncorrected meter reading. ^{1}H NMR spectra were recorded at 30° and 50° on a WM-500 Bruker spectrometer operating at 500 MHz. Chemical shifts are reported relative to 2,2-dimethyl-2-sila-5-pentane sulfonate, which was used as an external standard. NOE experiments were performed using various antibiotic to peptide ratios in a manner described in the preceding paper of this series (1). Presaturation times of 0.4 and 0.8 sec were used in the NOE experiments.

^{1}H NMR spectra recorded in 95% ^1H_2O were accomplished using the Redfield pulse sequence (2-1-4) for solvent suppression (8). The carrier frequency was set 4000 Hz downfield from the ^1H_2O signal. The P1 and P4 pulses were adjusted to give maximal solvent suppression.

RESULTS

UV. From the changes in the UV absorption of the antibiotics upon the addition of peptide, the binding curves of Fig. 2 were obtained. Binding constants were calculated as described under Experimental Procedures and appear in Table 1. β-avoparcin binds strongly to Ac-D-Ala-D-Ala ($K_a = 1.8 \times 10^5$ M^{-1}), whereas epi-β-avoparcin showed no measurable interaction with the dipeptide. However, epi-β-avoparcin did display appreciable binding ($K_a = 1.4 \times 10^5$ M^{-1}) to Ac_2-L-Lys-D-Ala-D-Ala, although the binding was stronger between β-avoparcin and the tripeptide ($K_a = 2.1 \times 10^6$ M^{-1}).

NMR. The ^{1}H NMR resonances of the β-avoparcin:peptide complexes were assigned from an analysis of

TABLE 1

Association constants for the binding of β-avoparcin and epi-β-avoparcin to Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala obtained by UV methods

	K_a	
	Ac-D-Ala-D-Ala	Ac_2-L-Lys-D-Ala-D-Ala
	M^{-1}	M^{-1}
β-Avoparcin	1.8×10^5	2.1×10^6
Epi-β-avoparcin	—[a]	1.4×10^5

[a] Binding was too weak to allow K_a to be measured.

the scalar coupling, ^{1}H-^{1}H NOE experiments, and, for protons in slow exchange, by saturation transfer experiments. Additional assignments were made for protons in fast exchange by following the chemical shift changes of the antibiotic with the addition of peptide. Substantial differences in chemical shift were observed in the NMR spectra of bound and free antibiotic. The ^{1}H chemical shifts of free and bound β-avoparcin are reported in Table 2. The chemical shift assignments of the peptide resonances are shown in Table 3.

The interactions of epi-β-avoparcin with Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala were also examined by NMR methods. No chemical shift changes were observed in the ^{1}H NMR spectrum of epi-β-avoparcin upon the addition of the dipeptide, as expected owing to the low association constant; however, several proton resonances shifted upon the addition of the tripeptide. Of particular interest was the observed change in chemical shift (0.21 ppm) for the NCH_3 protons of epi-β-avoparcin in this complex. The intermolecular NOEs that were detected in this antibiotic:peptide complex included an NOE between the CH_2 protons of the lysine side chain (1.55 ppm)[4] and the D_6 proton of epi-β-avoparcin and an NOE between the acetyl methyl protons of the lysine residue and the CH_3 protons of ristosamine (R^2-6).

In the ^{1}H-^{1}H NOE experiments performed with the β-avoparcin:Ac-D-Ala-D-Ala complex, an intermolecular NOE was observed between the *N*-acetyl methyl group of the dipeptide and the D_6 proton of β-avoparcin. Another NOE was observed between the NCH_3 group of β-avoparcin and the ^{1}CH proton of the D-Ala residue at the COOH terminus of the dipeptide. These were the only intermolecular NOEs that were detected. In contrast, several intermolecular NOEs were observed in the tighter complex formed between β-avoparcin and Ac_2-L-Lys-D-Ala-D-Ala. Table 4 depicts some of the NOEs obtained upon irradiation of the ^{1}H resonances of β-avoparcin and the tripeptide.

