THE CONFORMATION OF ALAMETHICIN

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The cyclic polypeptide alamethicin, when incorporated into a lipid bilayer imparts ion-gating properties resembling the electrical characteristics of natural membranes more closely than any other synthetic system. A model is described in which the molecule assumes a disc-like conformation, with the cyclic polypeptide structure extending around the circumference [1]. Physical studies [2, 3] indicate a high lipid solubility, and specific interaction with phospholipids in artificial systems. Following the determination of the amino acid sequence of alamethicin, the model has been modified to accommodate this and also the previously undetected cyclising γ-linked side chain, –Glu–Gln. The presumed ion binding groups have been located at the centre, in the form of a molecular pore [4]. We have examined the conformational properties of alamethicin in a variety of solvents by ORD and CD. The spectroscopic results indicate that the molecule assumes a compact conformation in hydrophobic environments. We propose that this conformation, which may contain up to 40% helical structure, is significant in the action of alamethicin on bilayer membranes.

The ORD and CD spectra are strongly solvent sensitive, showing greater amplitude with increasing hydrophobicity of the solvent. Fig. 1 shows the increase (more than 2-fold) in the negative troughs in CD at 207 nm and 220 nm on going from 10% ethanol in water to 100% ethanol. Similar effects have been found for methanol, dioxane and acetonitrile. Intensification of this spectrum is found in the presence of detergents such as 1% sodium dodecyl sulphate (fig. 1), 1% sarkosyl and 5% sodium deoxycholate, though in these cases the 207 nm trough is less pronounced.

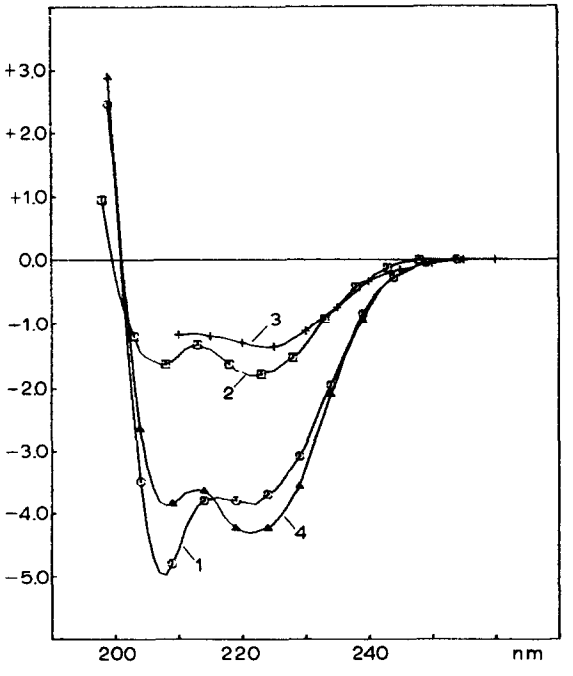

Fig. 1. Circular dichroism of alamethicin: 0.18 mg/ml, 1.00 mm path, 25°, mean residue MW 93; solvents 1, 100% ethanol; 2, 10% ethanol in water; 3, 6.0 M guanidine hydrochloride; 4, 1% sodium dodecyl sulphate (computer-drawn curves).

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The intensity of these spectra strongly suggests a highly ordered structure. In the absence of aromatic aminoacids, the CD is due entirely to peptide transitions, and the double negative trough resembles that of the right-handed α-helix. By comparison with the properties of poly L-glutamic acid (helical, pH 4.6 $\Delta\epsilon = -11.0$ at 220 nm; random, pH 7.5, $\Delta\epsilon = 0.9$ at 217 nm), measured under identical conditions, approximately 40% of the peptide residues in alamethicin would be in the helical conformation. The ORD spectra in ethanol show a minimum at 236 nm, and a maximum at 200 nm, similarly consistent with 40% of the peptide residues being in the helical conformation; the extrema are slightly red-shifted from those normally observed in helical polypeptides.

The spectrum in predominantly aqueous solution (i.e., 10% ethanol), indicates a less ordered structure. A similar spectrum is obtained in the presence of denaturants, such as 6.0 M guanidine hydrochloride (fig. 1) and 10 M urea, indicating that the structure is unusually resistent to randomisation by conventional methods.

Our interpretation of these results is that alamethicin exhibits conformational mobility dependent upon the nature of the environment. The spectrum in 100% ethanol, in which alamethicin is known to be monomeric [5], indicates that alamethicin assumes a specific structure in a hydrophobic environment. This structure must be maintained by intramolecular forces, such as the hydrogen bonding of a helical conformation, which would be enhanced in an apolar medium. The presence of the cyclic structure in the molecule means that a random conformation of the polypeptide chain in a normal sense is unattainable. The inclusion of a helical array within the cyclic peptide chain, would further restrict the degree of randomness attainable with concentrated denaturants.

These considerations suggest that alamethicin has a compact structure. Model building with CRE skeletal and CPK space-filling models shows that a region of α-helix, bounded by residues Pro-1 and Pro-13, can be accomodated in the structure. The remaining residues then form an extended chain, terminating in the γ-glutamyl-17-Pro-1 cyclising peptide link (fig. 2). This compact structure satisfies the need to include a high degree of order compatible with the spectroscopic results and a high degree of intramolecular stabilisation. The minimum cross-sectional area of this molecule is approximately 250 $Å^2$ in a plane perpendicular to the helix axis. This is in good agreement with the measurement of molecular area in a condensed monolayer at the air–water interface [2]. The amide residue of Gln-6 is in the same region of the molecule as the side-chains of Glu-17 and Gln-18.

