CIRCULAR DICHROISM AND OPTICAL ROTATORY DISPERSION OF GRAMICIDIN S IN AQUEOUS SOLUTION

Franco Quadrifoglio and Dan W. Urry

Institute for Biomedical Research
Education and Research Foundation
American Medical Association
Chicago, Illinois 60610

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Gramicidin S is a cyclic decapeptide whose primary structure consists of two identical pentapeptides closed into a ring.

$$\ulcorner\text{(L-Val - L-Orn - L-Leu - D-Phe - L-Pro)}_2\urcorner$$

Preliminary X-ray data (Schmidt et al., 1957) showed that the molecule in the crystal has a twofold symmetry axis and that the atoms are concentrated in layers 4.8 A apart along the c axis of the crystal, which is at right angles to the molecular symmetry axis. Previous infrared studies (Abbott and Ambrose, 1953) on crystals and optical rotatory dispersion measurements in aqueous solutions of gramicidin S (Ruttenberg et al., 1966) suggested that the molecule contains residues in α-helical conformation. Due to the cyclic nature of this antibiotic and to the presence of a twofold symmetry axis, conformational analysis calculations have been applied to this compound in an attempt to determine the lowest energy conformation. Two published results of such calculations show structures which are substantially different (Liquori et al., 1966; Vanderkooi et al., 1966).

In order to obtain information of the structure of Gramicidin S in aqueous solution and to assess the effect of chain length, circular dichroism (CD) and optical rotatory dispersion (ORD) measurements in the range 260-185 mμ have been carried out.

EXPERIMENTAL

Gramicidin S was obtained from Mann Research Laboratories, Inc. (Lot P1733). The compound was dissolved in water, centrifuged at 30,000 rpm for 10 minutes and then lyophilized. Amino acid analysis of this sample gave results in agreement with the reported composition of the antibiotic* (Table I).

TABLE I

Amino Acid Analysis of Gramicidin S

Amino Acid	Mole Ratio	Nearest Whole Number
Valine	0.93	1
Ornithine	1.07	1
Leucine	1.00	1
Phenylalanine	1.06	1
Proline	1.04	1

Paper chromatography using a solvent mixture of pyridine and 2-butanone (3:7) showed a single spot.* Nitrogen content using a Coleman 29 Nitrogen Analyzer gave a total of 13.1% in agreement with the reported composition of Gramicidin S. Dioxane was a spectroquality product (Burdick and Jackson Laboratories, Inc.). ORD and CD measurements were performed with a Cary 60 spectropolarimeter equipped with a Cary 6001 CD attachment.

A spectral bandwidth of 15 A was used. Unless otherwise stated, the temperature was 25°C. Scan speeds, pen period and time constants were chosen to maintain sufficient response time and to allow adequate signal-to-noise ratios. The CD unit was calibrated by using the Cary Model 1401 Circular Dichroism attachment for the Model 14. The standard used was an aqueous solution of d-10-camphor sulfonic acid (J. T. Baker, Lot No. 9-361) with an $\varepsilon_L - \varepsilon_R$ of 2.2 at 290 mμ. Cell path lengths were calibrated with solutions of chromate in 0.05N KOH.

* These analyses were carried out by Dr. Roderich Walter.

RESULTS AND DISCUSSION

The CD spectrum of Gramicidin S, as it appears in Fig. 1, shows two negative bands, the first at about 217 mμ and the second at 208 mμ, with ellipticity values on a mean residue basis of slightly more than 3×10^4 deg. cm^2/dmole, and a positive band at 185-186 mμ with an amplitude of about 3×10^4 deg. cm^2/dmole. The ORD curve shown in Fig. 2 displays a trough at 232-233 mμ with a molar rotation on a mean residue basis (uncorrected for the refractive index) of 18,000°, a shoulder at 210-212 mμ and a peak at 190-191 mμ with a mean molar rotation of about 60,000°. The ORD curve extrapolated to lower wavelengths gives a crossover at about 185-186 mμ, thus confirming the presence of a peak at these wavelengths in the CD spectrum. The position of this peak could otherwise be questioned on the basis of artifact at the short wavelength limit of the CD instrument.

The complexity of the spectrum and the large ellipticity values of the bands suggest a highly ordered structure for the antibiotic. Moreover the

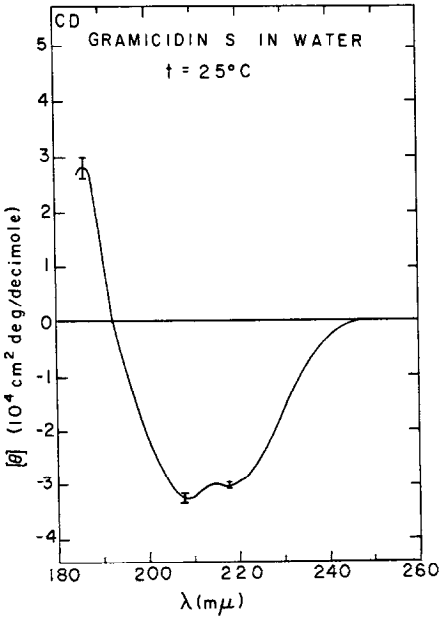

Fig. 1 - Circular dichroism of Gramicidin S.

