Preparation and Properties of *O*-Dansyltyrosine Gramicidin C†

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ABSTRACT: Gramicidins A, B, and C are a family of polypeptide antibiotics which facilitate the passive diffusion of alkali cations and protons through lipid bilayer membranes. It is clear that gramicidin forms a multimeric transmembrane channel and it has been suggested that the channel is an ion-conducting dimer in equilibrium on the membrane with nonconducting monomer. We describe the preparation and purification of a derivative of gramicidin C in which the phenolic hydroxyl of the tyrosine at position 11 has been esterified to 8-dimethylaminonaphthalene-1-sulfonate (dansyl). This derivative fluoresces strongly in the visible with an emission maximum in dioxane of 530 nm, an emission lifetime of 16 ns, and a quantum yield of 0.8. Veatch et al. ((1975), *J. Mol. Biol. 99,* 75) have shown this *O*-dansyltyrosine gramicidin C to be a fully active analogue of gramicidin A in artificial lipid bilayer membranes. We here utilize this derivative to further characterize the state of aggregation and rotational mobility of the four interconvertible conformational species formed by gramicidin in nonpolar organic solvents (Veatch et al. (1974), *Biochemistry 13,* 5249; Veatch and Blout (1974), *Biochemistry 13,* 5257). Fluorescence energy transfer from the tryptophans of gramicidin A to the *O*-dansyltyrosine of this derivative supports the conclusion that all of these gramicidin isolated species are aggregates. Decay of fluorescence polarization anisotropy measurements yield a rotational correlation time of 1 ns for the *O*-dansyltyrosine chromophore in ethanol in good agreement with the more detailed information previously obtained by ^{13}C-nuclear magnetic resonance for the monomer in dimethyl sulfoxide (Fossel et al. (1974), *Biochemistry 13,* 5264). However, it is likely that the chromophore has much more rotational mobility than the rest of the gramicidin molecule in the aggregated conformational states.

Gramicidins A, B, and C are a family of linear polypeptide antibiotics which have been separated by countercurrent distribution (Craig et al., 1949; Gross and Witkop, 1965). Gramicidin C has the following sequence (Sarges and Witkop, 1965):

1	2	3	4	5	6	7
Formyl-L-Val-	Gly-L-	Ala-D-	Leu-L-	Ala-D-	Val-L-	Val-

8	9	10	11	12	13	14	15
D-Val-	L-Trp-	D-Leu-	L-Tyr-	D-Leu-	L-Trp-	D-Leu-	L-Trp-

$NHCH_2CH_2OH$

Gramicidin A differs from gramicidin C by having an L-tryptophan at position 11, and gramicidin B has an L-phenylalanine. There is also a 10% substitution of L-isoleucine for L-valine at position 1 for each of gramicidins A, B, and C (Gross and Witkop, 1965). Almost all of the works characterizing antibiotic activity have used the naturally occurring mixture which is approximately 80% gramicidin A, 10% gramicidin B, and 10% gramicidin C. Gramicidin A facilitates the passive diffusion of alkali cations and protons through biological (Harold and Baarda, 1967; Chappell and Crofts, 1965; Harris and Pressman, 1967) and artificial lipid bilayer membranes (Mueller and Rudin, 1967; Myers and Haydon, 1972). There is direct evidence that this gramicidin-induced membrane conductance is mediated by the formation of transmembrane channels (Hladky and Haydon, 1970, 1972; Krasne et al., 1971); and there is some evidence that the ion-conducting channels are dimers in equilibrium in the membrane with nonconducting monomers (Tosteson et al., 1968; Bamberg and Läuger, 1973), although it has not previously been possible to directly measure the amount of gramicidin in these membranes.

