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Conformational analysis of gramicidin-gramicidin interactions at the air/water interface suggests that gramicidin aggregates into tube-like structures similar as found in the gramicidin-induced hexagonal H_{II} phase

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The energetics of interaction and the type of aggregate structure in lateral assemblies of up to five gramicidin molecules in the $\beta^{6.3}$ helical conformation at the air/water interface was calculated using conformational analysis procedures. It was found that within the aggregate two types of gramicidin interaction occur. One leading to a linear organization with a mean interaction energy between monomers of −6 kcal/mol and one in a perpendicular direction leading to a circularly organization with a lower mean interaction energy of −10 kcal/mol. Extrapolation towards larger gramicidin assemblies predicts that gramicidin itself could form tubular structures similar to those found in the gramicidin-induced H_{II} phase. The tryptophans appear to play an essential role in the tubular organization of the gramicidin aggregate, since they determine the cone shape of the monomer and contribute to the structure of the monomer and oligomer by stacking interactions. These results, which are discussed in the light of experimental observations of gramicidin self-association in model membranes and the importance of the tryptophans for H_{II} phase formation, further support the view (Killian, J.A. and De Kruijff, B. (1986) Chem. Phys. Lipids 40, 259–284) that gramicidin is a first example of a new class of hydrophobic polypeptides which can form cylindrical structures within the hydrophobic core of the membrane.

Introduction

Gramicidin A is a potent modulator of membrane lipid structure. In aqueous mixtures with lysophosphatidylcholine a bilayer is formed, despite the preferred micellar organization of the pure lipid [1–4]. Furthermore, it is the best-known example of a hydrophobic peptide, which can induce bilayer → hexagonal$_{II}$ phase transitions in model membranes composed of a variety of different phospholipids with acyl chain length in excess of 16 carbon atoms (for recent review, see Ref. 5) and even in the membrane of the human erythrocyte (Tournois, H., unpublished data). From DSC[6], NMR, [7,8] X-ray diffraction and sucrose

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidyl ethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine.

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density centrifugation experiments, it could be concluded that this pentadecapeptide has a tendency to aggregate in the bilayer and that this aggregation is a prerequisite for H_{II} phase formation. Interestingly, also for channel formation it appears that lateral aggregation of the peptide might be involved [10,18]. In the case of $18:1_c/18:1_c$ PC, a lipid which has been studied most thoroughly [7–9], the H_{II} phase is very rich in gramicidin (gramicidin/PC $\geqslant 1:7$, molar). The structural parameters of this phase seem to be mainly determined by the peptide itself [5], which because of its pronounced cone shape, due to the location of the four bulky tryptophan residues all at the C terminus of the peptide, seems to be ideally suited to fit in the tubes of which the H_{II} phase is formed. This shape of the peptide also is supposed to be the main determinant for bilayer formation with the oppositely shaped lysophosphatidylcholine [4].

In order to get a better theoretical understanding of the importance of gramicidin aggregation for hexagonal H_{II} phase formation, we report in this study the results of computations on the energetics of gramicidin–gramicidin interactions at the air/water interface. The most probable structure of the gramicidin aggregate was obtained using methods similar to those previously used to obtain insight in the conformation and interfacial location of pure lipid [11] and lysophosphatidylcholine-gramicidin aggregates [4].

Computational methodology

Gramicidin A was modelled according to the coordinates corresponding to the left handed $\beta^{6.3}$ helical structure [12]. This configuration of the monomer was maintained throughout the assemblage procedure. The molecule was oriented at the air/water interface as described earlier [4]. This orientation corresponds to the energetically most favorable orientation which has its C terminus position away from the aqueous phase [4] and in which the long helical axis is perpendicular to the interface. The procedure used to surround one gramicidin with other gramicidins is a modification of the method used to surround one gramicidin with lipid molecules [4] and can be described as follows (Fig. 1). After orienting one gramicidin

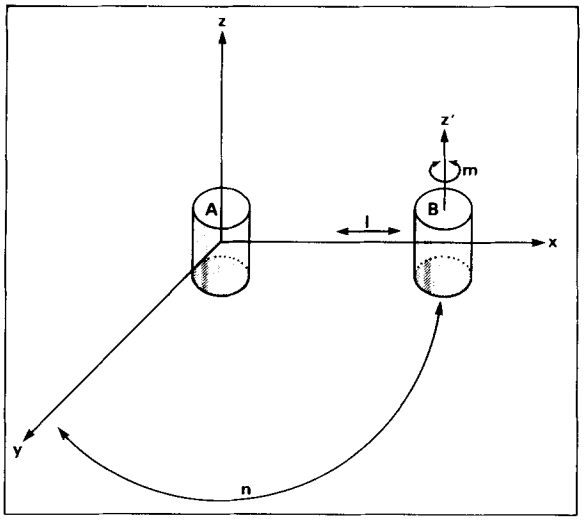

Fig. 1. Schematic representation of the packing procedure of gramicidin.

