VIBRATIONAL ANALYSIS OF THE STRUCTURE OF GRAMICIDIN A.

II. Vibrational Spectra

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ABSTRACT Raman and infrared spectra have been obtained of gramicidin A (GA) in the crystalline state both in the native form and complexed with CsSCN and KSCN, in solution in dioxane, and incorporated into lipid vesicles. Based on predictions from normal mode calculations of a number of relevant single- and double-stranded β-helix conformations (Naik and Krimm, 1986), it has been possible to assign the structures of GA that are present under the above conditions. In the crystalline state, native GA has a double-stranded $\uparrow\downarrow\beta^{5,6}$ structure, whereas complexes with CsSCN or KSCN adopt a $\uparrow\downarrow\beta^{7,2}$ structure. In dioxane solution, the $\uparrow\downarrow\beta^{5,6}$ structure predominates. In lipid vesicles, the single-stranded $\beta^{6,3}$-helix is found, which converts to a double-stranded helix on drying the sample. These results support our previous studies in showing that normal mode analysis can be a powerful technique in obtaining three-dimensional structural information from vibrational spectra.

INTRODUCTION

In the previous paper (Naik and Krimm, 1986) we presented the results of normal mode calculations on a number of single- and double-stranded β-helices that have been proposed for the structure of gramicidin A (GA) in a variety of environments. These predictions should permit the assignment of structure from observed vibrational spectra.

Here we present infrared and Raman spectra of GA in various states and discuss the structures that are indicated. Native and ion-bound GA have been examined in the crystalline state, and GA has been studied in dioxane and in lipid vesicles. The spectra are found to be dependent on the physical state of the GA, and structural assignments are possible based on the predictions from the normal mode calculations. A preliminary discussion of these assignments was given in Naik and Krimm (1984*b*).

MATERIALS AND METHODS

Gramicidin (a commerical mixture of ~85% GA and ~15% GB plus GC) was purchased from ICN Life Sciences Group (Plainview, NY) and used without further purification, as were the CsSCN and KSCN. GA was crystallized from ethanol or methanol using the methods of Koeppe et al. (1978) and Koeppe et al. (1979). The *N*-deuterated GA crystals were grown similarly from methanol-d or ethanol-d (Aldrich Chemical Co., Metuchen, NJ). The crystals of GA with cesium and potassium were also grown following the methods of Koeppe et al. (1978, 1979).

Liposomes were prepared following the procedures of Urry et al. (1983*b*). In brief, micelles were formed by dispersing lysolecithin in 0.5 mM NaCl in H_2O and sonicating the solution. A weighed amount of GA was added to these micelles to obtain a 10:1 molar ratio of phospholipid to GA, the mixture was shaken well, and was then further sonicated for several minutes. The sample was incubated in a thermostatted bath at 45°C for 10 min and a Raman spectrum was recorded. The remaining sample was incubated at 68°C for 8–15 h, and a Raman spectrum was again recorded. Finally, the sample was dried under reduced pressure and a Raman spectrum of this dried sample was recorded.

Infrared spectra were recorded in KBr disks using a Fourier Transform infrared instrument (model FTS-20C; Digilab Div., Bio Rad Laboratories, Cambridge, MA) equipped with a liquid-nitrogen–cooled HgCdTe detector and purged with dry air. The spectra were recorded with a nominal resolution of 1–2 cm^{-1}, by coadding 150–300 interferograms and with the use of a triangular apodization function.

Raman spectra were recorded using a double monochromator (model 1403; Spex Industries Inc., Edison, NJ) equipped with 10 cm^2 holographic gratings with 2,400 grooves/mm and a PMT (model 31034; RCA, Lancaster, PA). The excitation line used was 514.5 nm from a Spectra-Physics 165 Ar^+ laser. Most of the spectra were averaged over several scans by using a Datamate (Spex) microprocessor.

For solid samples, an incident laser power of ~150 mw was focused at the samples, sealed in glass capillaries. The spectral band pass was ~2 cm^{-1} at 514.5 nm, and the step resolution used was 1 cm^{-1} in all the spectra. For solution samples, the incident laser power of ~200 mw was focused at the capillaries containing the sample solutions. The spectral band pass was ~3 cm^{-1} at 514.5 nm. Because of the low concentration of the samples, the spectra were averaged over a large number of scans (~50) to obtain a better signal-to-noise ratio.

RESULTS

Infrared and Raman spectra of native GA and its *N*-deuterated derivative in the crystalline state are shown in

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Figs. 1 and 2. Table I lists the observed frequencies of GA together with tentative assignments for the identifiable modes of the side chains and the blocking end groups (Simons et al., 1972). Infrared and Raman spectra of GA and its Cs^+ and K^+ complexes in the crystalline state are shown in Figs. 3 and 4. Table II lists the amide I, II, III, and V frequencies of GA and GA-CsSCN crystals, together with calculated frequencies of the $\uparrow\downarrow\beta^{5,6}$ and $\uparrow\downarrow\beta^{7,2}$ structures (Naik and Krimm, 1986). The Raman spectrum of GA in dioxane is shown in Fig. 5, and Raman spectra of GA in membranes at various stages of incorporation are shown in Fig. 6.