TABLE 2

Chemical shift assignments of free and bound β-avoparcin

Peak	β-Avoparcin δ	β-Avoparcin + Ac-D-Ala-D-Ala[a] Δδ	β-Avoparcin + Ac_2-L-Lys-D-Ala-D-Ala[a] Δδ
	ppm	*ppm*	*ppm*
NCH_3	2.71	0.01	0.02
1′	5.25	0.09	0.12
6′	4.37	−0.41	−0.47
5′	4.76	−0.33	−0.29
R^2-1	5.09	−0.09	−0.12
R^3-1	5.21	−0.09	−0.09
2′	5.33	−0.38	−0.38
A_{BZ}	5.47	−0.11	0.21
R^4-1	5.54	0.24	0.26
C_{BZ}	5.54	0.04	0.02
D_6	6.58	0.02	0.07
D_4	6.61	−0.02	−0.01
E_3	6.96	0	0.02
E_2	7.05	−0.02	0.02
$G_{3,5}$	7.08	0.44	0.54
$G_{2,6}$	7.44	0.36	0.41
C_6	7.78	−0.07	−0.08
A_6	7.76	0.17	0.46
C_2	7.62	0.30	0.31
E_6	7.19	−0.44	−0.68

[a] Positive value denotes upfield shift.

DISCUSSION

β-Avoparcin:Ac_2-L-Lys-D-Ala-D-Ala. Several intermolecular NOEs were observed between β-avoparcin and Ac_2-L-Lys-D-Ala-D-Ala (Table 4) which aided greatly in defining the structure of the complex. For the COOH-terminal D-Ala residue of the tripeptide, NOEs were observed between the ^{1}CH proton and the NCH_3 and $G_{2,6}$ proton of β-avoparcin. These results indicate that the COOH-terminal end of Ac_2-L-Lys-D-Ala-D-Ala is in close proximity to the NH_2 terminus of β-avoparcin. Additional NOEs were observed between the $^2CH_3$ group from the middle amino acid residue (D-Ala) and the E_6 and 5′ proton of β-avoparcin. At the NH_2-terminal end of the tripeptide, an NOE was observed between the acetyl methyl proton and the anomeric (R^2-1) and methyl (R^2-6) protons of the ristosamine sugar of β-avoparcin. These results indicate that the tripeptide spans the entire length of the antibiotic. The structure of the complex was further defined by an additional NOE between the aliphatic side chain protons (CH_2) of Lys[4] and D_6 proton of β-avoparcin. In Fig. 3A, the tripeptide is shown, with the *dotted lines* in the figure indicating those protons that are dipolar-coupled. On the basis of the NOE data, a model was constructed for the complex, which is shown schematically in Fig. 3B.

Analysis of ^{1}H chemical shifts of the bound antibiotic lends further support to our proposed structure of the complex. However, it should be pointed out that structural arguments based on an analysis of the chemical shift data are more tenuous, since it is difficult to separate the contributions to the proton chemical shift changes observed upon binding from conformational changes of the antibiotic or a direct effect of the bound peptide. The structure of the COOH terminus of β-avoparcin (Fig. 1) contains a biphenyl unit (D- and E-rings) that is contained within a macrocyclic ring and is further tied together by another linkage between the A- and B-rings. Because of this bicyclic structure, it might be expected that the COOH-terminal end of β-avoparcin

[4] The chemical shifts for the β and γ protons of the lysine CH_2 side chain of Ac_2-L-Lys-D-Ala-D-Ala were not resolved. Therefore, the resonance(s) at 1.55 ppm which experience an NOE with the D_6 proton of β-avoparcin and epi-β-avoparcin in the antiobiotic:peptide complexes could be either the β and/or γ protons of the aliphatic lysine side chain.