We describe here the preparation of a derivative of gramicidin C in which the phenolic hydroxyl of the L-tyrosine at position 11 has been reacted with 8-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride). This *O*-dansyltyrosine gramicidin C fluoresces strongly with emission maximum near 530 nm. Recently, Veatch et al. (1975) have shown that this derivative is a fully active analogue of gramicidin A in artificial lipid bilayer membranes. They used the membrane fluorescence of this derivative to prove that the channels are dimers of the nonconducting species, and that under certain conditions most, if not all, of the gramicidin on the membrane is actually involved in these dimer channels.

Although membrane investigations such as these provided a major motivation for the preparation of *O*-dansyltyrosine gramicidin C, this derivative has also proved useful for further characterizing the aggregation and dynamics of gramicidin in solution. At sufficiently high concentration in nonpolar organic solvents, gramicidins A, B, and C each form at least four distinct conformational species (Veatch et al., 1974; Veatch and Blout, 1974). The interconversion rate between these conformational species is sufficiently slow for them to be physically isolated and individually characterized. They are denoted **1, 2, 3,** and **4** in order of their increasing mobilities on thin-layer chromatography (TLC). Species **1, 2,** and **4** are probably helical structures with largely parallel β-hydrogen bonds; species **1** and **2** have a handedness opposite to that of species **4.** Species **3** has largely antiparallel β-hydrogen bonding.

Here we exploit the fluorescence energy transfer from the tryptophans of gramicidin A to the *O*-dansyltyrosine of our derivative to demonstrate that those isolated species examined are gramicidin aggregates. The decay of fluorescence polarization anisotropy for the *O*-dansyltyrosine chromophore yields an estimate of the rotational correlation time of gramicidin monomer in more polar solvents which compares well with the

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more detailed ^{13}C nuclear magnetic resonance relaxation measurements of Fossel et al. (1974). However, the *O*-dansyltyrosine chromophore appears to have much more mobility than the rest of the gramicidin molecule in the dimer forms.

Methods and Materials

Dansylation of Gramicidin C. Gramicidin C was separated by countercurrent distribution from the commercially available mixture of gramicidins A, B, and C (Gross and Witkop, 1965), and we are indebted to Dr. Gross for performing the separation and supplying us with samples of each of the natural gramicidins. The gramicidin C sample was pooled from fractions 700–799 of a 500 tube distribution operated in the upper phase withdrawal mode. By amino acid analysis it was show to contain about 0.6 mol of tyrosine per 2 mol of alanine.

Typically, 8 mg of Na_2CO_3 in 1.0 ml of water was added with stirring to 2.0 ml of acetone containing 2 mg of gramicidin C and a 20-fold excess of dansyl chloride (8-dimethylaminonaphthalene-1-sulfonyl chloride, Sigma Chemical) (after Seiler, 1970). After 2 h at room temperature in the dark, the yellow color had disappeared; the solution was then evaporated, triturated with methanol, and chromatographed on a Sephadex LH-20 column (1 × 10 cm, methanol) to separate low molecular weight impurities from the gramicidin.

A tyrosine containing peptide, t-Boc[1]-Tyr-Gly-Pro-OH (supplied by Dr. Paul Young) was dansylated as described above, and TLC showed that all of the peptide was reacted. After evaporation the reaction mixture was dissolved in water, neutralized, and extracted three times with $CHCl_3$. The $CHCl_3$ was washed three times with 1×10^{-3} M HCl to remove dansylate. Preparative TLC was used for the final purification. The dansyl derivative of *N*-acetyl-L-tyrosinamide was also prepared and purified by silica TLC.

Amino Acid Analysis. The hydrolysis conditions were those of Gross and Witkop (1965), except that phenol was added to suppress chemical degradation of tyrosine. The analysis was carried out on a Beckman (Model 120B) amino acid analyzer. Hydrolysis of the tyrosine containing peptide, t-Boc-Tyr-Gly-Pro-OH, under these conditions yielded 0.88 mol of tyrosine per mol of peptide; after dansylation, as described above, 0.04 mol of tyrosine was found. Thus, the loss of tyrosine under these hydrolysis conditions is around 10% and *O*-dansyltyrosine is not converted into free tyrosine. The tryptophan values reported were obtained from the absorbance at 290 nm of the samples, prior to hydrolysis, in methanol using an extinction coefficient of 5600 cm^{-1} M^{-1} and correcting for the absorbance of *O*-dansyltyrosine at 290 nm.

