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Diffusion Experiments on Gramicidin S, Tyrocidine, and Gramicidin

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In connection with work on gramicidin S, tyrocidine and gramicidin, it was of interest to get an approximate idea of the molecular weights of these peptides. As it could be supposed that these would be of the order of some thousands, we decided to measure the diffusion constants for these peptides.

For an arbitrary molecule we have

$$f = \frac{RT}{D}$$

where f is the frictional coefficient per mol., R the gas constant ($8.314 \cdot 10^7$ ergs per °C per mol.), T the absolute temperature, and D the diffusion constant. For a spherical and unhydrated molecule the frictional coefficient, f_0, may be expressed in the following way

$$f_0 = 6\pi\eta N\left(\frac{3MV}{4\pi N}\right)^{1/3}$$

where η is the viscosity of the solution (in poises), N the Avogadro number, M the molecular weight and V the specific volume of the solute. By combining these two formulae and solving for M, we get

$$M\left(\frac{f}{f_0}\right)^3 = \frac{R^3T^3}{162\,\pi^2N^2\eta^3} \cdot \frac{1}{D^3V} = \frac{k}{D^3V}$$

where k is a constant depending upon the temperature and the solvent. For water at a temperature of 20° C, k has a value of $24.14 \cdot 10^{-15}$. For an un-

hydrated spherical particle $f/f_0 = 1$, in all other cases $f/f_0 > 1$. This means that diffusion experiments will furnish us with an upper limit for the molecular weight, if effects due to association or dissociation are disregarded.

Various solvents were used in the experiments, which were done at 20° C, but in order to get comparable values, the diffusion constants were all reduced to water at 20° C by means of the usual equation

$$D_1 = D_2 \cdot \frac{\eta_2}{\eta_1}$$

The relative viscosities of the various solvents used in the experiments were determined in an Ostwald viscosimeter using water as the reference substance. Potassium chloride was used with the peptide hydrochlorides studied, in order to eliminate effects due to diffusion potentials.

Table 1. Relative viscosity of solvents used in the diffusion experiments. Temperature: 20.0° C.

Solvent	Outflow time (in sec)				Average	Density	η/η_0
Water	46.7	46.8	46.7	46.6	46.7	0.998	1
0.1 *M* KCl in 50 per cent (v/v ethanol	94.7	94.2	94.6	94.6			
	94.5	94.8	94.6	94.9	94.6	1.062	2.16
Water	108.3	108.8	108.8	108.9	108.7	0.998	1
50 per cent (v/v) ethanol	326.6	326.1	326.0	326.0	326.2	0.928	2.78
70 » » » »		300.7	300.5	300.7	300.6	0.884	2.49
0.2 *M* KCl in 50 per cent ethanol		317.4	317.2	317.6	317.4	0.935	2.73

The diffusion was followed by means of the Lamm scale method [1]. The diffusion constant was calculated both according to the »area method» and according to the »moment method»[2]. In the first case the diffusion constant is defined in the following way

$$D_A = \frac{A^2}{4\pi t H^2}$$

where A denotes the area between the curve and the x-axis, H is the maximum height of the curve and t is the time after the formation of the boundary. In the second case the diffusion constant is calculated from the expression

$$D_m = \frac{m_2}{2\,t\,A}$$

where m_2 is the second moment of the curve about the vertical axis through the arithmetic medium of the curve.

The first experiments were carried out in the ordinary Lamm diffusion cell [1] with inner part of stainless steel. The peptides were dissolved in 50 per cent (v/v) aqueous acetic acid solutions 0.1 M in respect of potassium chloride. The diffusion cell was allowed to stand with this solution for some days before the diffusion experiments were started in order to remove any material from the cell that might disturb the measurements. Silicone was substituted for the wool-fat generally used as sealing-grease in this cell. Only a few, however, of the 18 experiments of this series could be used; all the other measurements were disturbed in some way or another by leakage and had to be rejected.

In the second series of experiments aqueous ethanol was used as solvent. As at that time the first Claesson diffusion cell [3] was put into use, this cell was used for the experiments S 20—S 28. It was found easier to make leak-free, although it sometimes gave trouble. As sealing-grease between the mirror-glass windows and the middle metal part of the cell wool-fat, several times extracted with acetone, was used. Recently it has been found that the Claesson cell worked exceedingly well when the utmost care was taken to make the surfaces of the metal part completely plane, and when, instead of ordinary mirror glasses, windows of specially ground plane parallel optical glass were used.

The length of the diffusion experiment varied from 20 hours to 40 hours.

The gramicidin S, tyrocidine, and gramicidin used in the diffusion experiments were the same preparations as those used by Synge and Tiselius [4]. The tyrocidine components I, II and III were prepared as described in that paper.

DISCUSSION

The general agreement between the results in acetic acid and ethanol solutions suggests that the molecular state of the peptides in the different solvents may not be greatly different and that the diffusion data may provide information concerning the molecular weights of the peptides. All the values lie, as might be expected, between those found for simpler peptides and those for proteins of low molecular weight.