TABLE 3

Chemical shift assignments of free and bound Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala

Peak[a]	Ac-D-Ala-D-Ala δ	β-Avoparcin + Ac-D-Ala-D-Ala Δδ	Ac_2-L-Lys-D-Ala-D-Ala δ	β-Avoparcin + Ac_2-L-Lys-D-Ala-D-Ala Δδ
	ppm	*ppm*	*ppm*	*ppm*
^1CH	4.33	0.30	4.31	0.27
$^1CH_3$	1.41	0.77	1.41	0.75
^2CH	4.33	—[b]	4.38	−0.52
$^2CH_3$	1.38	0.35	1.39	0.25
$COCH_3$	2.04	0.09	2.05	−0.05
ε-$^3CH_2$			3.18	−0.04
$COCH_3$ (Lys side chain)			1.99	0.02

[a] The amino acid residues were numbered beginning at the COOH-terminal end of the peptides.
[b] Unassigned in the complex.

is relatively inflexible and would exhibit very few conformational changes upon binding peptides. Indeed, the same NOEs were observed between the E_6, 5′, 6′, and A_6 protons (Table 4) in free and bound β-avoparcin, indicating that the conformation at the COOH-terminal end of β-avoparcin is similar in the free and bound state. The large downfield shifts (Table 2) observed for the 6′, 5′, and E_6 protons of β-avoparcin upon binding the tripeptide must therefore be explained by the proximity of these protons to the lysine residue, specifically the deshielding cone of the lysine carbonyl group. This orientation of the carbonyl moiety would place the A_6 proton of β-avoparcin in its shielding cone, which is consistent with the large upfield shift (0.46 ppm) of A_6. The little change observed in resonance position for the E_2 and E_3 protons of β-avoparcin in the complex results from the location of these protons on the opposite side of the proposed binding site.

In contrast, the NH_2-terminal end of β-avoparcin is less motionally restricted than is the COOH terminus. This is supported by the averaging of the $G_{2,6}$ and $G_{3,5}$ protons which appear as doublets in the proton spectrum of free β-avoparcin. In the β-avoparcin:peptide complexes, the $G_{2,6}$ and $G_{3,5}$ proton resonances appear as two doublets of doublets. These data indicate that, when β-avoparcin binds the peptide, a particular NH_2-terminal conformation is favored and now the G-ring is no longer able to reorient fast enough to average the ^1H chemical shifts. Stabilization of the NH_2-terminal conformation may, in part, be due to an electrostatic interaction be-

TABLE 4

Results of NOE experiments (0.4-sec presaturation) performed with a 2:1 complex of Ac_2-L-Lys-D-Ala-D-Ala and β-avoparcin (4 mM) at 30°

Peak irradiated	Peak affected
^1CH	$^1CH_3$, NCH_3 $G_{2,6}$(s)[a]
$^1CH_3$	^1CH
$^2CH_3$	^2CH, E_6, 5′(s)
$^3COCH_3$[b]	R^2-a, R^2-6
$^3CH_2$ (β+/or γ)	D_6, other $^3CH_2$ resonances
NCH_3	1′, $G_{2,6}$(s), ^1CH
$G_{2,6}$	1′, NCH_3, ^1CH(s)
E_6	5′, 6′, A_6(s), $^2CH_3$
R^2-1	A_6, $^3COCH_3$[b]

[a] s = small NOE (<5%).
[b] Attached to peptide chain.