molecule at the air/water interface its position and orientation were fixed. A second gramicidin molecule was oriented at the interface and it was allowed to move along the X-axis in steps of 0.05 nm. For each position the second molecule was rotated in steps of 30° around its long axis Z' and around the first molecule. l is the number of positions along the X-axis, m the number of rotations of the second molecule around the first one and n is the number of rotations of the molecule itself. For each set of values of l, m and n, the intermolecular energy of interaction was calculated as the sum of the London-Van der Waals energy of interactions, the electrostatic interaction and the transfer energy of atoms or groups of atoms from a hydrophobic phase to a hydrophilic phase as described earlier [11]. Then, the second molecule was allowed to move in steps of 0.05 nm along the Z'axis perpendicular to the interface and the position of the Z' axis was varied in steps of 5° with respect to the Z axis, such that for each set of values l, m and n, the lowest interaction energy state was obtained. This energy and the coordinates associated to each l, m and n combination were stored in a hyper matrix and were classified for decreasing values of the interaction energy. The position of the third gramicidin molecule is defined as the first energetically favorable orientation stored in the hyper matrix but taking into account the sterical and energetic

constraints imposed by the presence of the second molecule. Thus, orientations are disregarded in which overlap of atomic coordinates of two molecules occurs and in which the interaction energy between the two molecules was positive. In order to minimize further the conformational energy, the positions of the second and third molecule are then alternatively modified in steps according to the energy classification of the hyper matrix. For the fourth gramicidin molecule the same process is repeated but now the positions of the three surrounding gramicidins are modified alternatively in order to find the lowest energy state. In this calculation, the interaction energy between all gramicidin monomers in the aggregate are considered and minimalized till the lowest energy state of the entire aggregate is reached. The assemblage procedure is completed when the fixed gramicidin molecule is surround by four other gramicidin molecules. Due to sterical and energetic constraints, it was not possible to surround one gramicidin molecule with more than four other gramicidin molecules in direct interaction with the central molecule. The interaction energies between

