Use of model cell membranes to demonstrate templated binding of vancomycin group antibiotics

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In this paper we demonstrate the importance of binding geometry and dimerisation at the surface of model cell membranes in the mode of action of the clinically important glycopeptide antibiotics. This has been achieved through the use of model cell membranes (micelles and vesicles) to which cell wall analogues are anchored *via* a hydrophobic decanoyl chain. A number of –D-Ala-terminating cell wall analogues, ranging from two to six residues in length, have been used. Dipeptide, pentapeptide and hexapeptide display enhanced binding to the antibiotic at the model cell surface, but tripeptide and tetrapeptide do not. The possible implications of the observed binding geometries for bacterial systems are discussed.

Introduction

The vancomycin group of antibiotics kill Gram-positive bacteria by binding to cell wall precursors terminating in –Lys-D-Ala-D-Ala,[1] preventing peptidoglycan polymerisation and subsequent cross-linking, and in doing so weakening the cell wall, ultimately causing cell lysis.[2] Two members of the family, vancomycin and teicoplanin, are clinically important in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) and are currently the last line of defence against such infections.

The binding of the vancomycin group of antibiotics to model cell wall precursor peptides such as di-*N*-acetyl-Lys-D-Ala-D-Ala (Ac-tri-Ala‡) in free solution has been studied extensively and these studies provide valuable insights into their mode of action (Fig. 1).[3–5] We have also shown that almost all glycopeptide antibiotics dimerise, and that dimerisation of the antibiotic is, in all but one case, cooperative with ligand binding, *i.e.* the antibiotic dimerisation constant is higher in the presence of ligand than when free, and the ligand binds with a higher affinity to antibiotic dimer than to antibiotic monomer.[6] We therefore hypothesised that dimerisation might play an important role in the mode of action of these antibiotics, in that the second binding event between a dimer and the surface of a growing bacterium would be effectively intramolecular, thus allowing a chelate-like enhancement of binding.[7] We have subsequently provided evidence for this hypothesis through the use of *in vitro* bacterial assays, which reveal a correlation between dimerisation constant and the ability of the antibiotic to kill bacteria in the presence of competing peptides.[8]

To further demonstrate the importance of dimerisation in the mode of action of glycopeptide antibiotics, we have devised model membrane systems designed to mimic the growing bacterial cell wall, thus allowing for the expression of any binding enhancement due to dimerisation at a surface. In these models, the bacterial cell membrane is represented by sodium dodecyl sulfate (SDS) micelles or phosphatidylcholine (PC) vesicles and the cell wall precursors by cell wall peptide analogues with an *N*-terminal decanoyl chain, designed to insert into the model membranes. This *N*-terminal membrane anchor is similar to the C_{11} chain present in the antibiotic teicoplanin, which we have previously shown to associate with model membranes.[9] The whole arrangement of micelle/vesicle and anchored cell wall analogue is therefore similar to that at the bacterial cell surface, where cell wall precursors are anchored to the cell membrane *via* a C_{55} hydrocarbon chain.[10]

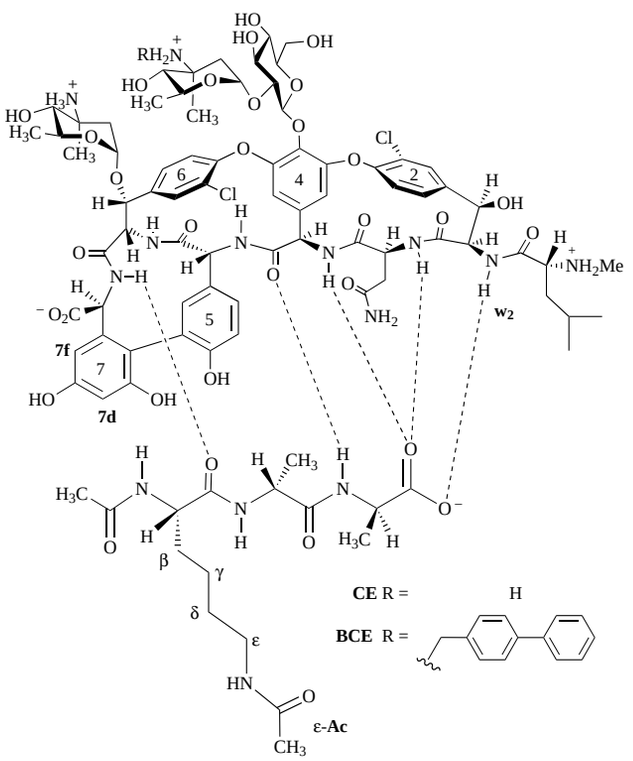

Fig. 1 Exploded view of the complex formed between chloroeremomycin (CE) or biphenylchloroeremomycin (BCE) (the two members of the vancomycin group of antibiotics studied in this paper) and di-*N*-acetyl-Lys-D-Ala-D-Ala. Protons referred to in the text are labelled. Aromatic rings are numbered according to residue number.

