THE CONFORMATION OF GRAMICIDIN-A IN SOLUTION

B.E. ISBELL, C. RICE-EVANS and G.H. BEAVEN
National Institute for Medical Research, Mill Hill, London, NW7 1AA, England

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1. Introduction

Two mechanisms have been proposed for the selective transport of ions across lipid bilayer membranes by antibiotics. In the carrier mechanism the ion is sequestered by the antibiotic and the complex diffuses through the hydrophobic interior of the membrane. The alternative mechanism is the formation of channels through the membrane, which provide pathways for ion transport. The hydrophobic, tryptophan-rich, linear gramicidin polypeptides were originally thought to function as ionophores [1–4], but recently emphasis has been directed to a possible channel-forming role [5, 6]. More specifically, Urry et al. [7–9] have suggested that a head-to head association of two gramicidin molecules, each in a novel $\pi_{L,D}$ helix conformation, provides a structure of the correct dimensions to span a lipid bilayer and form a channel for the transfer of ions.

The formation of a dimer species of gramicidin in the membrane appears to be an important feature of its mode of action. Both the kinetics and the concentration dependence of the increase in conductance of lipid membranes by gramicidin [10, 11] indicate that a dimer is the effective species, and the activity of the covalently bonded *N,N'*-dideformyl gramicidin-A-malonamide dimer tends to support this conclusion [8]. Here we report molecular weight and spectroscopic studies on the individual pure gramicidins in a variety of solvent systems, and their relevance to its possible conformation in hydrophobic environments, such as the lipid bilayer of a cell membrane.

2. Materials and methods

Primary structure of the gramicidins:

HCO-L-Val–Gly–L-Ala–D-Leu–L-Ala–D-Val–L-Val–
(5)
D-Val–L-Trp–D-Leu–L-(R)-D-Leu–L-Trp–D-Leu–
(10)
L-Trp-$NHCH_2CH_2OH$ (Gramicidin-A, R = Trp;
(15)

Gramicidin-B, R = Phe, Gramicidin-C, R = Tyr).

Pure samples of valine gramicidin-A [12], gramicidin-B [13] and gramicidin-C [14] were isolated from a W.H.O. reference sample [15] of the Dubos preparation [16] by means of a 1300-transfer steady state distribution, using the solvent system devised by Gregory and Craig [17]. Molecular weight data were measured in anhydrous organic solvents using a Perkin Elmer Hitachi vapour pressure osmometer. Circular dichroism (CD) measurements were made on a Roussel-Jouan 185 model II dichrograph over the wavelength range 185–320 nm, with nitrogen flushing for all measurements below 200 nm. Infrared (IR) spectra were measured in calcium and barium fluoride cells (0.20–0.025 mm) on a Perkin Elmer 457 grating spectrophotometer; solvent compensation was obtained with a variable pathlength cell in the reference beam.

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3. Results and discussion

The molecular weight data for valine–gramicidin-A in several organic solvents are listed in table 1. In the less polar solvents dioxan and ethyl acetate, the gramicidins attain a molecular weight which approaches the dimer value, even at the lower concentrations. In anhydrous aliphatic alcohols the proportion of monomer is higher, reflected by the generally lower $K_{association}$ values. The dimer species however is favoured by increasing size of solvent alkyl groups and decreasing temperature. In trifluoroethanol (TFE) over a wide concentration range the molecular weight was consistently near the monomer value. It may be expected that in less polar solvents such as lipids, the proportion of dimer will be appreciable, even at low total concentration. These results are therefore consistent with the studies of the gramicidin-mediated, ion transport across artificial lipid bilayers [10, 11] which indicate a dimer is the effective species.