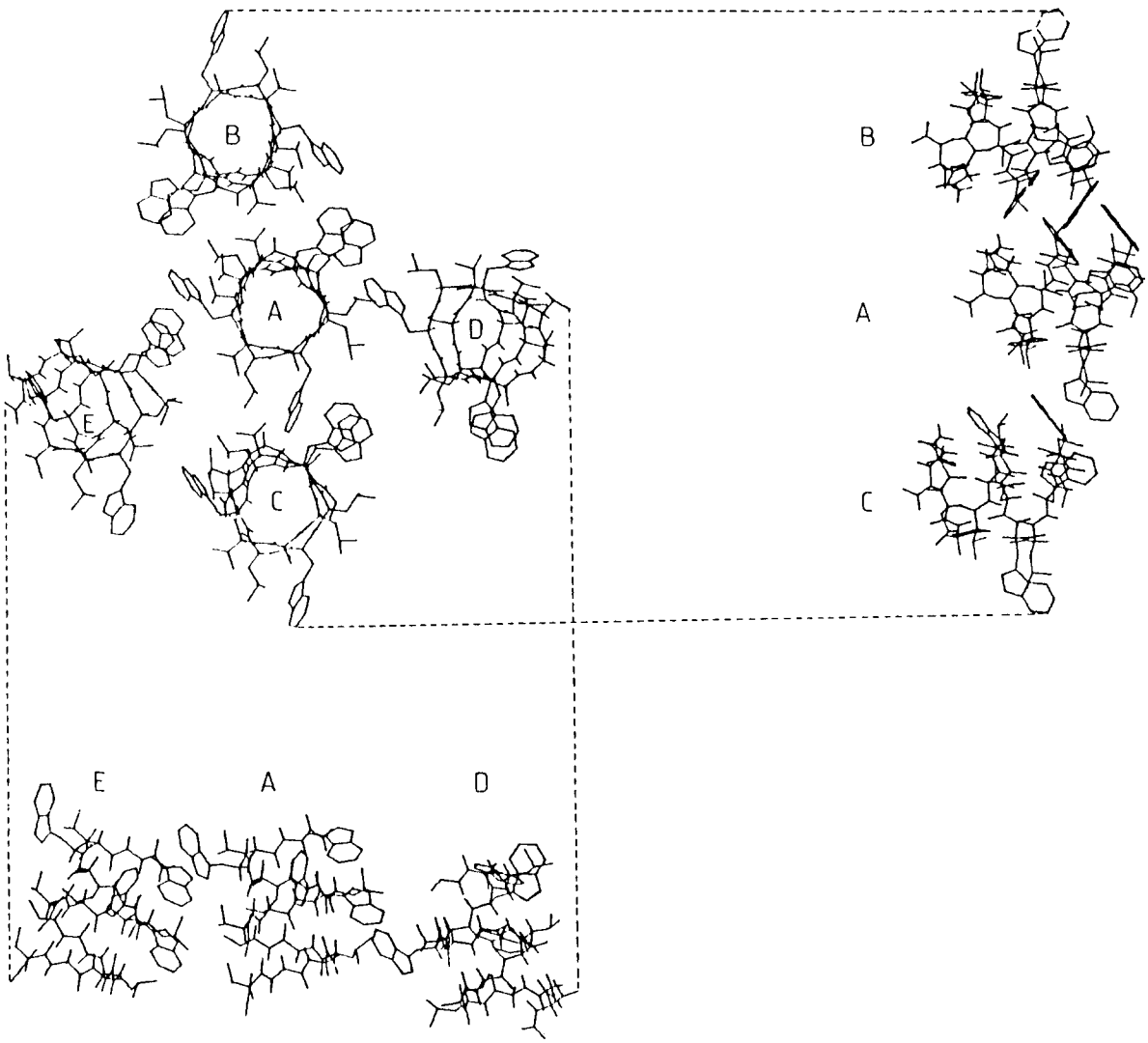

Fig. 2. Top and front view of four gramicidin molecules (B, C, D and E) which surround one gramicidin molecule (A). In the front view, for sake of clarity, only three molecules are shown.

gramicidin and $18:1_c/18:1_c$ PC and between two $18:1_c/18:1_c$ PC molecules were calculated as described before [4,11].

All calculations were performed on an Olivetti M24 using a 8087 processor and the PC-TAMMO procedure (Theoretical Analysis of Molecular Membrane Organization) which has been extensively described elsewhere [11]. Graphs were drawn with the PC-MGM (Molecular Graphics Manipulation) program.

Results

After assemblage of four gramicidin molecules around one fixed gramicidin molecule at the air/water interface, a lowest energy organization is obtained, which is as a top view depicted in the left-hand corner of Fig. 2. The channel present within the molecules is clearly visible. Due to the cone shape of gramicidin which is, for instance, apparent in the side view of molecule A, two kinds of assemblage occur in the pentameric aggregate. One results in a linear association such as is found in the direction of the molecules E, A and D (Figs. 2 and 3(B)) and one results in a curved association, such as is found in the direction of the molecules B, A and C (Figs. 2 and 3(A)). The mean energy of interaction between gramicidin molecules is higher along the BAC axis (mean interaction energy between monomers, -10 kcal/mol) than along the DAE axis (mean interaction energy between monomers, -6 kcal/mol). When these numbers are compared to the calculated interaction energy between two $18:1_c/18:1_c$ molecules (-6.8 kcal/mol) and between one gramicidin and one $18:1_c/18:1_c$ PC molecule (-6.5 kcal/mol) than it can be conclude that the curved self-association of gramicidin is the preferred organization even in a $18:1_c/18:1_c$ PC monolayer.

Interactions between tryptophans but also aliphatic amino acid side chains are responsible for the stronger interaction along the BAC axis. For instance, the energy of interaction between A and B is higher than the energy of interaction between A and D. This difference is the result of the close proximity of Trp^9 and Trp^{15} of molecule A and Trp^{13} of molecule B and of Trp^{13} associated with molecule A and Trp^9 and Trp^{15} associated