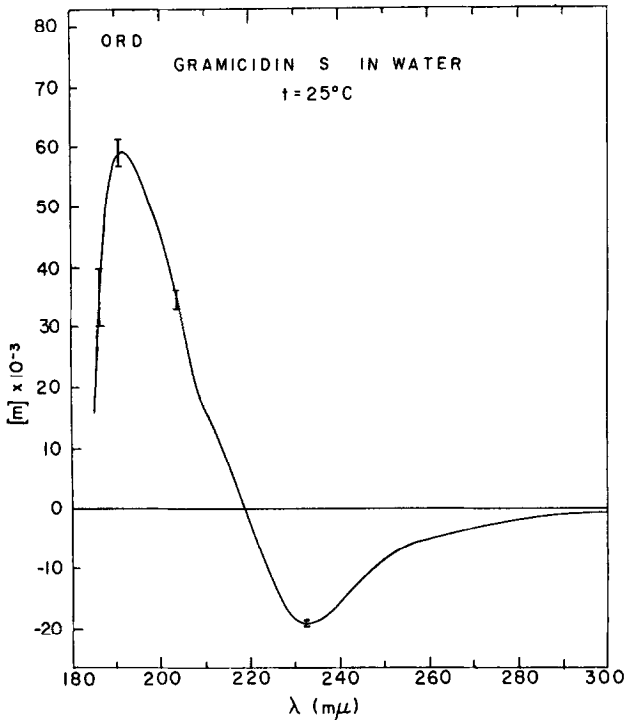

Fig. 2 - Optical Rotatory Dispersion of Gramicidin S.

position and sign of the bands are similar to those displayed by polypeptides in an α-helical conformation. The CD band found at 217 mμ may be attributed to the n-π* transition of the peptide chromophore, the shift from 222 to 217 mμ being in accord with the solvation of the peptide group by the water molecules. This shift has already been found in the case of aqueous solutions of random coil polypeptides, which display an n-π* band at 218 mμ (Holzwarth and Doty, 1965)

The two CD bands located at 208 mμ and 185 mμ would correspond to the exciton splitting of the NV_1 (π-π*) transition of the peptide chromophore as predicted from the theory for α-helical conformation of polypeptides and proteins (Moffitt, 1956). The relatively low amplitude of the positive band compared to that of the 208 mμ band is in qualitative agreement with the theoretical findings of Woody and Tinoco (1967) on the effect of chain length upon the rotatory parameters of α-helical and 3_{10} conformations. The results

of their calculations show that the 198 mμ ORD peak (which is the counterpart of the positive band in the CD spectrum) is much more dependent on the chain length than the 233 mμ trough. The low amplitude of the 186 mμ band and its occurrence at shorter wavelengths are also in agreement with preliminary results on short helices of poly-L-alanine in trifluoroethanol solutions (Quadrifoglio and Urry, unpublished results).

An increase of the temperature to 70°C in aqueous solutions of Gramicidin S does not appreciably change the CD spectrum. This demonstrates the high stability of the structure and rules out contributions to the spectrum which may arise from intermolecular association in aqueous solution. Differential dialysis studies (Craig, 1964) have indicated lack of association. The temperature studies also suggest the absence of substantial contribution to the rotation due to the aromatic rings of D-phenylalanine residues which may have been frozen in a particular conformation. On the other hand ORD studies of aqueous solutions of L-phenylalanine (Rosenberg, 1966) and of L-phenylalanine containing polypeptides (Sage and Fasman, 1966) show that the rotations attributable to the aromatic ring are of sufficiently low amplitude that the presence of two such residues would not greatly distort the optical rotation spectra of Gramicidin S. This is confirmed by the very low rotations observed at 260 mμ.

A study of the solvent effect employing dioxane and water mixtures showed no appreciable change in the CD spectrum up to 70% dioxane. These experiments again exclude contribution to the optical rotation properties due to intermolecular aggregation via hydrophobic interactions.

A comparison of these findings with the structures obtained by means of conformational analysis calculations (Liquori et al., 1966; Vanderkooi et al., (1966) favors the conformation calculated by Liquori et al. Their structure contains eight out of ten peptide linkages arranged in two separate turns of α-helix, whereas the structure proposed by Vanderkooi et al., is more similar to a β-pleated sheet. A calculation of the number of residues in α-helical

conformation based on the amplitude of the n-π^* band gave surprising agreement with the number of such residues present in the Liquori structure. If the agreement is not fortuitous one may conclude that a more meaningful estimate of the helix content in proteins should be based on the amplitude of the negative CD bands, whereas the positive band may give information concerning chain length.

In conclusion, the CD data, discussed on the basis of current knowledge of optical rotation of polypeptides and taken together with other observations (Abbott and Ambrose, 1953) support the presence in the Gramicidin S molecule of amino acids in helical conformation. Due to the chain length and cyclic nature of the antibiotic the extent of regular structures is limited. It may be concluded that just a few residues with the proper ψ and ϕ angles (Edsall et al., 1966) can give rise to large rotations which simulate those of long chain regular structures. This data provides clarification of the apparent disparity between the X-ray (Dickerson et al., 1967) and the optical rotation data of cytochrome c (Urry and Doty, 1965; Ulmer, 1965; and Urry, 1965). Proper comparison of solution structures as deduced from spectral measurements and crystal structures as determined by X-ray studies depends on sufficient resolution to give the ψ and ϕ angles. Furthermore there is, as yet, no basis on which one might conclude that the CD pattern of an α-helix cannot be simulated by residues related with ψ and ϕ angles other than those of the α-helix.

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