Using such a system we have demonstrated that, for a dimer–ligand complex, dimerisation and membrane anchoring result in enhanced binding affinity.[11] This was achieved by comparing the binding constants of ristocetin A (another member of the vancomycin group of antibiotics) to the cell wall analogues *N*-α-decanoyl-D-Ala-D-Ala (dec-di-Ala) and *N*-α-acetyl-D-Ala-D-

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‡ All peptides used in this study are abbreviated in this style in order to indicate the nature of their α-acyl chain (acetyl = Ac, decanoyl = dec, docosanoyl = docos), the number of residues (di, tri, tetra, penta or hexa, based on the sequence Gly-L-Ala-D-γ-Glu-Lys-D-Ala-D-Ala, counting from the *C*-terminus) and the identity of the *C*-terminal residue (–D-Alanine = Ala or –D-Lactate = Lac).

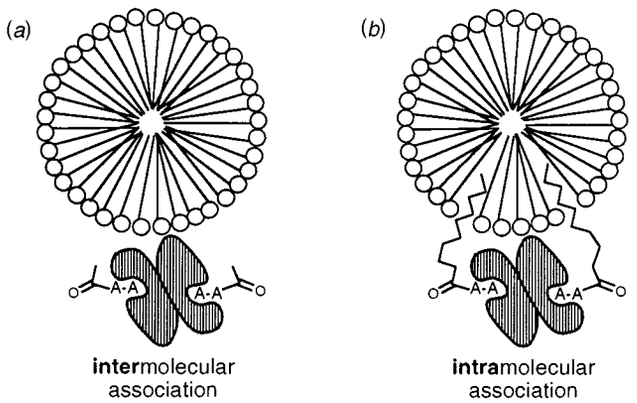

Fig. 2 Schematic illustration of a glycopeptide dimer binding cell wall analogues at the surface of a micelle. (*a*) The complex formed with Ac-di-Ala is intermolecular, whereas (*b*) that with dec-di-Ala is essentially intramolecular.

Ala (Ac-di-Ala) in the presence of SDS micelles. The complex formed between the antibiotic and Ac-di-Ala results from an intermolecular association [Fig. 2(*a*)], whereas the complex formed on the surface of a micelle with dec-di-Ala is essentially intramolecular [Fig. 2(*b*)]. However, inspection of Corey–Pauling–Kulton (CPK) models reveals that this peptide might be rather short and therefore limited in its ability to anchor to the model cell membrane when bound to the dimeric antibiotic. We have therefore decided to investigate the behaviour of a number of longer anchored peptides (ranging from two to six residues) in their complexes with antibiotic dimers in the model system, predicting increased anchoring from the longer peptides, which should act as better models of the natural peptides. The sequence followed for the peptides was that of the natural cell wall precursor, namely –L-Ala-D-γ-Glu-Lys-D-Ala-D-Ala, with an *N*-terminal glycine added as a spacer in the hexapeptide ligand (Fig. 3).

In this paper, we have therefore attempted to investigate the effect of the length of the peptide portions of –D-Ala-terminating cell wall precursor analogues on the enhancement of binding observed with glycopeptides at model cell surfaces. The influence that peptide length has on the geometry of binding to the antibiotic is also considered.

Results and discussion

Binding on a micelle surface

Initially, the binding of the ligands was assayed by a simple NMR method which involved measurement of the limiting chemical shift of the amide residue 2-proton (w_2) of the antibiotic (labelled in Fig. 1), under conditions where the antibiotic was >95% bound ($\delta^{lim}_{w_2}$). In the complex between antibiotic and ligand, w_2 is involved in a crucial hydrogen bond to the ligand carboxylate, such that its chemical shift moves dramatically downfield upon binding. We have previously observed a strong correlation between $\delta^{lim}_{w_2}$ and the free energy of ligand binding.[11–14] It is therefore possible to use $\delta^{lim}_{w_2}$ as a qualitative measure of binding affinity.

The glycopeptide antibiotic chloroeremomycin (CE, LY264826, Fig. 1) was chosen for these studies as it binds ligand strongly and has a high dimerisation constant.[6] It was therefore anticipated that it might exhibit a high degree of cooperativity when binding at a surface. The results obtained for $\delta^{lim}_{w_2}$ with this antibiotic upon binding to the decanoyl ligands in the presence of SDS micelles are shown in Table 1. It should be noted that additional *N*-terminal residues beyond lysine add a negligible amount to the intrinsic binding energy, but removal of the lysine *does* significantly reduce this intrinsic binding energy.[15]

The $\delta^{lim}_{w_2}$ for CE bound to Ac-tri-Ala in the presence of SDS micelles was observed to be 11.11 ppm. The value when bound to Ac-di-Ala in the presence of SDS was not observed due to exchange-broadening of the spectrum but is expected to be lower than 11.11 ppm as Ac-di-Ala binds more weakly to CE than Ac-tri-Ala. However, for CE bound to dec-di-Ala in the presence of SDS, $\delta^{lim}_{w_2}$ is 11.50 ppm—much further downfield than $\delta^{lim}_{w_2}$ when bound even to the longer peptide Ac-tri-Ala. This significant increase in $\delta^{lim}_{w_2}$ when bound to the decanoyl ligand is consistent with observations made previously with the antibiotic ristocetin A, *i.e.* an enhancement in binding affinity for the intramolecular system. However, the similarity of $\delta^{lim}_{w_2}$ for CE bound to *N*-α-decanoyl-*N*-ε-acetyl-Lys-D-Ala-D-Ala (dec-tri-Ala) and *N*-α-decanoyl-D-γ-Glu-*N*-ε-acetyl-Lys-D-Ala-D-Ala (dec-tetra-Ala) in the presence of SDS to that when bound to Ac-tri-Ala suggested a negligible enhancement of binding in these systems (11.13 and 11.15 ppm for dec-tri-Ala and dec-tetra-Ala, respectively, *vs.* 11.11 ppm for Ac-tri-Ala). The shifts when bound to *N*-α-decanoyl-L-Ala-D-γ-Glu-*N*-ε-acetyl-Lys-D-Ala-D-Ala (dec-penta-Ala) and *N*-α-decanoyl-Gly-L-Ala-D-γ-Glu-*N*-ε-acetyl-Lys-D-Ala-D-Ala (dec-hexa-Ala) (11.18 and 11.25 ppm, respectively), suggest that as the peptides become longer, there is some enhancement to the binding of the anchored ligand relative to the binding of the unanchored Ac-tri-Ala but substantially less enhancement than for binding to dec-di-Ala. The physical reasons for this enhancement to the binding of the anchored dipeptide ligand but not to the binding of anchored tripeptide are discussed later in this paper.