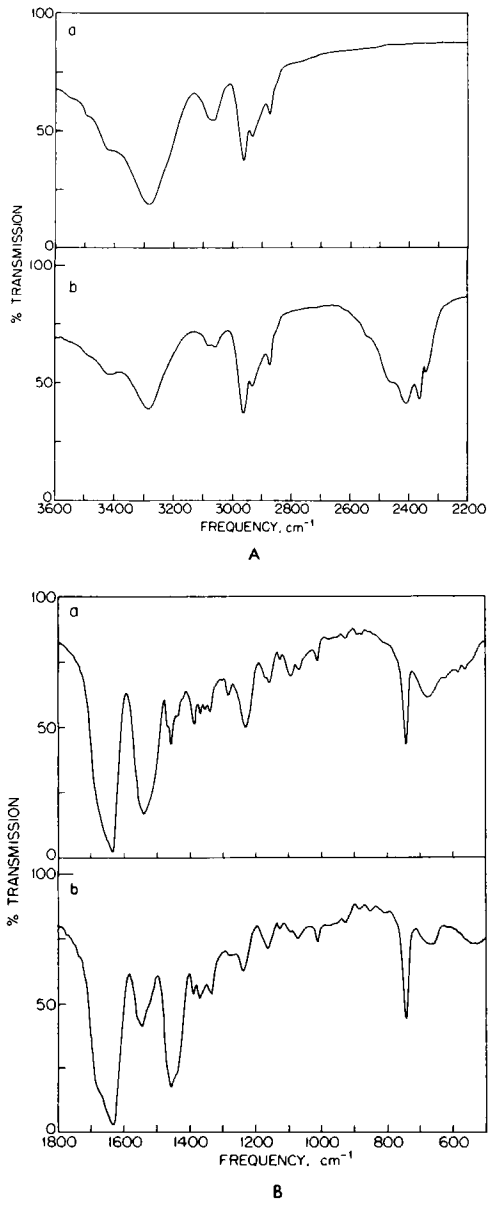


FIGURE 1 Infrared spectra of crystalline (*a*) gramicidin A and (*b*) its *N*-deuterated derivative. (*A*) 3,600–2,200 cm^{-1} region, (*B*) 1,800–500 cm^{-1} region.

DISCUSSION

Ion-free Crystalline GA

The amide A band is observed at 3,285S cm^{-1} in the infrared and at 3,274M cm^{-1} in the Raman. In addition to these bands, there are two infrared bands observed at 3,415 and 3,495 cm^{-1} and a Raman band at 3,418 cm^{-1}. These are due to NH groups of the trp side chain and OH groups

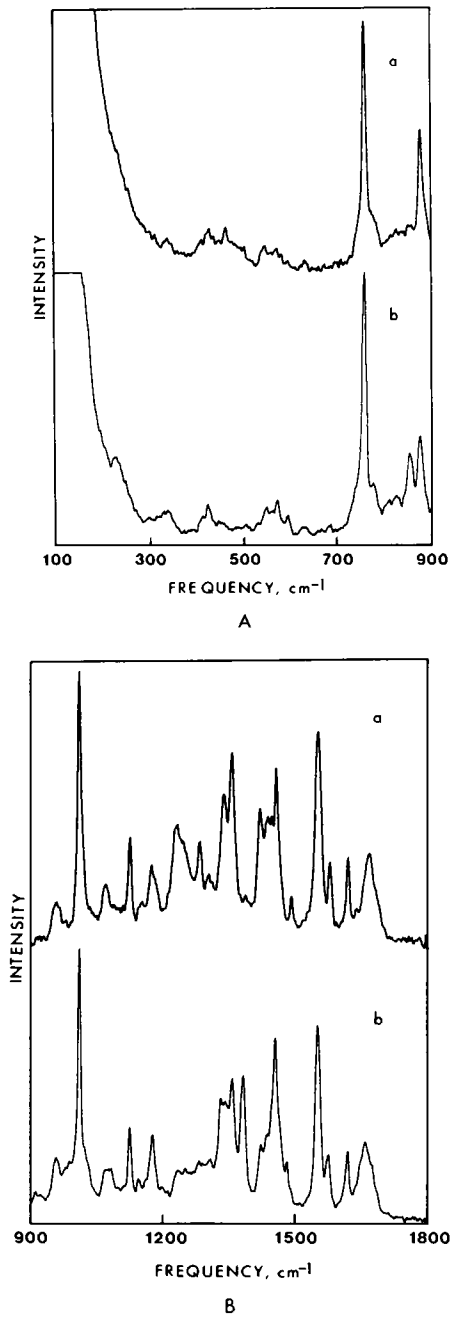


FIGURE 2 Raman spectra of crystalline (*a*) gramicidin A and (*b*) its *N*-deuterated derivative. (*A*) 100–900 cm^{-1} region, (*B*) 900–1,800 cm^{-1} region, and (*C*) 2,600–3,600 cm^{-1} region.