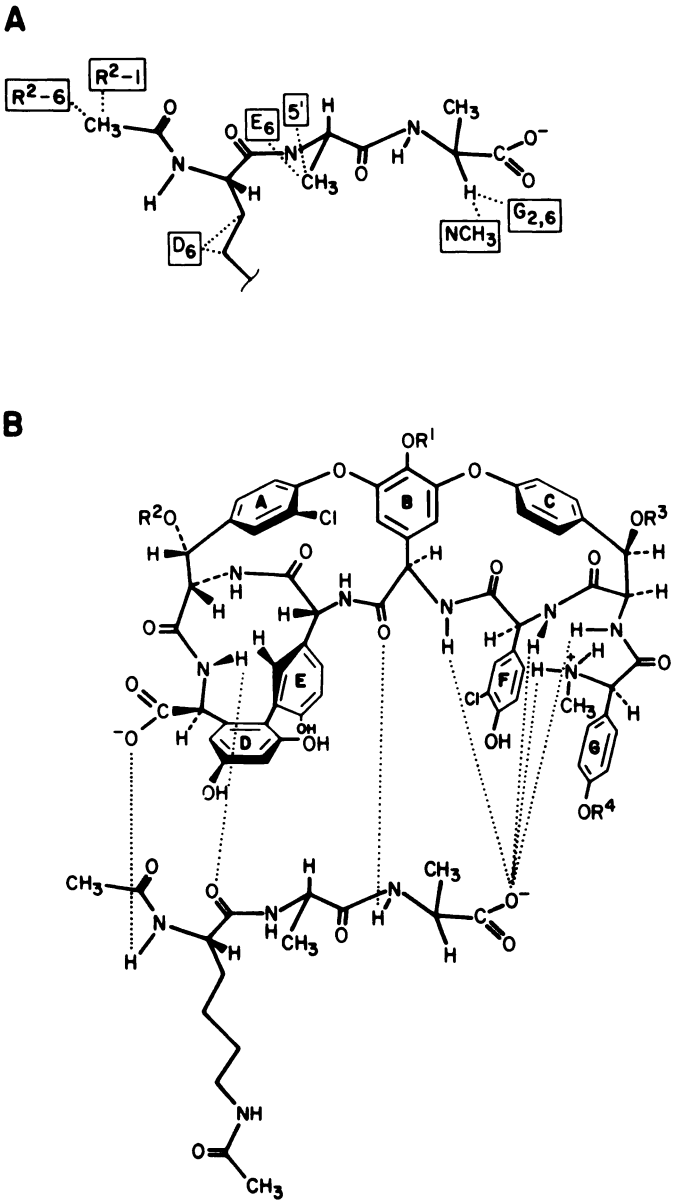

FIG. 3. *Intermolecular NOEs and proposed model of the complex*
A. Intermolecular NOEs (indicated by the *dotted lines*) between the protons of β-avoparcin and Ac_2-L-Lys-D-Ala-D-Ala. B. Proposed model of the structure of the β-avoparcin:Ac_2-L-Lys-D-Ala-D-Ala complex obtained from NOE and ^1H chemical shift data.

tween the protonated NH_2 terminus of the antibiotic and the carboxylate group of the peptide. This interaction is supported by an increase in pK_a (~1 pH unit) of the NCH_3 group observed in the β-avoparcin:Ac-D-Ala-D-Ala complex from that of the free antibiotic.

The ^1H chemical shifts of the resonances of Ac_2-L-Lys-D-Ala-D-Ala are also significantly different in the bound and free species (Table 2), as might be expected owing to the presence of the aromatic rings in the proposed binding site. For the methyl protons of the D-Ala residue at the COOH terminus of the tripeptide, the large upfield shift (0.75 ppm) observed upon binding may be explained by its proximity to the planar face of the B-ring of β-avoparcin, as has been proposed for other glycopeptide antibiotics. Furthermore, upfield and downfield shifts of the $^2CH_3$ and ^2CH protons, respectively, were observed, consistent with an orientation of the $^2CH_3$ protons in the shielding cone of the E-ring and the ^2CH proton of this residue in the deshielding cone of both the A- and E-rings.