Fluorescence Measurements. Unless otherwise noted, fluorescence measurements were carried out on a Hitachi Perkin-Elmer MPF-2A spectrofluorimeter with temperature control accessory. The excitation spectra were corrected using a rhodamine B quantum counter (Chen, 1967). The corrected emission spectra, the emission lifetime measurements, and the decay of fluorescence polarization anisotropy measurement were all carried out on equipment kindly made available by Dr. Lubert Stryer (Yale University). A Corning 7-54 filter was used for excitation, a 3-72 for emission and the temperature was controlled at 23 °C.

Results

Purification and Chemical Characterization. The dansylation of gramicidin C using Na_2CO_3, described under Methods and Materials, followed a suggestion of Seiler (1970) for the dansylation of phenols. The ratio of absorbance at 350 nm to that at 290 nm was used to quantitate the amount of *O*-dansyltyrosine per mole of gramicidin. Seiler (1970) determined the extinction coefficient to be 4050 cm^{-1} M^{-1} at 350 nm for dansylphenol in methanol. From the model *O*-dansyltyrosine peptide absorbance spectrum, the extinction coefficient at 290 nm would be 1500 cm^{-1} M^{-1}. Taking ϵ_{290} 5600 cm^{-1} M^{-1} for each of the three tryptophans in gramicidin C (in methanol, based on the amino acid analysis of gramicidin A), the expected A_{350}/A_{290} ratio is 0.22. After the dansylation the observed ratio was only 0.11, implying a 50% yield. When gramicidin A was allowed to react under the same conditions, about 4% dansylation was observed, presumably of the C-terminal hydroxyl.

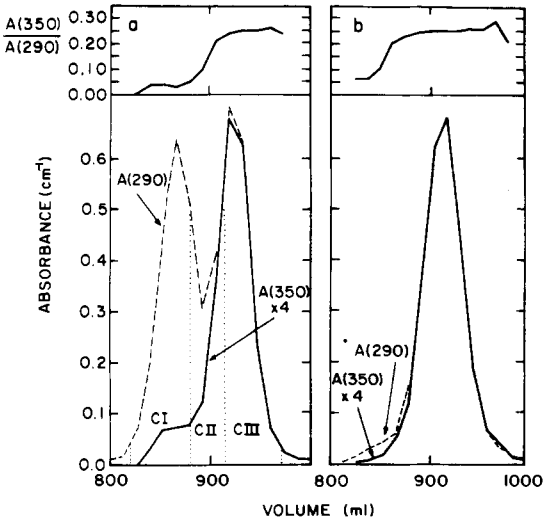

FIGURE 1: (a) Elution profile of dansylation products of gramicidin C on Sephadex LH-20 (150 × 4 cm) in methanol; (b) rechromatography of fraction CIII from a.

Separation of the dansylated gramicidin C from the undansylated material was achieved on a Sephadex LH-20 (150 × 4 cm, methanol) column. The elution profile is shown in Figure 1a. The A_{290} profile indicates that about 50% of the gramicidin was retarded relative to the rest, and that all of the dansyl absorbance was associated with the material in this peak. The ratio of A_{350} to A_{290} was proportional to the degree of dansylation and was reasonably constant over the retarded peak with an average value of 0.25. This value is uncorrected for the effect of double dansylation (tyrosine and C-terminal hydroxyl), but it is in reasonable agreement with the value of 0.22 expected for 100% dansylation. For a preparation (reacted with $NaHCO_3$ instead of Na_2CO_3) for which the yield of *O*-dansyltyrosine gramicidin C was only 10%, the retarded peak on the long column was correspondingly reduced, demonstrating that the separation was due to the presence of the dansyl group alone. The visible fluorescence of the gramicidin A product was dispersed widely on the long column and had an emission maximum at 460 nm, which is typical of free dansylate. These observations suggest decomposition of the C-terminal *O*-dansyl on the column. The *O*-dansyltyrosine seemed to be much more stable (if stored in methanol solution in the dark at 4 °C), though it did decompose slowly to form products with an emission peak at 460 nm at room temperature in the light. Fraction CIII (Figure 1a) was rechromatographed on the same column, yielding the profile in Figure 1B. The absorbance spectrum of *O*-dansyltyrosine gramicidin C is shown in Figure 2.