Table 1 Limiting chemical shifts of the residue 2 amide proton (w_2), $\delta^{lim}_{w_2}$, of CE with various decanoyl ligands in the presence of SDS at pH 4.5

Ligand	$\delta^{lim}_{w_2}$
dec-di-Ala	11.50
dec-tri-Ala	11.13
dec-tetra-Ala	11.15
dec-penta-Ala	11.18
dec-hexa-Ala	11.25

Binding on a vesicle surface

To further investigate these complexes, we used a model system in which the SDS micelles were replaced by PC vesicles, which are of a closer size, surface charge and curvature to the natural cell membrane. These features make them a better mimic of the bacterial membrane surface, which is composed of similar lipids. However, they are unsuitable for the w_2 binding assay described above since their large size results in a very long correlation time and consequently very broad NMR resonances. An antibiotic associated with the vesicle surface *via* binding to anchored ligands thus takes on the tumbling properties of the vesicle itself, with the effect that its resonances also appear as broad lines. Whilst this renders any high-resolution structural analysis almost impossible, we used this broadening to determine whether a complex was indeed anchored to the surface of the vesicle.

The results of these experiments are shown in Fig. 4. This shows the aromatic region of the ^{1}H NMR spectra of the complexes of CE with various length peptides in the presence of PC vesicles. For the complexes of CE with dec-di-Ala and dec-hexa-Ala, no signals were observed. This was interpreted as being due to tight binding of CE to the peptides on the surface of the vesicle resulting in fully broadened NMR resonances, *i.e.* all of the antibiotic was associated, *via* the bound peptide, to the surface of the vesicle. In these cases, the broadening was probably exacerbated by some aggregation of the vesicles; the sample became visibly turbid. The complex with dec-penta-Ala also shows some degree of broadening, but those with dec-tri-Ala and dec-tetra-Ala both result in well-resolved spectra. In these three cases (dec-tri-, -tetra- and -penta-Ala), the ligand