β-Avoparcin:Ac-D-Ala-D-Ala. The complex formed between β-avoparcin and Ac-D-Ala-D-Ala was found to be structurally similar to the β-avoparcin:tripeptide complex. NOEs were observed between the NCH_3 protons of β-avoparcin and the ^1CH proton of the COOH terminus D-Ala residue and between the acetyl methyl protons of the dipeptide and the D_6 proton of β-avoparcin. Both of these NOEs are consistent with the location of the dipeptide at the same binding site on the antibiotic as the tripeptide. Further evidence in support of this hypothesis lies in the similarities in some of the chemical shifts (C_2, C_6, $G_{3,5}$, $G_{2,6}$, E_2, E_3) observed for bound β-avoparcin in these two complexes. The proton resonances (E_6, A_6, A_{BZ}) that differ in their chemical shifts in these two complexes are located at the proposed binding site for the lysine residue of the tripeptide. The differences in chemical shift can best be explained by a difference in the orientation of the lysine carbonyl moiety as compared with the acetyl carbonyl group of Ac-D-Ala-D-Ala. For the β-avoparcin:Ac-D-Ala-D-Ala complex, the dipeptide binds to a site on the antibiotic which is similar in location to that found for ristocetin and vancomycin as determined from NMR studies (5, 7) of these antibiotic:Ac-D-Ala-D-Ala complexes in Me_2SO and Me_2SO/CCl_4 (70/30). In these studies, a number of hydrogen bonds were postulated in these complexes which we have incorporated in our model owing to the similarities in the binding sites. These include a hydrogen bond between the amide NH proton of residues 2, 3, and 4 of β-avoparcin and the carboxyl group of the peptide and between the amide proton of the COOH-terminal D-Ala residue and the carbonyl group of residue 4 (B-ring) of β-avoparcin. For the tripeptide complex, our NOE experiments indicate that the acetyl methyl protons of the lysine residue must be in close proximity to the ristosamine sugar of β-avoparcin, and the β and/or γ CH_2 protons of the lysine side chain must be located near D_6. The tripeptide is located in such a position as to be stabilized by a hydrogen bond between the NH proton of the lysine residue and the carboxylic acid of β-avoparcin as well as through Van der Waals interactions between the aliphatic lysine side chain and the D-ring of β-avoparcin. These additional interactions in the antibiotic:tripeptide complex are supported by our UV binding data in which β-avoparcin was found to have a higher affinity for the tripeptide as compared with the dipeptide (Table 1).

β-Avoparcin, vancomycin, and ristocetin. β-Avoparcin is structurally similar to vancomycin (9, 10) and ristocetin (11, 12), with the major differences in structure appearing at the NH_2-terminal end of the antibiotics. For ristocetin, the G-ring (see Fig. 1) is covalently linked to the F-ring by a phenyl ether linkage. In addition, ristocetin possesses a methyl ester moiety and a primary amine instead of a carboxylic acid and secondary amine, which are present in β-avoparcin. Vancomycin, on the other hand, has an *N*-methyl leucine and an asparagine residue in positions 1 and 3, respectively. Other differences in structure include the number and site of attachment of C1 and sugar residues.

As mentioned previously, vancomycin and ristocetin peptide complexes have been studied in Me_2SO and in an Me_2SO/CCl_4 (70/30) solvent mixture (5, 7). For the ristocetin:Ac-D-Ala-D-Ala complex, the observation of intermolecular NOEs plays an important role in defining the structure of the complex. As in the ristocetin study, we observed an NOE between the $^2CH_3$ protons of the D-Ala residue and E_6 proton of β-avoparcin. However, the NOE that we observed between the acetyl methyl protons of the dipeptide and D_6 proton of β-avoparcin was not obsereved in the ristocetin:Ac-D-Ala-D-Ala complex studied in Me_2SO (5). One would expect that, in aqueous solution, intermolecular hydrophobic interactions would be favored. Thus, in ^2H_2O, a Van der Waals interaction between the acetyl methyl protons of the dipeptide and the aromatic D-ring may act to stabilize the complex. In contrast, in nonaqueous solvents, these same interactions would no longer be favored. One of the reasons for performing NMR experiments in Me_2SO is that the amide protons do not undergo rapid exchange with the solvent and therefore may be observed directly. However, exchangeable protons can also be observed in the NMR spectra recorded in aqueous solution providing that the water solvent peak is suppressed and the exchange rate is slow enough on the NMR timescale. In the ^1H NMR spectrum of the β-avoparcin:peptide complexes obtained in water using Redfield solvent suppression techniques (8), four to six amide signals were observed, of which four were well resolved (9.21, 9.26, 9.87, and 11.60 ppm) in the dipeptide complex (30°), and five or six resonances (8.96, 9.15, 9.37 9.58, and 12.00 ppm) in the tripeptide complex (50°). Note the correspondence between the number of slowly exchanging amide resonances observed in the ^1H spectra and the number of hydrogen bonds postulated in the structures for the vancomycin and ristocetin dipeptide complexes (5, 7).