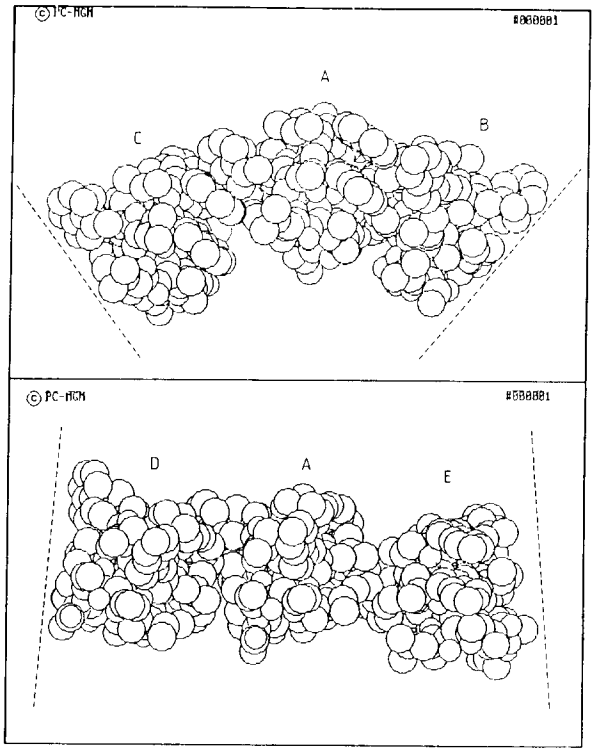

Fig. 3. Space-filling drawings of side views of the BAC and EAD assemblage.

with molecule B (Fig. 4). In the arrangement of molecules A and B there is also a strong possible interaction between the leucine side chains. Such a possibility does not exist in the AD association. Furthermore, in this case the Trp–Trp interaction is limited to Trp^{11} (of D) and Trp^9 and Trp^{15} (of A). The calculations further revealed the exact distances between the tryptophan residues, which are compared in Fig. 5.

Discussion

The two modes of organization calculated to be present in a pentameric lateral aggregate of gramicidin at the air/water interface provide new insight in the ability of gramicidin to induce the H_{II} phase in membrane systems. The curved association would be ideally suited to fit into a cylindrical structure such as those found in the gramicidin-induced H_{II} phase. The linear assemblage would then parallel the axis of the cylinder. Extrapolation of the association of the gramicidin molecules B, A and C (Fig. 2) towards a circular