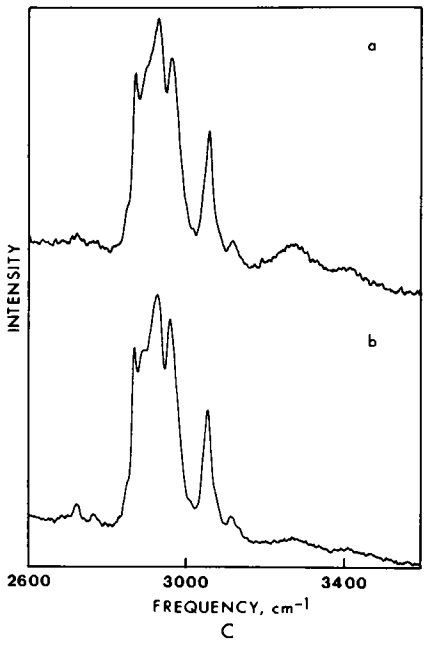


FIGURE 2 (Continued)

TABLE I
OBSERVED FREQUENCIES OF CRYSTALLINE NATIVE GRAMICIDIN A

Raman*	Infrared*	Assignment
	cm^{-1}	
	3,495 sh	NH stretch (trp)
3,418 W	3,415 M	OH stretch (ethanolamine, tyr)
3,274 M	3,285 S	amide A
3,121 M		CH stretch (ring)
	3,078 M	amide B
3,061 VS	3,064 M	CH stretch (ring)
2,963 VS	2,964 VS	CH_3/CH_2 stretch
2,931 VS	2,935 M	CH_3/CH_2 stretch
2,913 sh		
2,897 sh		
2,872 VS	2,874 M	$C^{\alpha}H^{\alpha}$ stretch
1,678 sh	~1,680 sh	amide I
1,666 S	1,638 VS	
1,619 S		ring stretch
1,578 S		ring stretch
1,551 VS		ring stretch
	1,542 VS	amide II
1,490 M		CH_3 antisymmetric bend
	1,466 sh	
1,457 VS	1,460 S	CH_2 bend (side chain)
1,449 S		
1,435 S	1,440 sh	CH_2 bend (side chain)
1,422 VS		NH bend (trp)
1,387 VW	1,388 M	CH_3 symmetric bend
	1,370 W	
1,357 VS	1,355 W	
1,338 VS	1,341 W	
1,303 W	1,304 VW	
1,284 M	1,285 W	amide III
1,258 W		
1,243 sh		
1,233 S	1,233 S	

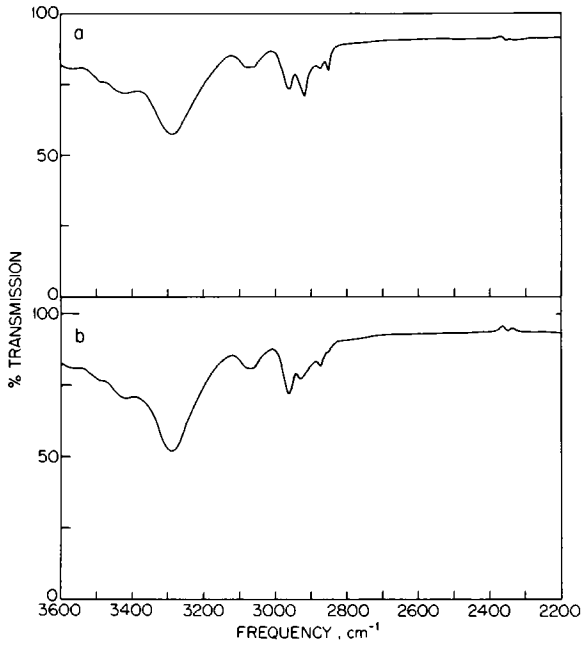
TABLE I (Continued)

Raman*	Infrared*	Assignment
1,191 W		
1,174 M	1,171 sh	COH (ethanolamine)
	1,161 M	
1,147 VW		
1,126 S	1,128 VW	
	1,097 M	
1,069 M	1,070 W	COH (ethanolamine)
1,019 sh		
1,011 VS	1,013 W	
978 WV	984 VW	
965 sh	965 VW	
957 M		
924 W	927 W	
	893 VW	
876 S	879 VW	
	858 VW	
827 W		
799 VW	808 VW	
758 VS		
	743 S	
684 W	676 M	amide V
641 VW		
629 VW	626 W	
593 VW		
	584 VW	
	567 VW	
547 W		
490 W		
460 W		
423 W		
337 VW		
306 VW		
292 VW		
225 M		