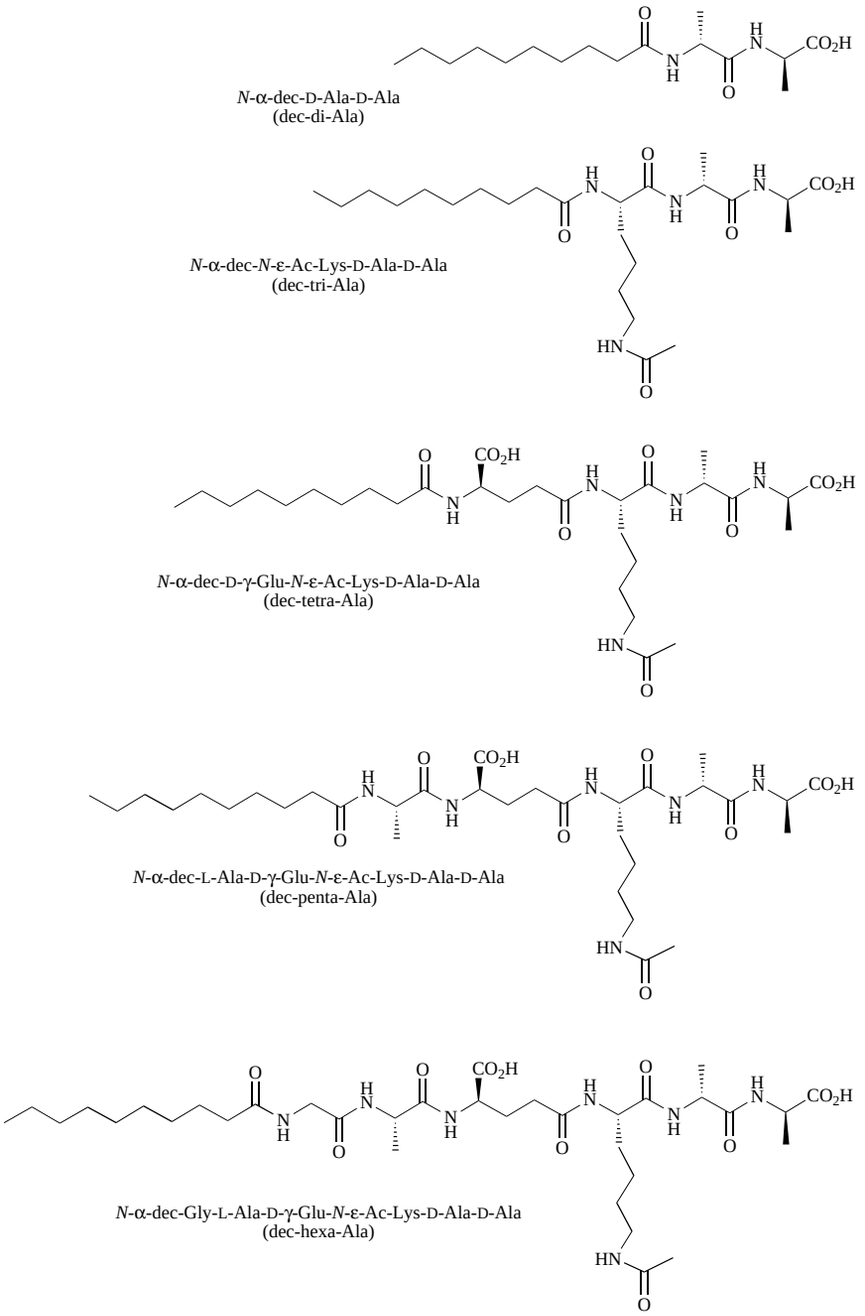

Fig. 3 Structures of the Ala-terminating decanoyl peptides used in these studies

was confirmed to be bound to the antibiotic by the downfield shift of the proton w_2, indicative of ligand binding. However, the lack of broadening of the resonances for the complexes with dec-tri-Ala and dec-tetra-Ala suggested that even though the ligand was bound to the antibiotic it was not simultaneously associated with the vesicles. In the case of dec-penta-Ala the partial broadening observed indicates that the antibiotic–ligand complexes are in slow exchange on the NMR timescale between being vesicle-associated and free in solution.

Competition experiments

We have also used a competition strategy, illustrated schematically in Fig. 5, to estimate a binding constant of dec-hexa-Ala to CE on the surface of vesicles. In these experiments, a non-anchored cell wall analogue, Ac-tri-Ala (1.0 mM), was added to a solution of CE (0.5 mM) bound to dec-hexa-Ala (1.0 mM) on the surface of vesicles. The results of this experiment, and a control experiment in the absence of dec-hexa-Ala, showed (Fig. 6) that even in the presence of an excess of the antagonist, most of the antibiotic appears to remain in an anchored complex on the surface of the vesicle. Based on the relative peak integrals in the presence and absence of dec-hexa-Ala [Figs. 6(*d*) and (*b*)] and concentrations of added Ac-tri-Ala in these two solutions, a binding enhancement of approximately one hundred-fold is estimated for the anchored hexapeptide assembly over the non-anchored Ac-tri-Ala/CE complex. This is similar to the four hundred-fold binding enhancement due to templating measured for ristocetin A binding to dec-di-Ala in the presence of SDS micelles.[11]

Therefore, the above observations can be rationalised by the hypothesis that dec-tri-Ala and dec-tetra-Ala, when bound to the antibiotic, are simply not long enough for the alkyl chain to insert into the model membrane [Fig. 7(*a*)], whereas dec-penta-Ala and, more effectively, dec-hexa-Ala are of sufficient length [Fig. 7(*b*)]. If this is the case, the seemingly anomalous cooperativity exhibited by the dec-di-Ala/CE complex when anchoring to micelles or vesicles might arise as a result of some alternative binding geometry.

It was anticipated that when binding *in vivo* the antibiotics would normally interact with cell wall peptides at the bacterial