[1] Abbreviation used: t-Boc, *tert*-butoxycarbonyl.

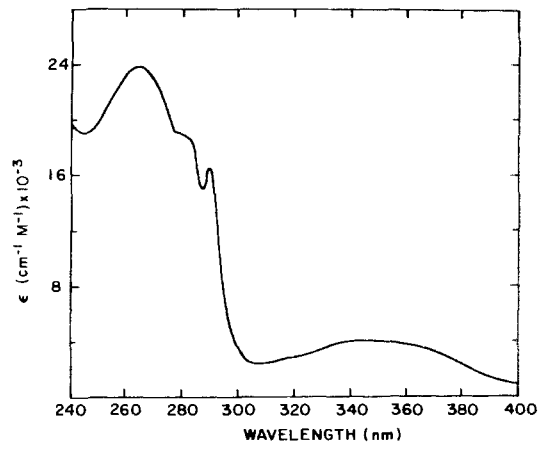

FIGURE 2: Absorbance spectra of *O*-dansyltyrosine gramicidin C (in methanol).

The material from the long LH-20 chromatography was pooled into three fractions for amino acid analysis (CI, CII, and CIII, in order of increasing dansyl content as shown in Figure 1a). Control experiments described under Materials and Methods showed, under the hydrolysis conditions employed, about 90% recovery of tyrosine and little or no conversion of *O*-dansyltyrosine to free tyrosine. For each of the fractions the free tyrosine recovery was less than 0.1 mol of tyrosine per mol of peptide, compared with 0.5 mol for the starting material. The amino acid compositions of fractions CI and CIII are shown in Table I along with that of the gramicidin C starting material. The tryptophan and *O*-dansyltyrosine values were obtained from absorbance before hydrolysis and the "total tyrosine" is the sum of *O*-dansyltyrosine before hydrolysis and free tyrosine after. The valine values may be low in part due to incomplete hydrolysis of the Val-Val-Val sequence in gramicidin (Gross and Witkop, 1965). While fraction CIII is significantly too high in Gly and too low in Val, it has better values than the starting material in Trp and particularly in total Tyr. The undansylated fraction, CI, probably consists of two components lacking tyrosine—part gramicidin A and part the more polar "gramicidin D" (see Table II of Gross and Witkop).

O-dansyltyrosine gramicidin C yields a 4 × 4 interconversion pattern on two-dimensional TLC (see Veatch et al., 1974) with an additional fastest moving spot remaining on the diagonal. This fastest spot may be gramicidin C dansylated at both tyrosine and the C-terminal hydroxyl.

Fluorescence in Organic Solvents. The wavelength of maximum emission, the quantum yield, and mean emission lifetime of the *O*-dansyltyrosine chromophore in *O*-dansyltyrosine gramicidin C are tabulated in Table II for solvents of varying polarity, corresponding to various gramicidin conformational states. The mean emission lifetime was also measured for the model compound *N*-acetyl-(*O*-dansyltyrosin)amide in the same solvents for comparison. In dioxane the decay of emission was that of a single exponential, as shown in Figure 3, for each of the gramicidin isolated species containing *O*-dansyltyrosine gramicidin C. All of the species had very nearly equal lifetimes, and the lifetime of the model compound was nearly equal to that of the gramicidin species. This result suggests that, for all of these conformations, the *O*-dansyltyrosine chromophore is relatively fully accessible to the solvent.