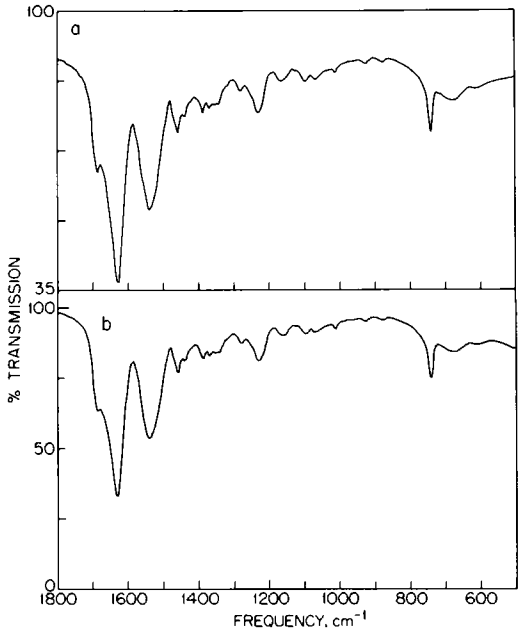
*S = strong, M = medium, V = very, W = weak, and sh = shoulder.

of ethanolamine and tyr that are present in GC. The amide B region overlaps that associated with CH stretching frequencies of the trp and phe rings, and this makes it hard to discern the amide B band. However, from intensity decreases on *N*-deuteration, a band at 3,078 cm^{-1} in the infrared of undeuterated GA can be assigned to the amide B mode.

In the amide I region there is a very strong infrared band with a peak at 1,638 cm^{-1}, accompanied by medium intensity absorption near 1,660–1,670 cm^{-1} terminating in a shoulder near 1,680 cm^{-1}. In the Raman spectrum, the strong amide I band is observed at 1,666 cm^{-1} with a shoulder at ~1,678 cm^{-1}. In terms of the predicted normal modes (Naik and Krimm, 1986), this frequency pattern is inconsistent with single-stranded structures, which predict a maximum infrared splitting of 17 cm^{-1} and Raman modes below 1,654 cm^{-1}. It is most consistent with the $\uparrow\downarrow\beta^{5,6}$ structure, whose strong infrared band is calculated at 1,636 cm^{-1}, with medium intensity bands predicted at 1,666, 1,669, and 1,675 cm^{-1} (see Table II). The observed



A



B

FIGURE 3 Infrared spectra of crystalline (*a*) gramicidin A-CsSCN complex and (*b*) gramicidin A-KSCN complex. (*A*) 3,600–2,200 cm^{-1} region, (*B*) 1,800–500 cm^{-1} region.

Raman band at 1,666 cm^{-1} falls at the midpoint of the range of predicted frequencies after exclusion of the infrared-intense mode, namely, 1,656–1,675 cm^{-1}. The $\uparrow\downarrow\beta^{7.2}$ structure is more favored for the GA-CsSCN complex (see below), and the parallel double-stranded helices are disfavored on the basis of their infrared frequencies and splittings.

We also note that our calculations predict very well the

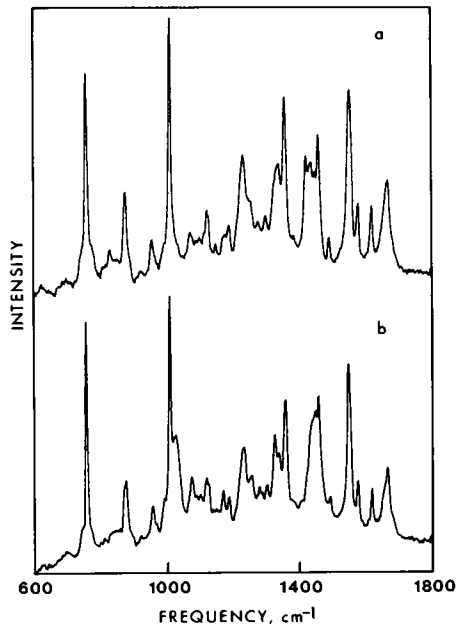


FIGURE 4 Raman spectra of crystalline (*a*) gramicidin A-CsSCN complex and (*b*) gramicidin A-KSCN complex in the 600–1,800 cm^{-1} region.

observed infrared amide I band and its dichroic ratio in the $\uparrow\downarrow\beta^{5.6}$ structure of poly(γ-benzyl-L,D-glutamate) (Lotz et al., 1976). These authors have characterized poly(γ-benzyl-L,D-glutamate) in the $\uparrow\downarrow\beta^{5.6}$ conformation by x-ray and electron diffraction studies, and they find a strong parallel-polarized infrared amide I band at 1,632 cm^{-1} with a dichroic ratio of 2.0. Our predicted frequency at 1,636 cm^{-1} and dichroic ratio of 1.3 (Naik and Krimm, 1986) are in good agreement with these observed values.