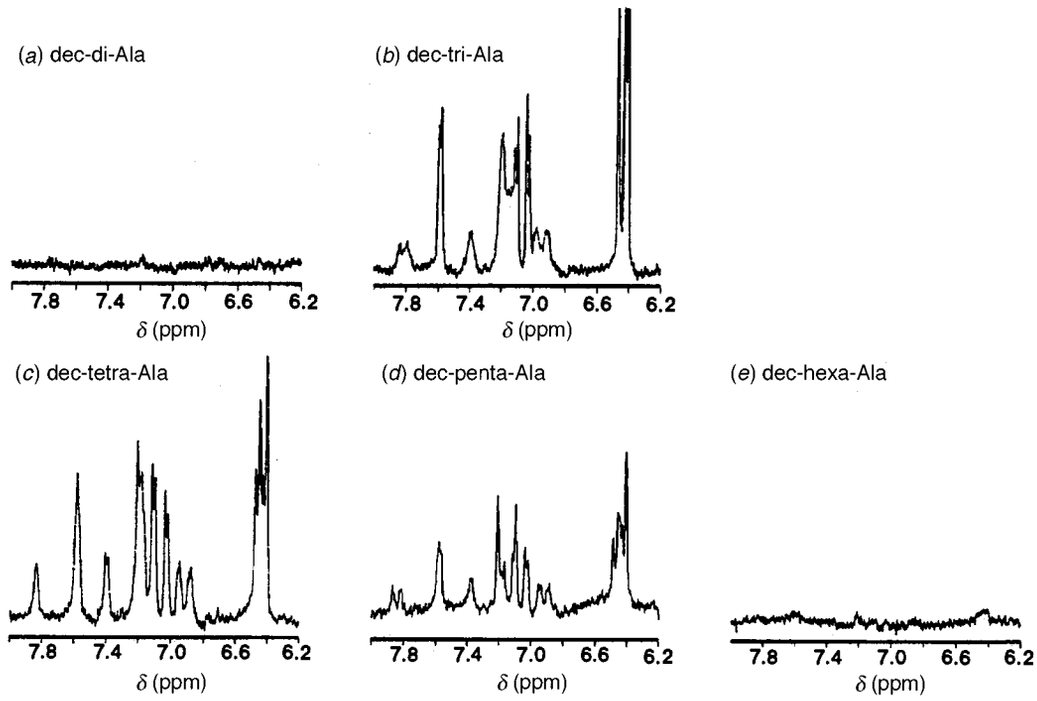

Fig. 4 Aromatic region of the 500 MHz ^{1}H NMR spectra (D_2O, pD 6.2) of CE (0.5 mM) with the various decanoyl peptides (1 mM) in the presence of vesicles (10 mM). In the case of dec-di-Ala and dec-hexa-Ala the signals from the complex are broadened due to anchoring to the vesicle.

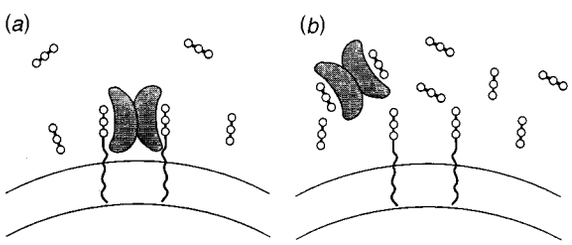

Fig. 5 Schematic illustration of the competition experiment in which antibiotic dimer bound to anchored ligands is displaced from the surface of a vesicle

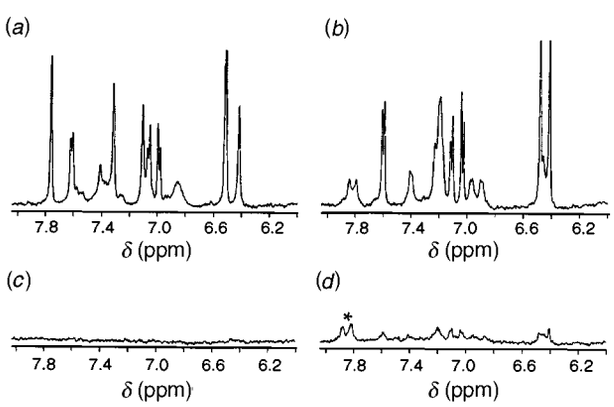

Fig. 6 Aromatic region of the 500 MHz ^{1}H NMR spectra (D_2O, pD 6.2) of (*a*) CE (0.5 mM) in the presence of vesicles (10 mM) and (*b*) following the addition of Ac-tri-Ala (1 mM). (*c*) The same region of the spectrum of CE (0.5 mM) in the presence of vesicles (10 mM) and dec-hexa-Ala (1 mM) and (*d*) following the addition of Ac-tri-Ala (4 mM) (* these resonances result from residual amide protons of the added ligand).

cell surface with their ring-4 sugars [the disaccharide glucose:4-*epi*-vancosamine in CE (Fig. 1)] in contact, or near to, the cell surface. This supposition was based on the fact that teicoplanin[9,16] and the semi-synthetic glycopeptide biphenylchloroeremomycin (BCE, LY307599, Fig. 1)[17] have putative membrane anchors attached to their ring-4 sugars. Additionally, the

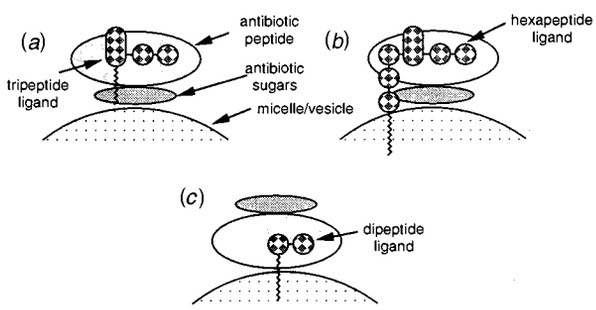

Fig. 7 Schematic representation of the hypothesis which explains lack of templating for dec-tri-Ala and dec-tetra-Ala in the presence of vesicles. (*a*) Dec-tri-Ala is not long enough to reach the surface of the model membrane when bound to antibiotic, whereas (*b*) dec-hexa-Ala is able to both anchor and bind antibiotic. (*c*) Dec-di-Ala can both anchor to the model membrane and bind antibiotic but only when oriented 'upside down' (a geometry not accessible to longer peptides because of the presence of the lysine side chain).