The preferential penetration of the lipid interior of monolayers by alamethicin has been inferred from surface measurements [2]. Similarly alamethicin forms spread monolayers with the hydrophobic contacts optimised. We consider the hydrophobic environment of the organic solvents and the apolarity of SDS micelles to afford environments similar to these layers which are, in turn, analogous to the bilayer. Specific effects of the polypeptide upon lipids have been shown by NMR: in the presence of low molar ratios of alamethicin, the mobility of the hydrocarbon side-chains in phosphatidyl choline and phosphatidyl serine liposomes is severely restricted [2, 3]. On the proposed model, alamethicin within the lipid bilayer would be in the compact and possibly monomolecular form described. Examination of the space-filling model shows that, in the helical region, the apolar side-chains of alanyl, amino-isobutyryl, leucyl and valyl residues occur in extended left-handed helices, forming chains of hydrocarbon residues resembling the lipid hydrocarbon side-chains. These arrays would provide suitable binding sites for the interaction with lipids and detergents.

The mode of action of alamethicin, whether as a pore or as a carrier of ions, in unclear. No effects of monovalent cations on the conformation of alamethicin have been observed by NMR [2] or in the present work. The conformational properties of alamethicin may rather be expected to be determined by the location of the molecule in the bilayer. In the aqueous phase, aggregation is to be expected and aggregation has been implicated in the function from the high order of the kinetics of ion transport [1]. At the interface conformational changes are to be anticipated with the change of medium. Within the bilayer, the lipid-soluble form capable of interacting with lipid side-chains would, from the present evidence, be the compact monomer observed in ethanol. Utilising the distinctive properties of the molecule in the different loci, alamethicin could function as a dynamic pore, incorporating features of both the pore and carrier mechanisms.

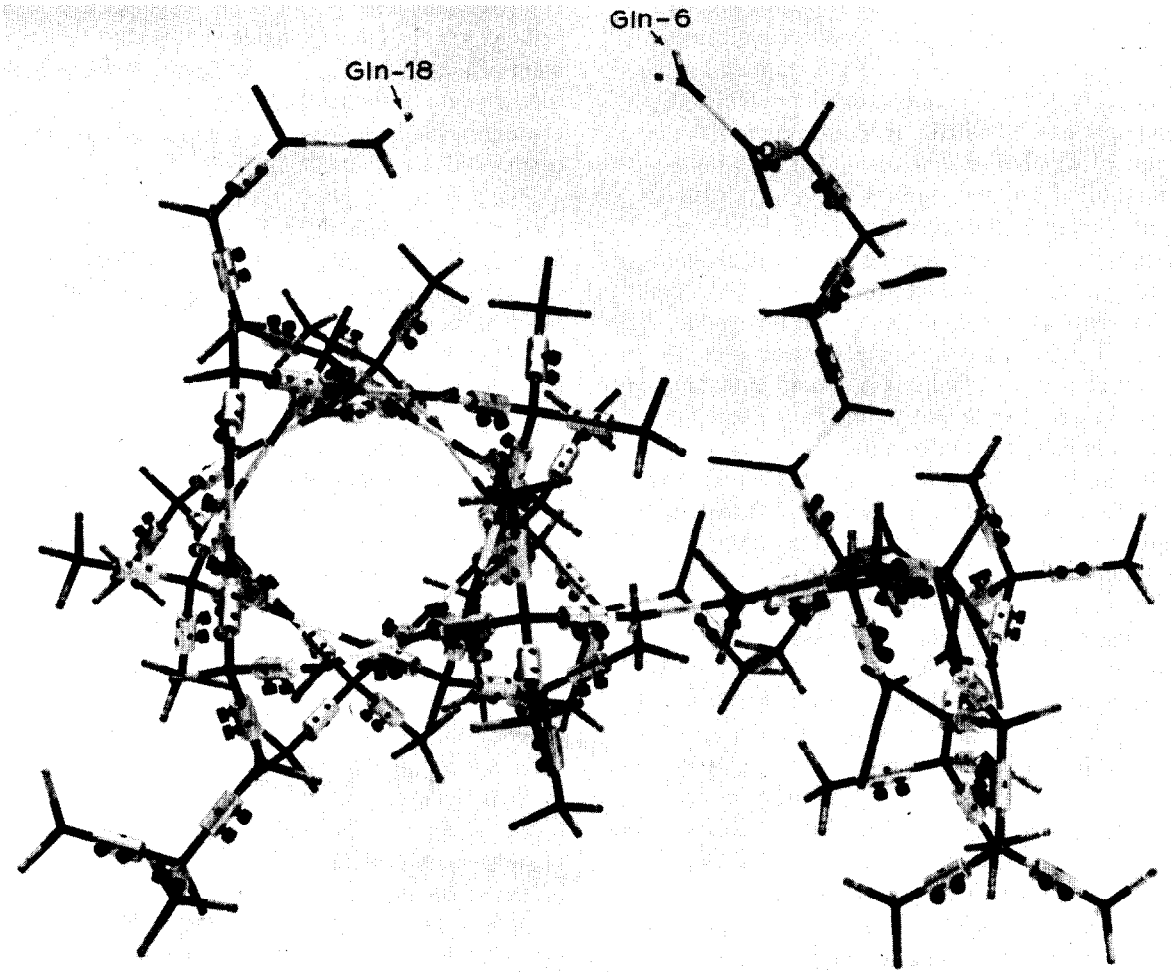

Fig. 2. CRE (Cambridge) molecular model of alamethicin illustrating the potential for α-helical formation by up to a maximum of 60% of the residues; being those 11 residues located between the two prolines including Gln-6.

Acknowledgement

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