The amide II band is observed at 1,542 cm^{-1} in the infrared, which is consistent with the calculated range of 1,553–1,541 cm^{-1} for $\uparrow\downarrow\beta^{5.6}$ (Naik and Krimm, 1986). Until reliable intensities are calculated for amide II frequencies, this mode will not be very useful in discriminating between structures.

In the amide III region there are four Raman bands, at 1,284, 1,258, 1,243, and 1,233 cm^{-1}, that lose intensity on *N*-deuteration. In the infrared there are only two such bands, at 1,285 and 1,233 cm^{-1}. For the $\uparrow\downarrow\beta^{5.6}$ structure, modes with large NH ib contributions are calculated at 1,238–1,236 (E_1, E_2,) cm^{-1}, which accounts well for the lowest frequency band; with a smaller NH ib contribution at 1,240 (*A*) cm^{-1}, that matches the 1,243 cm^{-1} band; with similar contributions at 1,267 (E_1) and 1,265 (E_1) cm^{-1}, assignable to the 1,258 cm^{-1} band; and with comparable NH ib contributions at 1,288 (*A*) and 1,284 (*A*) cm^{-1}, to which the 1,284 cm^{-1} band can be well assigned (see Table II). Comparable agreement is not obtained, for example, with the $\uparrow\downarrow\beta^{7.2}$ or the parallel double-stranded structures.

In the amide V region there are two bands in the infrared, at 676 cm^{-1} (medium, broad) and a weak band at 626 cm^{-1}, that weaken on *N*-deuteration. In the Raman

TABLE II
OBSERVED AMIDE FREQUENCIES OF GRAMICIDIN A AND ITS CsSCN COMPLEX, AND CALCULATED FREQUENCIES OF $\uparrow\downarrow\beta^{5.6}$ AND $\uparrow\downarrow\beta^{7.2}$ HELICES

Mode	Gramicidin A			Gramicidin A-CsSCN		
	Raman*	Infrared*	$\uparrow\downarrow\beta^{5.6}$	Raman*	Infrared*	$\uparrow\downarrow\beta^{7.2}$
I	1,678 sh	1,680 W	1,675 M		1,685 W	1,686 VW
		1,670-60M	1,669-66 MS		~1,670 MW	1,677-74 M
	1,666 S		1,666‡	1,668 S		1,669‡
		1,638 VS	1,636 VS		1,630 VS	1,632 VS
II		1,542 S	1,553-41		1,539 S	1,556-49
III	1,284 M	1,285 W	1,288 1,284	1,279 VW	1,280 VW	1,274 1,273
	1,258 W		1,267 1,265	1,256 W		1,266 1,265
	1,243 sh		1,240			
	1,233 S	1,233 M	1,238-36	1,233 S	1,230 M	1,246-44
V		676 M	692		680 M	680
		626 W	640		617 VW	634-17

*S = strong, M = medium, W = weak, V = very, sh = shoulder
‡See text

spectrum no such clearly identifiable bands are seen. Calculated frequencies that can be assigned to these bands are predicted by the $\uparrow\downarrow\beta^{5.6}$ structure at 692 (A) and 640 (E_1) cm^{-1}. In this case, the predictions for $\uparrow\downarrow\beta^{7.2}$ are comparable, if not better, but the predicted amide V modes for the parallel double-stranded helices are inconsistent with the observed bands.

In summary, native GA in the crystalline state has a vibrational spectrum most consistent with a $\uparrow\downarrow\beta^{5.6}$ structure.

Ion-bound Crystalline GA

The spectra of Cs^+ and K^+ complexes of GA are very similar (see Figs. 3 and 4). In what follows we will focus on the spectra of the GA-CsSCN complex.

The amide A mode is observed at 3,285 cm^{-1} in the infrared and 3,280 cm^{-1} in the Raman; the amide B mode is found at 3,078 cm^{-1}. In the amide I region the very strong infrared band is now found at 1,630 cm^{-1}, shifted down from the 1,638 cm^{-1} of native GA. There is only modest absorption near 1,670 cm^{-1}, and a clear weak band is seen at 1,685 cm^{-1}. This frequency shift, splitting, and intensity pattern is most consistent with a $\uparrow\downarrow\beta^{7.2}$ structure, the changes from the $\uparrow\downarrow\beta^{5.6}$ structure all being in the predicted directions (Naik and Krimm, 1986; see Table II). We also find in this case that the strong Raman band,

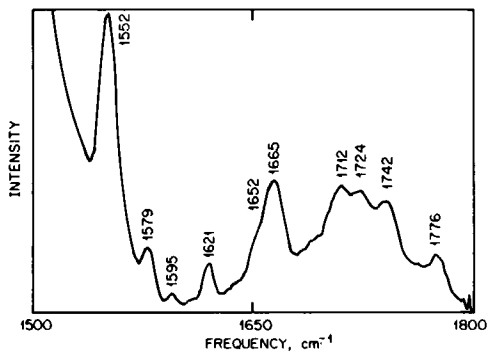


FIGURE 5 Raman spectrum in the 1,500–1,800 cm^{-1} region of gramicidin A in dioxane.