β-Avoparcin and epi-β-avoparcin. The association constants (K_a) for the binding of β-avoparcin and epi-β-avoparcin to Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala were measured by UV methods. β-avoparcin exhibited appreciable binding to Ac-D-Ala-D-Ala ($K_a = 1.8 \times 10^5$ M^{-1}), whereas the association constant for the epi-β-avoparcin:dipeptide interaction was too small ($<10^3$ M^{-1}) to be measured by this method. This result can be explained by the different NH_2-terminal conformations of the two compounds, which affect their ability to bind the

peptide and are consistent with the model proposed for the structure of the antibiotic:dipeptide complex in which the antibiotic binding site of Ac-D-Ala-D-Ala is mainly composed of the NH pocket at the NH_2 terminus. The importance of these interactions at the NH_2 terminus is partially overcome in the complex with the tripeptide, where several additional interactions are possible. In agreement with this hypothesis is the larger association constant measured for the epi-β-avoparcin:tripeptide complex.

As in our NMR studies of β-avoparcin:Ac_2-L-Lys-D-Ala-D-Ala, intermolecular NOEs were observed between the CH_2 protons of Lys and the D_6 proton of epi-β-avoparcin and between the R^2-6 methyl protons of ristosamine and the acetyl methyl protons of the tripeptide in the epi-β-avoparcin:Ac_2-L-Lys-D-Ala-D-Ala complex. However, no intermolecular NOEs could be detected at the NH_2 terminus of epi-β-avoparcin. Furthermore, a large chemical shift change was observed for the NCH_3 protons of epi-β-avoparcin upon binding the tripeptide that was not observed in the β-avoparcin:tripeptide complex. These results suggest that the binding site for the tripeptide at the COOH-terminal end of epi-β-avoparcin and β-avoparcin is the same, and the differences in the binding site occur at the NH_2-terminal end of the antibiotics; this suggestion agrees with the differences in NH_2-terminal conformation found for the two antibiotics.

CONCLUSIONS

On the basis of our NOE and chemical shift data, a model was proposed for the structure of the β-avoparcin:peptide complexes. For the β-avoparcin:Ac_2-L-Lys-D-Ala-D-Ala complex, the COOH-terminal end of the tripeptide is located near the NH_2 terminus of the antibiotic, with the tripeptide extending across the peptide backbone of β-avoparcin toward its COOH-terminal end. In our proposed structure, the three amino acid residues of the peptide span the entire length of the antibiotic, and the aliphatic side chain of the lysine residue extends over the D-ring of β-avoparcin.

Epi-β-avoparcin, which was shown in the preceding paper to have a different conformation at the NH_2 terminus as compared with β-avoparcin, exhibited no measurable association to Ac-D-Ala-D-Ala but had a significant association with the tripeptide, suggesting the importance of the interactions provided by the lysine residue in stabilizing the COOH-terminal end of the antibiotics in the complex. Although the structures of the antibiotics of this class may differ significantly at the NH_2 terminus, the structural and conformational features at the COOH-terminal end for the avoparcin antibiotics, as well as for other members of this class, are very similar (1, 2, 9, 11) and appear to be important for their antibacterial activity.

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