Table 2. Diffusion data for gramicidin S, tyrocidine, and gramicidin.

Substance	Experiment no.	$D_m \cdot 10^7$	$D_A \cdot 10^7$	Concentration g per 100 ml solution	Solvent
Gramicidin S (HCl)	S23A	25.9	27.0	1	0.2 *M* KCl in
	S23B	24.4	25.3	1	50 per cent ($^v/_v$)
	S23C	25.9	27.0	1	aqueous ethanol
	S25B	27.1	27.2	0.5	
	S25C	26.4	27.1	0.5	
	S4	26.3	25.5	1	0.1 *M* KCl in
	S2	27.1	25.1	1	50 per cent ($^v/_v$)
	S6	29.0	30.2	0.5	aqueous acetic
	S18	24.5	25.8	0.3	acid
Tyrocidine (HCl) (unfractionated)	S26 II	18.4	17.4	1	0.2 *M* KCl in
	S20B	20.4	19.6	0.5	50 per cent ($^v/_v$)
	S20A	20.5	19.2	0.5	aqueous ethanol
	S20C	22.7	21.6	0.5	
	S3	20.8	19.8	1	0.1 *M* KCl in
	S1	20.0	19.7	1	50 per cent ($^v/_v$)
	S14	20.4		0.7	aqueous acetic
	S5	18.3	19.3	0.5	acid
Tyrocidine fraction					
I »colorless»	S24C	21.2	22.9	0.5	0.2 *M* KCl in
II »pink»	S28A	20.3	21.8	0.5	50 per cent ($^v/_v$)
	S28C	20.9	21.5	0.5	aqueous ethanol
	S28B	20.6	21.7	0.5	
III »blue»	S27B	16.5	20.2	0.5	
	S27A	17.9	20.1	0.5	
Gramicidin	S21A	18.4	19.2	1	70 per cent ($^v/_v$)
	S21B	17.8	18.3	1	aqueous ethanol
	S21C	16.2	18.2	1	
	S22A	18.7	19.4	0.5	
	S22B	18.8	19.2	0.5	
	S22C	19.7	19.4	0.5	

The X-ray [5] and chemical [6] data on *gramicidin S* indicate that it must be either a cyclopentapeptide (MW of free unhydrated base 570) or a cyclodecapeptide (MW of free unhydrated base 1140). If a spherical unhydrated mole-

cule is assumed having partial specific volume 0.81 (calc. according to Cohn and Edsall) [7], the diffusion constants correspond to molecular weights in the range (1080—1880). This result suggests that gramicidin S has the cyclodecapeptide structure. It would be necessary to postulate considerable departure from the spherical form or extensive solvation or association to reconcile the diffusion data with the cyclopentapeptide structure. The cryoscopic data for gramicidin S in camphor [8] are the only other available indications and they gave a molecular weight of 1060—1340, which appears to be in agreement with our results. Employing the arithmetic mean of our values for gramicidins ($D_{20} \cdot 10^7 = 26.5$), f/f_0 is 1.12 for the cyclodecapeptide molecule.

If the same degree of asymmetry/solvation is assumed for *tyrocidine* as for gramicidin S and the partial specific volume is taken to be 0.75, the diffusion data suggest a molecular weight in the range (1900—5100). This agrees reasonably with a molecule such as that postulated by Hotchkiss [9] — a 19-peptide with a molecular weight of 2473 for the free unhydrated base. The different components of »tyrocidine»[4] separated by adsorption on charcoal and differing in tryptophan content do not differ much from one another in their diffusion characteristics, and are evidently, as suggested by the chemical evidence, rather similar substances. The small differences in their diffusion constants may turn out to be real; it is perhaps significant in this connection that of all the materials here studied only unfractionated »tyrocidine» gave evidence of polydispersity, D_m being systematically, though only slightly, higher than D_A. The differences in D for the different tyrocidine components could be attributed to association effects, increasing with increasing tryptophan content.

The diffusion of *gramicidin* has only been studied in 70 per cent (*v/v*) ethanol. Here, making the same assumptions as with tyrocidine, but assuming partial specific volume 0.80, the MW would lie in the range (2800—5000). This is in fair agreement with the MW determinations based on the vapour pressure lowering in methanol and the diffusion in butanol. Cryoscopic determinations in camphor, phenol and cyclohexanol give lower values (for references see Hotchkiss [9]). It is hoped to report before long new stoichiometric and crystallographic data bearing on this problem.

SUMMARY

1) The diffusion constants for gramicidin S, tyrocidine components and gramicidin have been determined in aqueous acetic acid and ethanol solutions.

2) From the diffusion constants maximum values for the molecular weights of the peptides have been calculated.

3) The results suggest that gramicidin S has the cyclodecapeptide structure. The values for tyrocidine components lie in the range 1900—5100 and those for gramicidin in the range 2800—5000.

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