natural precursor peptide analogue penta-Ala has been shown to bind CE with its *N*-terminal L-alanine residue adjacent to the ring-4 sugars.[18] Inspection of CPK models suggested that if the decanoyl chain of dec-di-Ala were to pass over ring-7, rather than adjacent to ring-6 (which is the orientation necessary if the ring-4 sugars are to interact with the micelle surface), there would be more of the hydrocarbon chain available to insert into the micelle. We therefore hypothesised that in binding to the anchored dec-di-Ala ligand, CE might be oriented 'upside down', *i.e.* with the sugars projecting away from the micelle [Fig. 7(*c*)], thus allowing the decanoyl chain to pass over ring-7 of the antibiotic and insert into the membrane. Such a conformation is not available to longer peptides because the lysine side chain occupies this position over ring-7.[5,18–21] With the lysine side-chain positioned over ring-7, the L-stereochemistry of the lysine forces the additional residues of the longer peptides, and indeed the alkyl chain, into an orientation in which they are directed toward the ring-4 sugars. In the case of binding by dec-tri-Ala and dec-tetra-Ala, the alkyl chain is then not long enough to be able to insert into the membrane [Fig. 7(*a*)]; it is only for dec-penta-Ala and dec-hexa-Ala that this is possible [Fig. 7(*b*)], as observed experimentally.

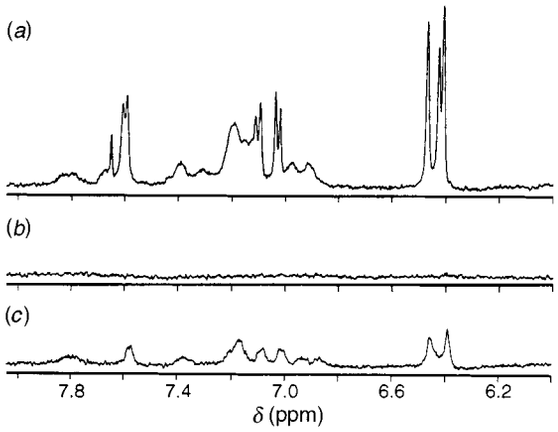

Fig. 8 Aromatic region of the ^{1}H NMR spectra (D_2O, pD 6.2) of (*a*) CE (0.5 mM) in the presence of vesicles (10 mM), (*b*) following the addition of docos-tri-Ala (1 mM) [*cf.* Fig. 6(*b*)] and (*c*) after the addition of Ac-tri-Ala (2 mM)

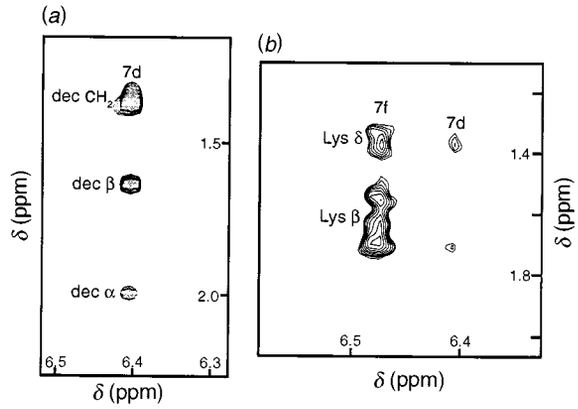

Fig. 9 Portions of the NOESY spectra of the complexes formed, in the presence of micelles, between CE and (*a*) dec-di-Ala and (*b*) dec-tri-Ala, illustrating the cross peaks from ring-7 of the antibiotic to ligand protons. In (*a*), 'dec CH_2' is used to signify those methylene groups of the decanoyl chain which have unresolved chemical shifts.

To test the validity of this hypothesis, experiments were performed with two new ligands, *N*-α-decanoyl-Gly-D-Ala-D-Ala and *N*-α-docosanoyl-*N*-ε-acetyl-Lys-D-Ala-D-Ala (docos-tri-Ala, docosanoyl = C_{22}) binding to CE in the presence of vesicles. It was anticipated that for the former ligand, the absence of the lysine side chain would allow it to take up a conformation in the binding pocket similar to that of dec-di-Ala, with the decanoyl chain passing over ring-7, so that binding to the antibiotic on the vesicle surface would be facilitated. For docos-tri-Ala it was predicted that the binding geometry would be the same as for dec-tri-Ala but that the much longer hydrocarbon chain would now be able to reach the membrane, again allowing binding of the antibiotic to the vesicle surface. The results were exactly as anticipated, with the complexes of CE bound to each ligand in the presence of vesicles resulting in substantially broadened antibiotic ^{1}H NMR signals. Also, the addition of Ac-tri-Ala did not result in the antibiotic becoming displaced from its complexes with the anchored ligands on the surface of vesicles (Fig. 8 shows the aromatic region of the spectra obtained with docos-tri-Ala).

One possible alternative explanation for the difference in binding to dec-di-Ala, dec-penta-Ala and dec-hexa-Ala compared to dec-tri-Ala and dec-tetra-Ala could lie in the relative abilities of the peptides to form self-micelles or self-vesicles. This self-association could thus be responsible for the observed ^{1}H NMR line broadening and enhancement of binding of the first three named peptides to CE, instead of the templated binding with antibiotics on PC vesicle surfaces, as described above. However, ^{1}H NMR spectra of these ligands in solution with vesicles, at the same concentration to that used in the binding experiments, show that their signals all broaden to a similar degree (although, predictably, the *C*-terminal signals of the longer peptides are sharper than their *N*-terminal signals), indicating that all of the ligands associate with the PC vesicles to a similar extent. Additionally, given the relatively low concentration of ligands used in these experiments (typically twice that of antibiotic), aggregates composed entirely of such ligands would possess only sufficient surface area to bind a small fraction of the antibiotic present and therefore could not lead to the complete broadening of signals observed particularly for dec-di-Ala and dec-hexa-Ala.