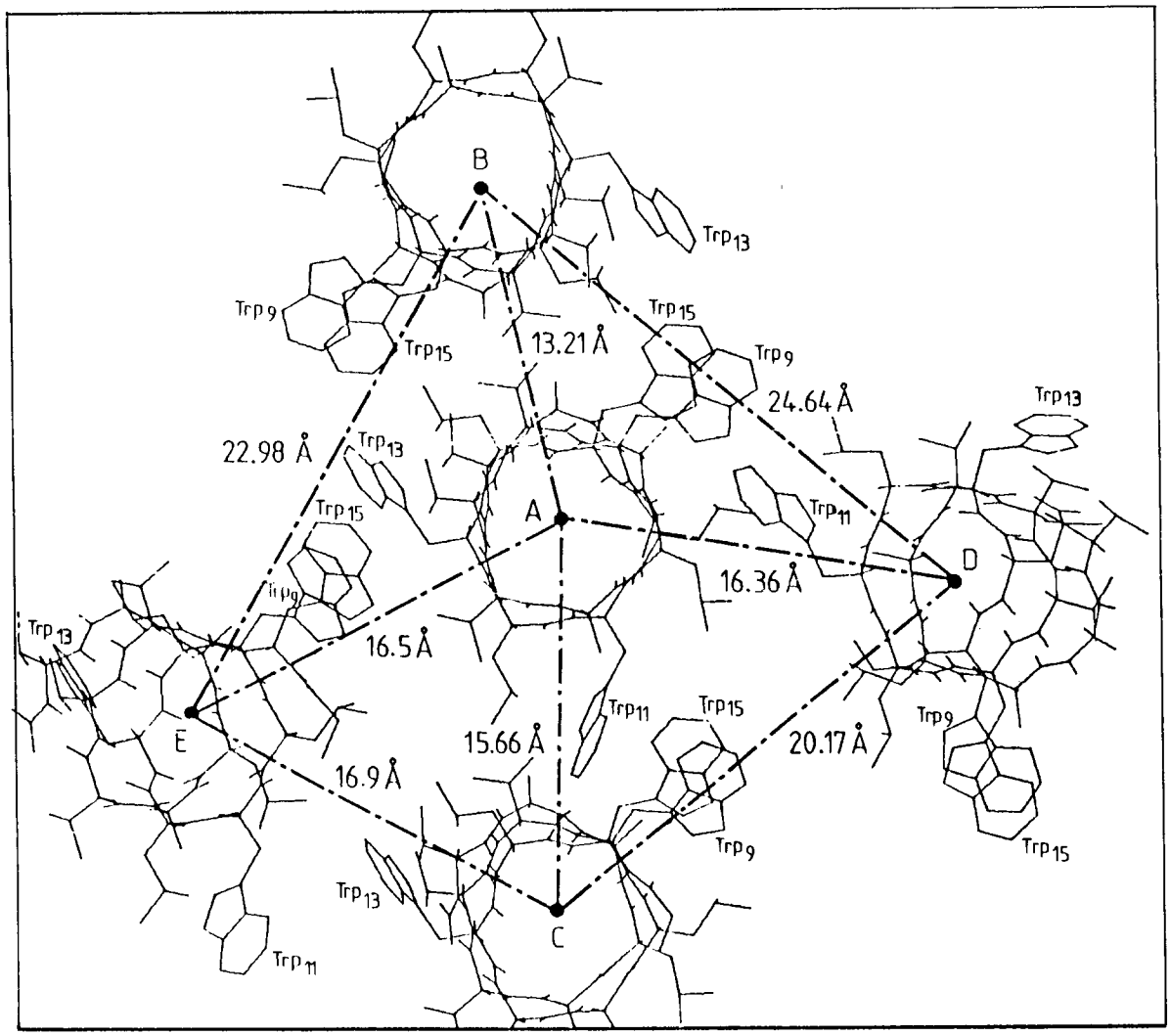

Fig. 4. Top view of the pentameric gramicidin assemblage with location of tryptophans and distances between gramicidin molecules within the aggregate. The labelling of each gramicidin is the same as used in Fig. 2.

arrangement requires 12 gramicidin molecules. From this arrangement and the length of the gramicidin monomer in the $\beta^{6.3}$ helical conformation it can be estimated that the outer diameter of such a circular arrangement is 70 Å. This value is very close to the tube diameters reported for a number of gramicidin-induced H_{II} phases which are summarized in Table I. The relative insensitivity of the diameter of the gramicidin-containing tubes with respect to the nature of the membrane lipid constituents together with the remarkably temperature insensitivity of the tube diameter (see Table I) and acyl chain order [8] as opposed to the strong temperature-dependent tube diameter and acyl chain order in the hexagonal H_{II} phases formed by phosphatidylethanolamine [6,8] support the view [7] that gramicidin in a laterally aggregated manner forms the structural backbone of the H_{II} phase.

Because phospholipids can rapidly diffuse around the tubes present in the gramicidin-induced H_{II} phase (as inferred from ^{31}P [7] and ^{2}H-NMR [8] data) and acyl chain dynamics are decreased by the gramicidin–18 : 1/18 : 1_c PC in-