In more polar solvents the emission decay deviated significantly from that expected for a single lifetime. In ethanol the decay could be accurately fitted by assuming that about 10% of the chromophores had an emission lifetime about twice that of the majority of the chromophores. The value given in Table II represents the mean lifetime. The emission decay of the model compound was accurately a single lifetime even in the more polar solvents. The relatively low level of heterogeneity in the emission lifetime of *O*-dansyltyrosine gramicidin C could have two possible sources: chemical heterogeneity or a physical heterogeneity of the environment of the chromophore. Although the emission heterogeneity was constant across the elution profile in Figure 1a, it is still not possible to exclude the possibility of chemical heterogeneity.

In ethanol the mean lifetime of *O*-dansyltyrosine in gramicidin is about twice that of the model compound, suggesting that in the monomer state in ethanol the chromophore is at least partially shielded from the solvent. If this shielding of the chromophore varied significantly for different monomer conformations (e.g., helices of opposite handedness), it might explain the heterogeneous emission lifetime observed in ethanol. The low amount of emission heterogeneity observed would have negligible effect on the measurements of fluorescence polarization anisotropy in this solvent.

Corrected excitation and emission spectra of *O*-dansyltyrosine gramicidin C in the monomer state are shown in Figure 4. Fluorescence energy transfer is evident from the component of tryptophan absorbance seen in the excitation spectrum of

Table I: Amino Acid Analysis of Chromatographic Fractions from Dansylation of Gramicidin C.

Amino Acid	Moles Per Mole of Peptide[a]		Fraction[b]	
	Gramicidin C Theor	Original Gramicidin C	CI	CIII
Gly	1	1.3	1.5	1.4
Ala	2	1.9	1.5	2.2
Val	4	2.4	2.1	2.3
Leu	4	3.9	4.1	3.8
Trp	3	3.6	4.7	2.9
O-Dansyltyrosine			0.0	1.0
Total Tyr	1	0.5	0.1	1.1

[a] Moles of peptide is the ratio of observed to theoretical averaged over all amino acids except Tyr and *O*-dansyltyrosine. [b] See Figure 1a.

Table II: Emission Lifetime and Quantum Yield of Dansyltyrosine in *O*-Dansyltyrosine Gramicidin C.

Gramidicin State		Solvent	Q	Emission Max (nm)	Emission Lifetime τ (ns)	
					O-Dansyltyrosine Gramicidin C	Model Compd[b]
Isolated species[a]	1	Dioxane	0.8	530	16.1	15.3
	2	Dioxane			15.7	
	3	Dioxane			15.0	
	4	Dioxane			15.6	
Monomer		Ethanol	0.2	555	5	2.4
		Methanol	0.1	565	3	1.6

[a] Prepared from *O*-dansyltyrosine gramicidin C–gramicidin A (1:5). [b] *N*-Acetyl(*O*-dansyltyrosin)amide.

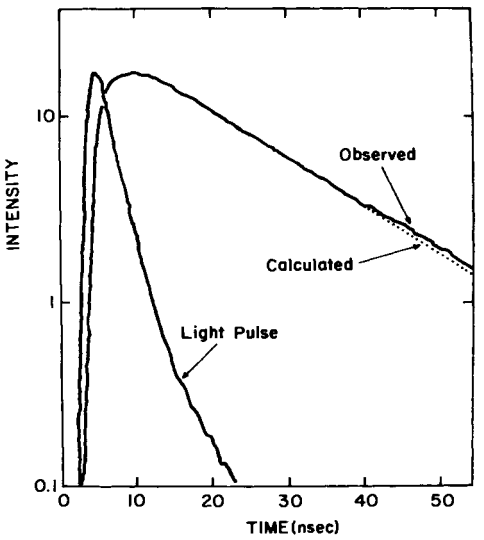

FIGURE 3: Time course of dansyltyrosine fluorescence emission in dioxane. The dotted curve is that calculated for an emission lifetime of 15.7 ns.