Confirmation of ligand orientation

To further support the hypothesis outlined in the previous section, two-dimensional NOESY spectra were acquired in order to identify the orientation of the decanoylated peptides with respect to the antibiotic in the presence of SDS. For the complex of CE with dec-di-Ala, where the decanoyl chain was anticipated to pass over ring-7 in the 'upside down' arrangement, NOESY cross peaks were observed between the aromatic protons of ring-7 of the antibiotic and the methylene protons of the decanoyl chain [Fig. 9(*a*)]. From this data, we conclude only that the decanoyl chain of dec-di-Ala must lie over ring-7. A more precise interpretation of the data does not seem to be warranted because of (i) the anticipated dynamic behaviour of this portion of the ligand, and (ii) spin diffusion. Thus, the decanoyl chain projects from the antibiotic toward the micelle (or vesicle) in an orientation which places the ring-4 sugars away from the surface of the model membrane. For the complex with dec-tri-Ala, NOESY cross peaks were observed from the lysine side chain to ring-7 [Fig. 9(*b*)] and the methylene groups of the decanoyl chain gave cross peaks to residue 6 of the antibiotic. This positions the decanoyl chain such that it could anchor the complex to a membrane in the case of either the longer peptides (dec-penta-Ala and dec-hexa-Ala) or the tripeptide with a longer acyl chain (docos-tri-Ala), while simultaneously positioning the ring-4 sugars adjacent to the membrane surface. Thus, the orientation of the ligands that was predicted by the results of the $\delta_{w_2}^{lim}$ measurements and vesicle binding experiments was shown to exist by two-dimensional NMR spectroscopy. Three-dimensional representations of the complexes formed between CE and dec-di-Ala and dec-tri-Ala are illustrated in Fig. 10(*a*) and 10(*b*), respectively.

Conclusions

We have employed two model membrane systems in an attempt to establish the optimum conditions for the expression of cooperativity due to binding of ligands to glycopeptides on a surface. Each system has its advantages and disadvantages. SDS is available in deuterated form and the micelles it forms are small in diameter. These features enable complexes formed on the surface of micelles to be studied by high-resolution ^{1}H NMR spectroscopy, providing a wealth of structural information. However, the small diameter of the SDS micelles (approximately 25–30 Å) results in a high degree of curvature at the surface, leading perhaps to a non-ideal binding geometry with certain ligands. PC vesicles are much larger in diameter (1000–10 000 Å), and are thus expected to overcome any problems related to surface curvature. The size of PC vesicles precluded a detailed study of vesicle-bound complexes by NMR spectroscopy, but allowed a qualitative determination of the extent to which templated binding was achieved for CE binding to a series of decanoylated ligands.

We have thus been able to demonstrate in a direct manner the importance of binding geometry and dimerisation in the mode of action of these antibiotics. We believe that the origin of this enhanced binding lies in the chelate-like enhancement conveyed

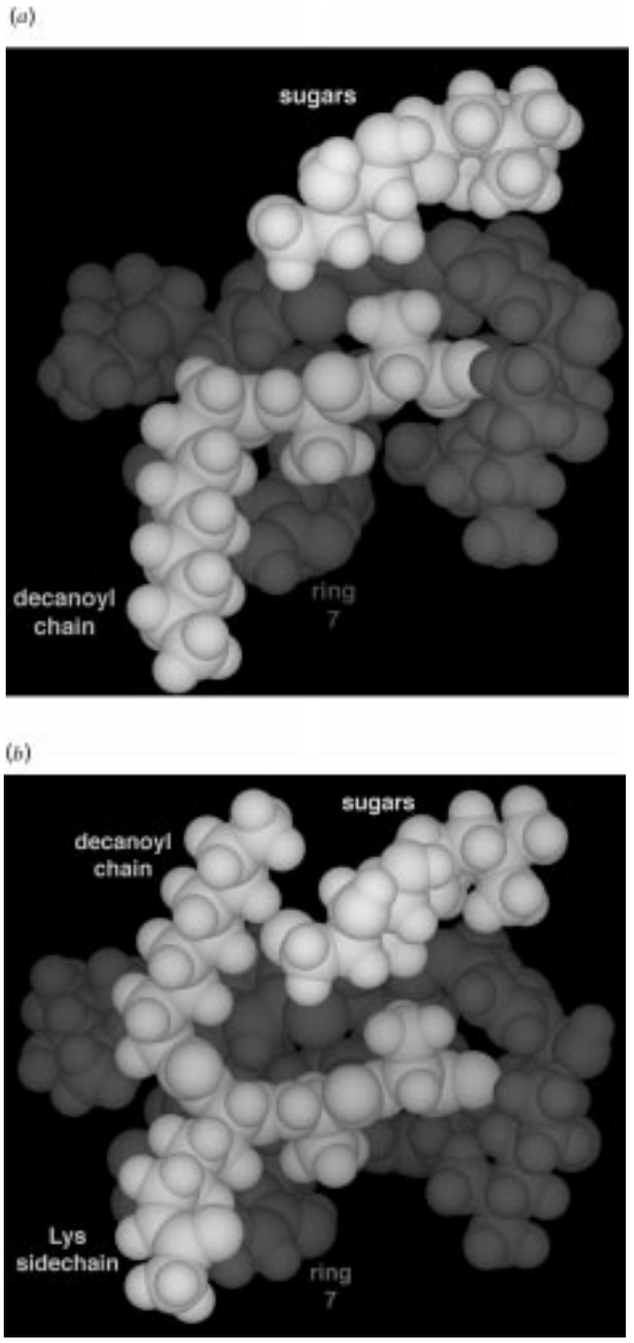

Fig. 10 Three dimensional representations of half of the dimeric complexes formed between CE and (*a*) dec-di-Ala and (*b*) dec-tri-Ala. Note how the orientation of the decanoyl chain attached to the dipeptide results in more exposure of hydrocarbon than the chain attached to the tripeptide.