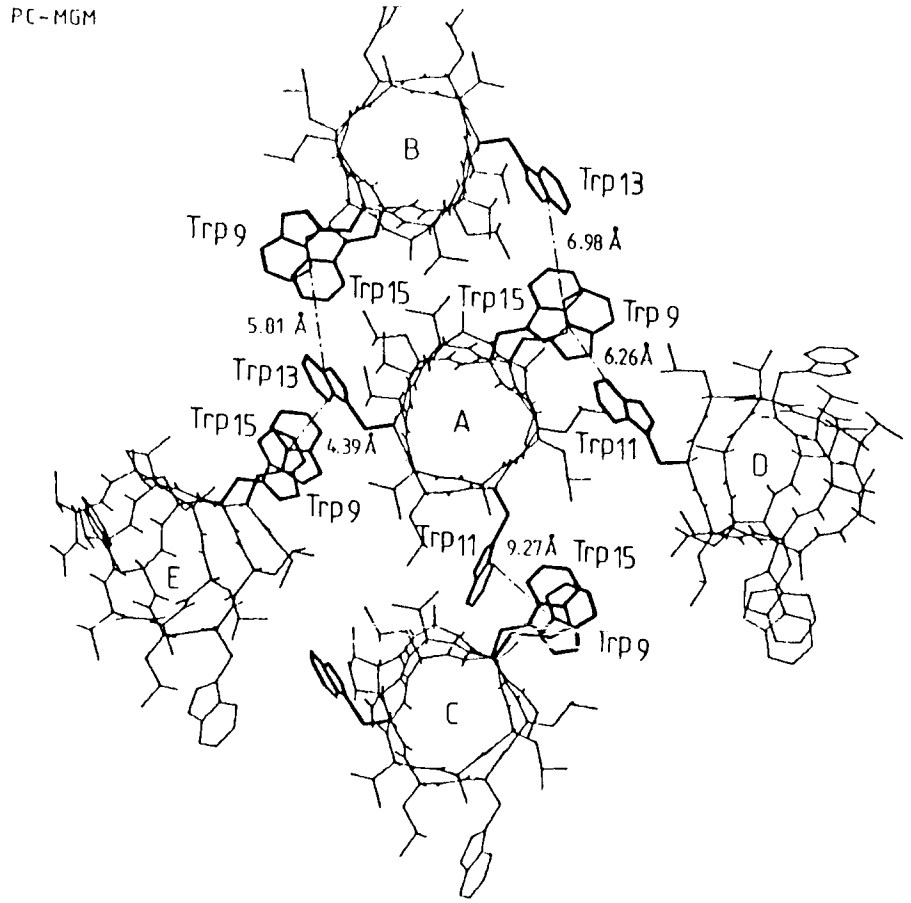

Fig. 5. Distances between atomic centers of tryptophans in the pentameric gramicidin aggregate. The labelling of each gramicidin is the same as used in Fig. 2.

teraction in this phase [8], we have to propose that the tubes of the H_{II} phase are composed of both cylindrical aggregates of gramicidin of the kind shown in Fig. 2 and phospholipids. A schematic representation of the peptide and lipid organization in a tube is shown in Fig. 6.

The calculated importance of intermolecular Trp–Trp interactions for stabilization of the structure of the cylindrical gramicidin aggregate supports recent experimental observations that *N*-formylation of these residues completely blocks H_{II} phase formation [15] and that replacement of Trp^9 or Trp^{11} by a phenylalanine residue results in a large decrease in extent of H_{II} phase formation [9]. Both types of modification are expected to cause considerable changes in possibilities for aromatic–aromatic interactions. The importance of intramolecular Trp–Trp interactions for the structure of the gramicidin monomer is indicated by the close proximity of Trp^9 and Trp^{15} in the $\beta^{6.3}$ helical configuration. For the other main functional abilities of gramicidin, e.g., channel formation [16] and involvement in DNA transcription [17] the tryptophans also appear to play an essential role. A detailed model which relates the gramicidin-induced channel and hexagonal H_{II} phase formation will be published elsewhere.

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TABLE I

TUBE DIAMETER OF H_{II} PHASES INDUCED BY GRAMICIDIN IN DIFFERENT (MODEL) MEMBRANE SYSTEMS

The tube diameter is calculated as 2-times the second-order $(1/\sqrt{3})$ reflection in small-angle diffraction patterns of the samples. For details see original references.

	Tube diameter (Å)	Ref.
$18:1_t/18:1_t$ PE	74 (40–60 °C)	6
$18:1_c/18:1_c$ PC	71 (20 °C)	8
	70 (70 °C)	8
$18:1_c/18:1_c$ PG	72 (25 °C)	13
$18:1_c/18:1_c$ PS	70 (25 °C)	13
Cardiolipin (beef heart)	72 (25 °C)	13
$22:1_c/22:1_c$ PC		
PC/gramicidin = 5, molar	86	14
PC/gramicidin = 2.5, molar	72	14
Total lipid extract		
Human erythrocytes	69 (37 °C)	– [a]
Human erythrocytes ghosts	64 (37 °C)	– [a]

[a] Tournois, H. (unpublished data).

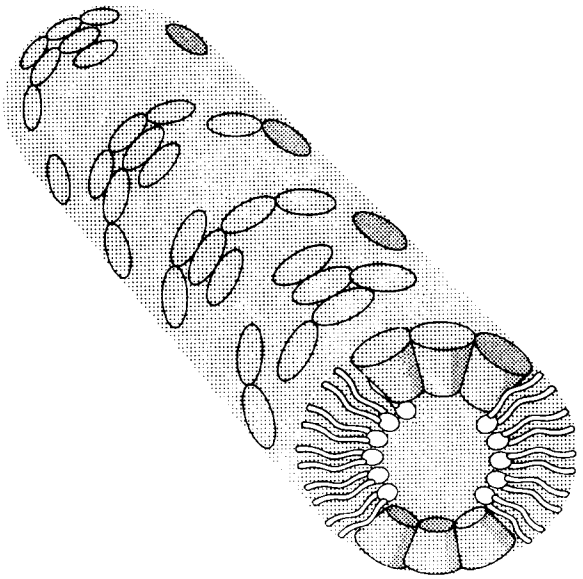

Fig. 6. Schematic representation of one tube of the gramicidin-induced H_{II} phase in $18:1_c/18:1_c$ PC.

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