The interpretation of the CD spectra of gramicidin-A and its congeners is complicated by the possibility of contributions from the high concentrations of aromatic side chains, the association equilibrium and the presence of both D and L residues. The circular dichroism of valine–gramicidin-A in anhydrous *n*-propanol (fig. 1, curve 1), which is concentration-independent, shows intense bands centred at about 230 and 195 nm with $[\theta]_{res}$ values of about -1.6 and $+3.9 \times 10^4$, respectively. Apart from the resolved

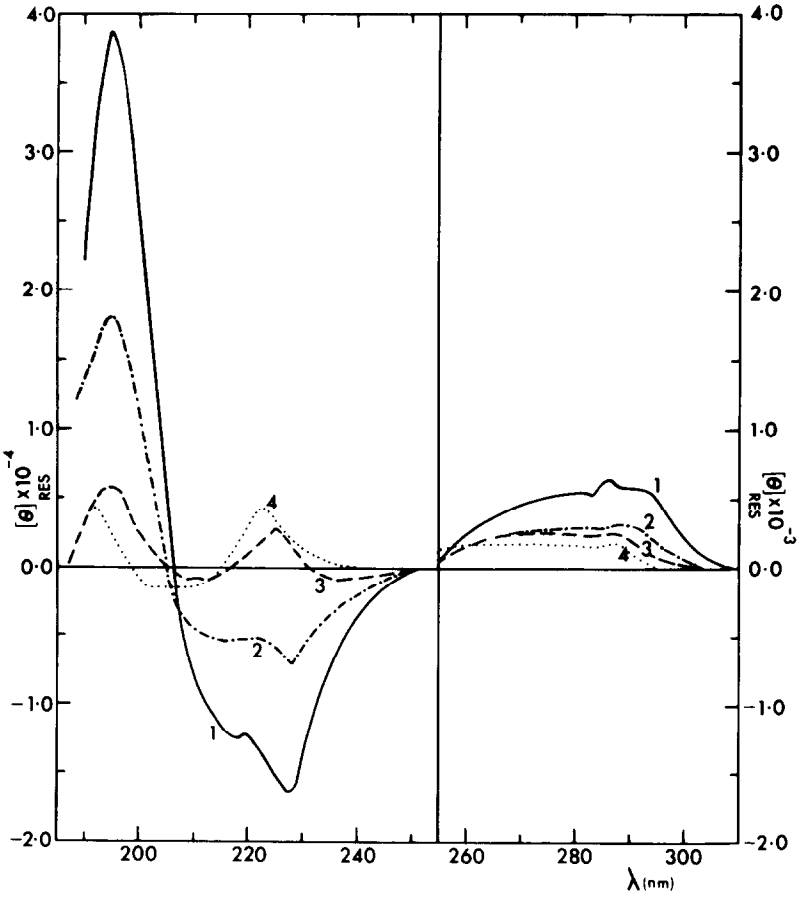

Fig. 1. Circular dichroism of valine–gramicidin-A at 22°. 1) *n*-propanol, 1.00 mg/ml; 2) 95% v/v *n*-propanol:water, 0.94 mg/ml; 3) 75% v/v *n*-propanol:water, 0.75 mg/ml; 4) TFE, 1.12 mg/ml.

Table 1
The number average molecular weight ($\bar{M}_n$) of valine–gramicidin-A in anhydrous organic solvents.

Solvent	$T°$	Conc. (mg/ml)	$\bar{M}_n$	Conc. (mg/ml)	$\bar{M}_n$	Approx. $K_{association}$ (mole^{-1}/kg)
Dioxan	40	9.5	3480	14.9	3530	10^4
	60	9.0	2990	15.3	3090	10^3
Methanol	30	5.0	1860	30.0	2390	50
	57	5.0	1880	40.0	1900	1–5
Ethanol	30	5.0	2340	30.0	3150	5×10^2
n-Propanol	60	5.0	2530	26.6	2840	2×10^2
Ethyl acetate	40	1.4	2080	7.8	3010	10^3

monomer: 1880, dimer: 3760.