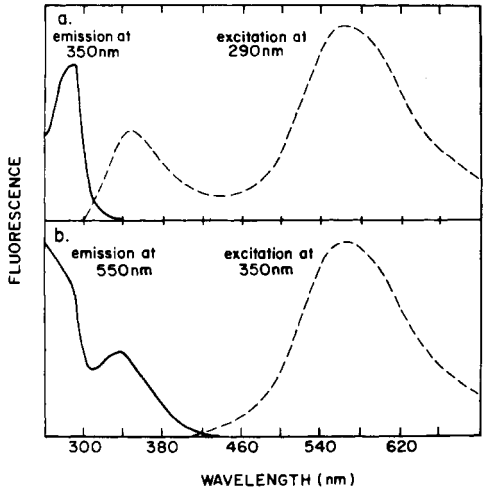

FIGURE 4: Corrected excitation and emission spectra of *O*-dansyltyrosine gramicidin C in methanol. (a) Excitation spectrum for emission at 350 nm (—); emission spectrum for excitation at 290 nm (- - -). (b) Excitation spectrum for emission at 550 nm (—); emission spectrum for excitation at 350 nm (- - -). Excitation and emission slit widths were both 4 nm.

O-dansyltyrosine emission at 550 nm (Figure 4). The measured A_{290}/A_{350} ratio for purified *O*-dansyltyrosine gramicidin C is 4.0 and, if the energy transfer from tryptophan to *O*-dansyltyrosine were 100% efficient, then the corrected excitation spectrum of *O*-dansyltyrosine would have the same A_{290}/A_{350} ratio. If the efficiency of transfer were 0%, then the ratio would be 0.4 because of the extinction coefficient of *O*-dansyltyrosine at 290 nm (see Materials and Methods).

The energy transfer evident in Figure 4 is necessarily intramolecular since under these conditions the *O*-dansyltyrosine gramicidin C is in the monomer state ($\sim 1^{-5}$ M in methanol, see Veatch and Blout, 1974). The addition of an equal amount of gramicidin A would not alter the excitation spectrum; however, at equilibrium in the aggregated state, adding gramicidin A nearly doubles the excitation ratio. The upper part of Table III shows the excitation ratio for a mixture of isolated species **1** and **2** and for species **3** (prepared from *O*-dansyltyrosine gramicidin C as described by Veatch et al., 1974). The average efficiency of energy transfer has been calculated. The small difference between the two species for

Table III: Energy Transfer Parameters for Gramicidin Isolated Species in Ethyl Acetate.

Species	Dansyltyrosine 290 nm/350 nm Excitation Ratio	Tryptophan Emission Relative to Gramicidin B	Fluorescence Energy Transfer
	O-Dansyltyrosine Gramicidin C Alone		
1 + 2	2.9	6%	66%
3	2.8	9%	63%
	O-Dansyltyrosine Gramicidin C–Gramicidin A (1:9)		
1 + 2	4.0–4.9		
3	3.3–3.9		
4	3.9–4.7		

the energy transfer and tryptophan quenching may not be significant. Over 90% of the tryptophan fluorescence is quenched (relative to gramicidin B, an ideal model for the three tryptophans in the absence of the *O*-dansyltyrosine), while only 60% is transferred to *O*-dansyltyrosine. Such a result could be obtained for multiple donors if the donors with higher quantum yields (in the absence of the acceptor) transferred more than those with lower quantum yields. Alternatively, an additional source of tryptophan quenching by dansyl has been reported in another system (Conrad and Brand, 1968), though no specific mechanism was proposed. In the absence of such low donor quantum yields, one would expect nearly 100% efficient transfer from tryptophan to the *O*-dansyltyrosine within 10 Å along the chain, unless the chromophores were rigidly held in very unfavorable relative orientations.