by the ability of these antibiotics to dimerise at the cell surface, resulting in a tightening of all interactions within the complex thus giving rise to enthalpic as well as entropic gains.[22]

The greatest degree of templated binding was achieved with the longest and also, paradoxically, the shortest of the anchored ligands studied. We have shown, semi-quantitatively, that the enhancement to binding to CE due to templating for dec-hexa-Ala is similar to that for dec-di-Ala, measured previously.[11] We have put forward a physical model, with accompanying evidence, which accounts for this anomaly.

These results provide a rationale as to why membrane anchors on naturally occurring glycopeptide antibiotics are located on the ring-4 sugars,[16] and why membrane anchors on the most active semi-synthetic antibiotics are similarly located.[17] The L-stereochemistry of the lysine residue present in cell wall peptides directs the antibiotic to bind in a fashion that places the residue-4 sugars in close proximity to the bacterial cell membrane. Nature takes advantage of this by placing a locating device (in the case of teicoplanin, a C_{11} acyl chain) at precisely this point. Despite this rationale, we do not preclude the possibility of enhancement of antibiotic action through the location of hydrophobic chains at alternative sites.

The model systems studied in this work thus present a more detailed picture of how the vancomycin group antibiotics function in biological systems. The results support the hypothesis that these antibiotics bind to nascent bacterial cell walls with their ring-4 saccharides adjacent to the cell membrane, and the parallel nature of these saccharides in the antibiotic dimers may reflect a similar parallel arrangement of the peptidoglycan strands of growing cell wall.

Experimental §

Preparation of phosphatidylcholine vesicles

Type XV1-E L-α-phosphatidylcholine from fresh egg yolk (Sigma, 80 mg) was dissolved in chloroform (2 ml) which had been rendered ethanol-free by passage through a column of activated alumina. The solution was then evaporated under reduced pressure to yield a thin film on the wall of the flask. The flask was flushed with nitrogen followed by addition of D_2O (5 ml) or 50 mM NaH_2PO_4, pH 6.2 buffer (5 ml). The mixture was shaken for 20 min, then sonicated for 90 min to yield a slightly turbid suspension of vesicles (20 mM phosphatidylcholine).

^{1}H NMR spectroscopy

Sodium [$^2H_{25}$]dodecyl sulfate (SDS; 98 atom%D) was purchased from Euriso-top. All ^{1}H NMR spectroscopy experiments were performed on 500 MHz Bruker DRX-500 and AM500 spectrometers at 300 K. Suppression of the solvent resonance was achieved using WATERGATE[23] or pre-saturation. One-dimensional spectra were recorded using 32k complex data points. In two-dimensional experiments, 4k complex points were acquired in f_2, with 512 increments in f_1. TPPI was used to achieve quadrature detection in the indirect dimension. Data was processed with XWIN-NMR software, using a sine-squared window function and zero-filling in f_1 up to 1k or 2k points. Two-dimensional NOESY experiments employed mixing times ranging through 50–150 ms, and were used to confirm all w_2 assignments. In experiments involving micelles or vesicles, the ligand was added to the vesicle/micelle solution and the mixture was sonicated to facilitate insertion. Experiments involving SDS employed a concentration of 70 mM SDS (above the SDS critical micelle concentration), 5 mM antibiotic and 10 or 20 mM ligand. These concentrations ensured that a high (>90%) proportion of antibiotic was bound by ligand but also that the SDS was not 'overloaded' with ligand. Only when dissolution was complete was the antibiotic added. In the vesicle experiments, vesicles were prepared as described above and used as a 10 mM solution; concentrations of antibiotics used were as described in the individual figure legends. In the competition experiments, the unanchored ligand was added to the NMR tubes as a concentrated solution (50 mM), so as not to change the concentration of the contents of the tube significantly, and to allow accurate concentrations to be achieved on the addition of the appropriate volume.

Acknowledgements

Eli Lilly and Co. are thanked for generously providing samples of CE (LY264826) and BCE (LY307599). The EPSRC (G. J. S.),

§ Details of the synthesis of the peptides are available as supplementary material (SUP 57275; 33 pp.) deposited with the British Library. Details are available from the editorial office.

Xenova (A. C. T.), The Wellcome Trust (R. J. D.), the EPSRC and Roussel (B. B.) and the BBSRC and Roussel (R. M. H. E.) are thanked for financial support. We also thank the Biomedical NMR Centre, NIMR, Mill Hill, London, for access to NMR equipment.

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Paper 7/01880K
Received 18*th March* 1997
Accepted 19*th June* 1997