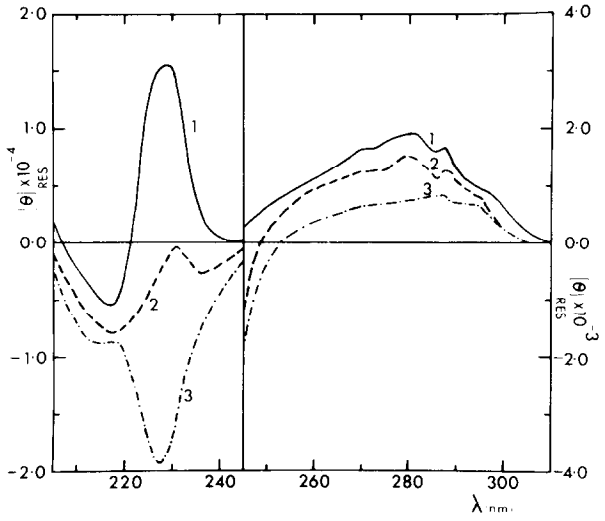

Fig. 2. The concentration dependence of the circular dichroism of valine–gramicidin-A in anhydrous dioxan at 22°; 1) 4.86 mg/ml; 2) 1.16 mg/ml; 3) 0.80 mg/ml.

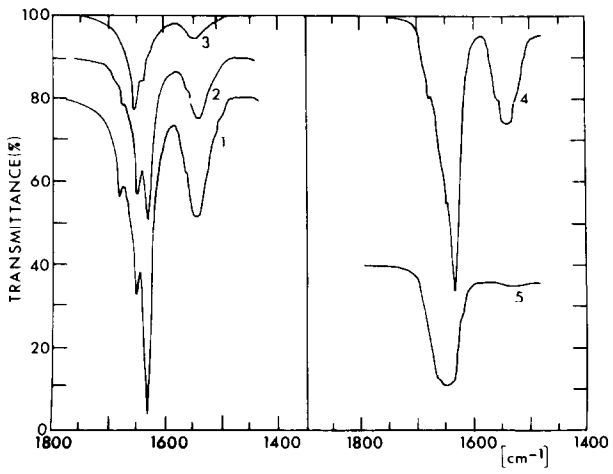

Fig. 3. The infrared spectra of valine–gramicidin-A; 1) 4.85 mg/ml; 2) 2.03 mg/ml; 3) 0.80 mg/ml; all in dioxan; 4) *n*-propanol, 6 mg/ml; 5) *n*-propanol-D_1:D_2O, 75:25 v/v, 30 mg/ml.

shoulder on the shortwave side of the 230 nm band, this dichroism shows considerable similarity with respect to sign and magnitude to the spectra of model homopolymers in the β-conformation [18, 19]. In conjunction with the positive bands in the aromatic region (255–310 nm) the spectrum indicates a largely structured form. The most likely explanation for the complexity of the negative CD band is that it contains a contribution from the tryptophan residues in this ordered conformation. The entire CD spectrum is progressively reduced by addition of water (fig. 1, curves 2, 3) and the residual dichroism at limiting water content can be almost entirely accounted for, with respect to band location, sign and magnitude, by intrinsic tryptophan contributions, as given, for example, by *N*-acetyl tryptophan amide in the same solvent system. The spectrum in TFE is closely similar to that of the unstructured form (fig. 1, curve 4).

The dichroism of gramicidin-A in anhydrous dioxan is concentration-dependent. At low concentrations the spectrum (fig. 2, curve 3) is similar to that of the structured form in aliphatic alcohols, except that the shoulder at about 215 nm is more prominent. At the highest concentration studied, the spectrum is inverted (fig. 2, curve 1) and closely resembles that of poly-L-tryptophan, which arises from strong coupling interactions between tryptophan chromophores [20]. Curves 1 and 3 of fig. 2 appear to represent the extremes of a conformational equilibrium, as changes in concentration beyond these limits were without effect. Furthermore, the spectrum at an intermediate concentration (curve 2) can be matched by adding approximately equal contributions of the two extrem forms. The aromatic Cotton effects in the 280 nm region in dioxan are also very concentration-dependen In this solvent gramicidin is substantially present as the dimer species at concentrations higher than 1 mg/ml, and the concentration dependence of the CI spectrum appears to be related to the dimerisation. The same type of inverted spectrum (fig. 2, curve 1) also has been observed by us for gramicidin-A solubilised in detergent micelles and in liposome preparations of natural membrane components. Gramicidin-B and C give similar spectra, but with reduced intensities consistent with one less tryptophan residue.