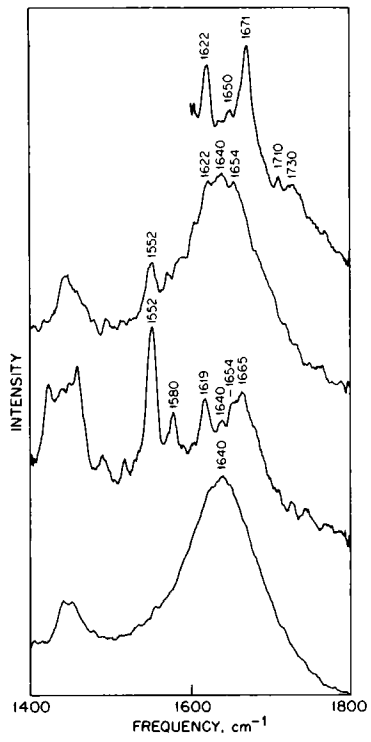


FIGURE 6 Raman spectra in 1,400–1,800 cm^{-1} region of gramicidin A in vesicles. (*a*) Vesicles in water, (*b*) gramicidin A in vesicle suspension after 10 min incubation at 45°C, (*c*) gramicidin A in vesicle suspension after 8–15 h incubation at 60°C and (*d*) dried sample of *c*.

at 1,668 cm^{-1}, is near the midpoint of the range of predicted frequencies after exclusion of the infrared-intense mode, namely, 1,651–1,686 cm^{-1}.

The observed amide II mode, at 1,539 cm^{-1}, has shifted down slightly from its value in native GA (1,542 cm^{-1}). The general range for $\uparrow\downarrow\beta^{7.2}$ is slightly higher than for $\uparrow\downarrow\beta^{5.6}$ (see Table II), but since we do not know at present which E_1 mode to assign to the strong amide II band, it is difficult to assess this small discrepancy.

In the amide III region, the highest frequency bands shift down from ~1,285 cm^{-1} in native GA to ~1280 cm^{-1} in GA-CsSCN. If these modes remain the same in character, namely, mixed H^{α} bend plus NH ib, then this is consistent with $\uparrow\downarrow\beta^{5.6}$ modes at 1,288 and 1,284 cm^{-1} shifting down to $\uparrow\downarrow\beta^{7.2}$ modes at 1,274 and 1,273 cm^{-1} (Naik and Krimm, 1986; see Table II). The lowest frequency band, near 1,233 cm^{-1}, remains essentially unchanged; a small increase in this mode is predicted for the $\uparrow\downarrow\beta^{7.2}$ structure (see Table II).

The amide V modes are well accounted for by the $\uparrow\downarrow\beta^{7.2}$ structure (see Table II), although the changes from $\uparrow\downarrow\beta^{5.6}$ are explained only for the lower frequency mode.

In sum, considering the greater certainty for predictions involving amide I, the vibrational spectra strongly indicate that the GA-CsSCN complex has a $\uparrow\downarrow\beta^{7.2}$ structure. Our predictions are also consistent with observations on the $\uparrow\downarrow\beta^{7.2}$ structure of poly(γ-benzyl-L,D-glutamate) (Lotz et al., 1976): the strong infrared band is observed to decrease to 1,628 cm^{-1} and the observed dichroic ratio is observed to increase to 4.9 (cf. our predicted value of 5.7 for the latter, Naik and Krimm. 1986).

These conclusions are also consistent with the x-ray results. The observed dimer length decreases from 32 to 26 Å and the channel diameter increases from 5 to 6.8 Å in going from the ion-free to the ion-bound form (Koeppe et al., 1978, 1979). Chandrasekaran and Prasad (1978) have calculated these parameters for various structures formed by 15 amino acid residues of a poly(L,D-Ala) chain. The lengths for the $\uparrow\downarrow\beta^{5.6}$ and $\uparrow\downarrow\beta^{7.2}$ structures were found to be ~32 and ~25 Å, and the channel diameters were ~1.8 and ~4.0 Å, respectively. (Note that these diameters represent the actual diameters of an ion that passes unhindered through the central hole, while those quoted in the x-ray work represent the average diameter of the peptide main chain atoms.) This is further supported by the fact that the ion-bound conformation is the same for the Cs^+, K^+, and Tl^+ GA, all of which have different ionic radii. However, Li^+-bound GA is believed to have the same dimensions, dimer length, and core radius as that of the ion-free GA crystals (B.A. Wallace, personal communication). This is because the inner core radius of the $\uparrow\downarrow\beta^{5.6}$ structure is large enough so that it can hold a Li^+ ion whose radius is 0.6 Å. (The ionic radii of other ions are: Na^+, 0.95 Å; Tl^+, 0.95 Å; K^+, 1.33 Å; and Cs^+, 1.48 Å.) It is obvious that the $\uparrow\downarrow\beta^{7.2}$ structure, which has an inner core radius of ~2 Å, can hold any of these ions while the $\uparrow\downarrow\beta^{5.6}$ structure can hold only the Li^+ ion. It should also be mentioned that, on the basis of the dimer channel length and inner core radius, the structure of ion-bound GA is also consistent with a dimeric structure consisting of two $\beta^{6.3}$ helices aggregated end-to-end. The structure that is proposed for GA in membranes is the so-called head-to-head dimer formed by two $\beta^{6.3}$ helices (Urry et al., 1971). This structure has a dimer length of ~26 Å and an inner-core radius of ~3.4 Å (Chandrasekaran and Prasad, 1978). However, our calculations eliminate such a structure for GA in the ion-bound GA crystals.