The lower part of Table III shows the excitation ratios for the isolated species prepared from a *O*-dansyltyrosine gramicidin C diluted with a ninefold excess of gramicidin A. The increases in the ratio upon dilution imply that species **3** and species **1** and/or species **2** are aggregates. Species **4** appears only very weakly on one-dimensional TLC of *O*-dansyltyrosine gramicidin C by itself as monitored by the *O*-dansyltyrosine fluorescence, whereas it is much stronger in the gramicidin A dilution. This increase in the fraction of *O*-dansyltyrosine gramicidin A in species **4** caused by the addition of gramicidin A is most simply consistent with species **4** containing at least one molecule each of *O*-dansyltyrosine gramicidin C and gramicidin A in the mixture. The high absolute value of the excitation ratio is also consistent with, but does not prove, that species **4** is also an aggregate.

In dioxane the mean fluorescence polarization anisotropy for all of the gramicidin isolated species was 0.01–0.02, too small for decay of anisotropy measurements. In the monomer state achieved at low concentrations in ethanol (Veatch and Blout, 1974), the emission lifetime of 5 ns was short enough to result in a mean fluorescence polarization anisotropy of 0.05. A decay of fluorescence polarization anisotropy measurement (Yguerabide et al., 1970) yielded a rotational correlation time of about 1 ns for the *O*-dansyltyrosine chromophore.

If gramicidin equilibrated at high concentration in ethanol is diluted to low concentration, then the isolated species present break down into monomers with half-times from 0.5 to 2 h (Veatch and Blout, 1974). With pure *O*-dansyltyrosine gramicidin C, the excitation ratio associated with energy transfer does not change much during this decay, but the tryptophan emission appears to increase from 5 to 20% (relative to gramicidin B). This change may be partially due to a small percentage impurity of gramicidin A. In mixtures with excess gramicidin A there is, of course, a steady decrease in the

excitation ratio which can be used to follow the kinetics of the disaggregation.

Discussion

O-Dansyltyrosine gramicidin C has been prepared in highly purified form. After the second chromatography, shown in Figure 1b, the purity of the fractions of the slower half of the peak can be conservatively estimated to be greater than 90%. Such fractions have been shown to be fully active relative to gramicidin A on artificial lipid bilayer membranes (Veatch et al., 1975).

The increase in the excitation ratio upon dilution with gramicidin A proves that gramicidin isolated species **3** and one or both of species **1** and **2** are aggregates. These results further support the conclusion of Veatch and Blout (1974) that all of the isolated species are aggregates. It is not possible to infer the order of the aggregates from the energy transfer results, although Veatch and Blout (1974) have suggested that all of the species are dimers.

The fluorescence parameters of the gramicidin isolated species containing *O*-dansyltyrosine gramicidin C, reported in Tables II and III, are remarkably insensitive to the conformational differences among the species. It seems likely that the emission lifetimes are largely determined by the environment provided by the solvent. The similarities among the energy transfer parameters are due in part to the fact that fluorescence energy transfer is effective over relatively long distances (about 30 Å for these chromophores; see Stryer, 1968). Consequently, it is likely that fluorescence energy transfer experiments from gramicidin A to dansyl gramicidin C (tryptoptan to *O*-dansyltyrosine) should be feasible in artificial lipid bilayer membranes. Veatch et al. (1975) have used simultaneous conductance and fluorescence measurements with *O*-dansyltyrosine gramicidin C alone to prove that the ion-conducting channels are dimers of the nonconducting gramicidin state in the membrane. But is the nonconducting state a monomer? An intermolecular energy transfer experiment on the membrane would provide an unambiguous answer.