The infrared absorption spectra of gramicidin-A in anhydrous dioxan are shown in fig. 3 (curves 1–3). From comparison with reference data [21, 22] the decrease in relative intensity of the 1650 cm^{-1} band and the increase in that of the 1630 cm^{-1} band with increasing concentration, and therefore formation of the dimer species, could be interpreted as a transition to β-structure [23]. At concentrations higher than 10 mg/ml the spectrum in dioxan is closely similar to that in the anhydrous aliphatic alcohols (fig. 3, curve 4), and also of gramicidin solubilised in detergent micelles. This structure is characterised by a strong 1630 cm^{-1} band and a resolved 1680 cm^{-1} band, indicative of antiparallel β-structure; there is also a shoulder at 1650 cm^{-1}. The broad amide II transition centred at 1545 cm^{-1} could represent an α-helical structure [21], but its evident complexity and the possibility of solvent effects makes this assignment uncertain. The spectrum in aqueous-organic solvents (fig. 3, curve 5) is characterised by a very broad amid I band centred at 1655 cm^{-1}, indicative of an unstructured state [22]. The disappearance of the amide II band on deuteration confirms its assignment and the absence of other overlapping bands. The similarity of the IR spectra of gramicidin in anhydrous *n*-propanol and in dioxan is in contrast with the differences in the CD spectra, and strongly suggest that the gramicidin–solvent interactions are qualitatively different in these two solvents. Our own studies on th CD spectrum of the tryptophan chromophore in alcohol and dioxan-based solvent systems support thi view.

In conjunction with the IR spectra, the CD results indicate that in some anhydrous solvents and in lipid systems, the gramicidins exist in a substantially ordered conformation, possibly of antiparallel-β type. The tryptophan interchromophore interactions, which occur at high concentrations in dioxan and in lipid solvent systems, presumably provide additional stabilisation for the structured conformation. The behaviour of gramicidin in solution is similar in many respects to that of the polypeptide hormone glucagon, which can assume α-helical, β or random coil conformations under appropriate conditions, and also forms trimers and higher associated species [24].

Urry and coworkers have adduced evidence from proton magnetic resonance [25] and spectroscopic [7, 9, 26] studies in support of their $\pi_{L,D}$ dimer model for gramicidin. Hydrogenated gramicidin, in which the tryptophan chromophores have been eliminated, has also been studied by Urry [9, 26]. The CD spectra in TFE were interpreted as indicative of a left-handed helix. This result may not be relevant to the conformation of gramicidin itself in this solvent, since the reduction of aromatic groups is known, in at least one case, strongly to stabilise the helical conformation [27], presumably due to increased hydrophobic interactions between the hydroaromatic side groups. In this solvent gramicidin itself also differs from its hydrogenated derivative in showing a weak positive CD band rather than a strong negative CD band below 200 nm. On the basis of our present work it appears that gramicidin itself is unstructured in TFE.

The results reported here provide no direct evidence for or against the $\pi_{L,D}$ dimer model, since the optical properties of the gramicidins appear to be largely explicable in terms of established polypeptide chain conformations. They do however emphasize the importance of a dimer species in many solvents and presumably also in lipid systems, and also show that the conformation of the individual gramicidins are similar and highly solvent-dependent. Furthermore the structured form is markedly destabilised by water but is stable in low polarity solvents and lipid environments.

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