Finally, we note that bands near 1,422, 1,174, and 1,069 cm^{-1} are affected in intensity on complexation with Cs^+. The 1,422 cm^{-1} band is assignable to the NH in-plane motion of the trp side chain on the basis of *N*-deuteration studies. The bands at 1,174 and 1,069 cm^{-1} are probably due to the C-O-H group of ethanolamine. The intensity changes observed in these bands suggest that the cations are bound close to these residues. This means they are likely to be bound at 2.5 Å from the ends of the channel. This is one of the two possible sites suggested by the x-ray work (Koeppe et al., 1979).

It is interesting to note that a recent molecular model fit to a 1.8 Å map of a GA-CsCl complex shows that the backbone is a left-handed antiparallel-chain double-stranded helix analogous to $\uparrow\downarrow\beta^{7.2}$ (Wallace, 1986). However, in these crystals the cesium sites are found at 7.1 Å from each end and separated by 11.8 Å. These crystals have a space group different from that of the CsSCN crystals studied by us and Koeppe et al. (1979). It is possible that the differences in space groups and cesium sites may be a consequence of the different counter ions present (Wallace, 1986).

GA in Solution and in Membranes

The structure of GA in dioxane solution has been reported to be $\uparrow\downarrow\beta^{5.6}$ on the basis of a two-dimensional NMR study (Arseniev et al., 1984). We have recorded a Raman spectrum in dioxane in the 1,500–1,800 cm^{-1} region (Fig. 5), and we find the strong amide I band to be at 1,665 cm^{-1}. On the basis of our normal mode analysis (Naik and Krimm, 1986), and the above discussion of the spectrum of native crystalline GA, we would also assign the predominant structure of GA in dioxane as $\uparrow\downarrow\beta^{5.6}$. The weak shoulder at 1,652 cm^{-1}, not seen in native crystalline GA, may be associated with another structure, which, as will be seen below, is likely to be $\beta^{6.3}$.

There have been a number of infrared studies of GA in membrane preparations (Sychev and Ivanov, 1982; Ovchinnikov and Ivanov, 1982; Urry et al., 1983*b*; Nabedryk et al., 1982). In liposome preparations with GA to dimyristoylphosphatidylcholine (DMPC) ratios of 1:35 to 1:350, Sychev and Ivanov (1982) observed a band at 1,631 cm^{-1}, and a band at 1,630 cm^{-1} was observed by them in vesicles with GA to dipalmitoylphosphatidylcholine (DPPC) ratios of 1:75 to 1:150. Based on these observa-

tions, they proposed a β_{DL}-hairpin conformation of GA, in other words, an extended GA chain bending back on itself through a type II' DL-turn. In membranes with a GA to DPPC ratio of 1:310, where they observed an amide I frequency of 1,634 cm^{-1}, they assigned a $\uparrow\downarrow\beta^{5.6}$ structure to GA. However, they used D_2O in their studies, and the exchange of NH to ND leads to a decrease in the amide I frequency, as reported in our earlier work (Naik and Krimm, 1984*a*, *b*). Urry et al. (1983*b*) also studied GA in membranes prepared in D_2O. They observed an amide I band at 1,633 ± 1 cm^{-1}. Based on this, they claimed that the amide I frequency could not be used to distinguish between single- and double-stranded structures. All these results neglected the effect of *N*-deuteration on the amide I frequency and this leaves these interpretations from infrared spectra questionable.

It is well known that the deuteration of the peptide group causes the amide I frequency of β structures to decrease (Dwivedi and Krimm, 1982*a*, *b*), and these studies indicate that a decrease of ~6 cm^{-1} is likely in the present case. Thus, the unshifted amide I mode for GA in vesicles should be at ~1,640 cm^{-1}. In fact, Nabedryk et al. (1982), from polarized infrared studies of GA in DMPC liposomes, showed that the amide I frequency is at 1,638 cm^{-1}; they also noted the absence of any shoulder near 1,685 cm^{-1}.