We have obtained an estimate of the rotational correlation time for the *O*-dansyltyrosine chromophore of 1 ns in the monomer state in ethanol. Fossel et al. (1974) have used ^{13}C nuclear magnetic resonance relaxation measurements to measure rotational correlation time of 2 ns in the monomer state in dimethyl sulfoxide for both the tryptophan indole ring and α carbons in gramicidin. When account is taken of the twofold greater viscosity of dimethyl sulfoxide, the agreement is very good. The ^{13}C NMR measurements also showed the tryptophan side chains to be held relatively rigidly even on a 20-ns time scale in the dimer state; however, it is possible that rotation about the sulfonic ester linkage might allow the naphthalene ring of the *O*-dansyltyrosine chromophore much more rotational mobility than the sterically hindered tryptophan side chains. In fact, the low mean fluorescence polarization anisotropy values for the dansyltyrosine emission (0.01–0.02) for all of the isolated species in dioxane imply a correlation time about an order of magnitude faster than the nearly 20 ns inferred for the tryptophan from the ^{13}C NMR measurements in methanol.

Summary

1. Gramicidin C has been specifically O-dansylated on the L-tyrosine at position 11 and this *O*-dansyltyrosine gramicidin C has been chromatographically purified.

2. In dioxane, *O*-dansyltyrosine gramicidin C has an emission maximum of 530 nm, an emission lifetime of 16 ns, and a quantum yield of 0.8. All three parameters are sensitive to the polarity of the environment of the *O*-dansyltyrosine moiety.

3. Fluorescence energy transfer from the tryptophans of gramicidin A to the *O*-dansyltyrosine of the derivative supports the conclusion that all of the gramicidin isolated species are aggregates.

4. Decay of fluorescence polarization anisotropy measurements yields a rotational correlation time of 1 ns for the *O*-dansyltyrosine chromophore in the monomer state of *O*-dansyltyrosine gramicidin C in ethanol. It is likely that the chromophore has more rotational mobility than the rest of the molecule in the dimer state.

Acknowledgments

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References

Bamberg, E., and Läuger, P. (1973), *J. Membr. Biol. 11,* 177.
Chappell, J. B., and Crofts, A. R. (1965), *Biochem. J. 95,* 393.
Chen, R. F. (1967), *Anal. Biochem. 20,* 339.
Conrad, R. H., and Brand, L. (1968), *Biochemistry 7,* 777.
Craig, L. C., Gregory, J. D., and Barry, G. T. (1949), *Cold Spring Harbor Symp. Quant. Biol. 14,* 24.
Fossel, E. T., Veatch, W. R., Ovchinnikov, Y. A., and Blout, E. R. (1974), *Biochemistry 13,* 5264.
Gross, E., and Witkop, B. (1965), *Biochemistry 4,* 2495.
Harold, F. M., and Baarda, J. R. (1967), *J. Bacteriol. 94,* 53.
Harris, E. J., and Pressman, B. C. (1967), *Nature (London) 216,* 918.
Hladky, S. B., and Haydon, D. A. (1970), *Nature (London) 225,* 451.
Hladky, S. B., and Haydon, D. A. (1972), *Biochim. Biophys. Acta 274,* 294.
Krasne, S., Eisenman, G., and Szabo, G. (1971), *Science 174,* 412.
Mueller, P., and Rudin, D. O. (1967), *Biochem. Biophys. Res. Commun. 26,* 398.
Myers, V. B., and Haydon, D. A. (1972), *Biochim. Biophys. Acta 274,* 313.
Sarges, R., and Witkop, B. (1965), *Biochemistry 4,* 2491.
Seiler, N. (1970), in *Methods Biochem. Anal. 18,* 259.
Stryer, L. (1968), *Science 162,* 526.
Tosteson, D. C., Andreoli, T. E., Tieffenberg, M., and Cook, P. (1968), *J. Gen. Physiol. 51,* 373S.
Veatch, W. R., and Blout, E. R. (1974), *Biochemistry 13,* 5257.
Veatch, W. R., Fossel, E. T., and Blout, E. R. (1974), *Biochemistry 13,* 5249.
Veatch, W. R., Mathies, R., Eisenberg, M., and Stryer, L. (1975), *J. Mol. Biol. 99,* 75.
Yguerabide, J., Epstein, H. F., and Stryer, L. (1970), *J. Mol. Biol. 51,* 573.