Normal mode calculations (Naik and Krimm, 1986) show that a distinction between $\uparrow\downarrow\beta^{5.6}$ and $\beta^{6.3}$, a structure convincingly demonstrated in vesicles (Urry et al., 1983*a*), may indeed be difficult on the basis of the position of the strong infrared band alone: the predicted frequencies of 1,636 and 1,643 cm^{-1}, respectively, are not unambiguously separable within the uncertainties of the calculations (Krimm, 1983). A more compelling distinction can be made on the basis of splittings in the amide I modes, 39 cm^{-1} being predicted for $\uparrow\downarrow\beta^{5.6}$ and 9 cm^{-1} being predicted for $\beta^{6.3}$. The absence of any large splittings for GA in vesicles (Nabedryk et al., 1982; Urry et al., 1983*b*) thus indicates the likely presence of a single-stranded structure. However, a distinction seems possible on the basis of the Raman-active amide I mode. This mode is found at 1,666 and 1,668 cm^{-1} for the $\uparrow\downarrow\beta^{5.6}$ and $\uparrow\downarrow\beta^{7.2}$ structures, respectively, and its frequency corresponds to the midpoint of the range of predicted frequencies after excluding that of the intense infrared mode (Naik and Krimm, 1986). On this basis, we would expect the Raman mode of $\beta^{6.3}$ to be found at ~1,650 cm^{-1}. This is significantly separated from those for the double-stranded helices, suggesting that the Raman spectrum should permit a distinction to be made between these structures.

Raman spectra of GA incorporated into vesicles are shown in Fig. 6. The spectrum of vesicles alone (Fig. 6 *a*) shows a single band at 1,640 cm^{-1} due to H_2O. In the early stages of incorporation (Fig. 6 *b*), a band is seen at 1,665 cm^{-1}, accompanied by a weak band at 1,654 cm^{-1}. In the conducting state, in other words after extended incubation (Fig. 6 *c*), the former band has disappeared and only the 1,654 cm^{-1} band is present. When the vesicles are dried (Fig. 6 *d*), a strong band returns at 1,671 cm^{-1}, with only a weak band remaining at ~1,650 cm^{-1} (the 1,640 cm^{-1} band due to H_2O is, as expected, essentially absent).

These results suggest the following sequence of events. The initial structure of GA is $\uparrow\downarrow\beta^{5.6}$, or $\uparrow\downarrow\beta^{7.2}$ if complexing with Na^+ occurs, with a characteristic Raman band near 1,667 cm^{-1}. As incorporation proceeds, the structure converts to $\beta^{6.3}$, with a characteristic Raman band near 1,650 cm^{-1}. On drying, the GA converts back substantially to a double-stranded structure. Of course, this last result does not establish whether the GA is still incorporated in the membrane. In any case, it appears that an interconversion between single- and double-stranded helical structures can occur relatively easily under these conditions. Incidentally, the mixed structure found in the dried state can account for the complex band observed in the infrared spectrum of the dried film (Urry et al., 1983*b*), whereas a simple band is seen in the infrared spectrum of the liposome system (Sychev and Ivanov, 1982).

CONCLUSION

From the infrared and Raman measurements, in conjunction with the normal mode calculations, we have been able to assign the structure of GA in ion-free and various ion-bound states, and in dioxane and membrane preparations.

The structure of GA in the crystalline state is cation-size dependent. In the native crystals GA has an antiparallel-chain double-stranded helical structure of the $\uparrow\downarrow\beta^{5.6}$ type. On binding Cs^+, K^+, or Tl^+, GA assumes a $\uparrow\downarrow\beta^{7.2}$ structure, which has an inner radius of 2 Å and can accommodate any of these ions. The $\uparrow\downarrow\beta^{7.2}$ structure for ion-bound GA (Naik and Krimm, 1984*b*) is also supported by recent x-ray studies (Wallace, 1986). Our results also favor one of the two choices for the ion-binding sites indicated by earlier x-ray work on the CsSCN complex (Koeppe et al., 1978, 1979), although these sites seem to be different from those in the CsCl complex (Wallace, 1986).

In the liposome suspension in H_2O the structure of GA is that of a $\beta^{6.3}$ helix. In the dried membrane preparations two structures exist, with the double-helical structure being prominent.

Our results show that the $\uparrow\downarrow\beta^{5.6}$ and $\beta^{6.3}$ structures have a similar infrared amide I frequency, at ~1,638 cm^{-1}. This points up the difficulty in assigning the structures on the basis of the infrared amide I mode alone, as was done in some of the earlier studies (Sychev and Ivanov, 1982; Ovchinnikov and Ivanov, 1982). However, a combination of Raman and infrared measurements together with normal mode calculations clearly permit more definitive assignments to be made of the structure of GA in various environments.

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