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The tryptophans of gramicidin are essential for the lipid structure modulating effect of the peptide

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It is shown that N-formylation of the tryptophan residues of gramicidin completely and reversibly blocks the hexagonal H_{II} phase-inducing ability of the peptide in dioleoylphosphatidylcholine model membranes.

Many membrane lipids show polymorphism, in that dependent on the environmental conditions, they can adopt a variety of phases [1]. In recent years strong indications have been obtained that non-bilayer lipid structures such as the hexagonal H_{II} phase, the related 'lipidic particles' [2–6] and cubic phases [7] may be of importance for the structure of specialized membrane systems, such as the tight junction [8,9] and the prolamellar body [7,10] as well as for general membrane functions, e.g. fusion [11] and transmembrane transport (for a general recent overview on polymorphism in relation to membrane function, see Ref. 12).

From the functional point of view it can be inferred that mechanisms should exist by which the lipid structure in biological membranes can be locally modulated. Specific proteins are likely candidates to be involved in the formation of non-bilayer lipid structures. The antibiotic peptide gramicidin, which has been studied as a model for the hydrophobic part of intrinsic membrane proteins [13,14], was shown to have, besides for its channel-forming capacity [15,16] and its interaction with RNA polymerase [17], the ability to act as a profound modulator of the structure of lipids in aqueous dispersions. It triggers a micellar to bilayer transition in lysophosphatidylcholine [18,19], drastically lowers the bilayer to hexagonal H_{II} transition temperature in phosphatidylethanolamines [20], and most interestingly, induces an H_{II} phase in phosphatidylcholines (PC) with a chain length in excess of 16 carbon atoms [20–22]. The structure of the gramicidin molecule appears to be essential for this latter effect, as for instance the hydrophobic membrane-spanning part of glycophorin in contrast stabilizes bilayer structure in H_{II} phase-preferring systems [23]. Gramicidins are linear poly-L- and D-amino acids with blocked C- and N-terminal ends. The structure of gramicidin A, which amounts to 80% in the natural mixture, is:

1 2 3 4 5 6 7 8 9
formyl-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-

10 11 12 13 14 15
-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-ethanolamine

The unusual concentration of tryptophans near the C-terminal end of the molecule and their importance for channel formation [24] and for interaction with RNA polymerase [25] prompted us to focus on their significance for the lipid structure-modulating activity of the polypeptide. The approach we have chosen was to formylate the tryptophans (replace the > N-H by > N-CHO) and to compare the N-formylated gramicidin with the

unmodified peptide in its effect on the structure of aqueous dispersions of dioleoylphosphatidylcholine (DOPC).

Gramicidin (Sigma, St. Louis, MO, U.S.A.) was formylated in 4 h at room temperature in a 4 mg/ml solution of formic acid saturated with HCl gas [26]. In this time, the tryptophan fluorescence was completely lost. The reaction was stopped by the addition of an excess of ice-cold methanol (2 ml per mg gramicidin), whereafter the polypeptide was isolated by extraction according to Bligh and Dyer [27]. In order to further remove traces of the original acidic solvent, the combined chloroform phases of three extractions were washed three times with an equal volume of H_2O. 200 MHz ^{1}H-NMR and 50.3 MHz ^{13}C-NMR measurements demonstrated the complete loss of the resonance from the indol N-H protons (10.8 ppm downfield TMS) and the proportional appearance of the characteristic ^{13}C *N*-formyl signal (161.8 ppm downfield of TMS). These and other changes in the tryptophan part of the spectra were completely reversed upon replacing the formyl groups again by protons in methanol/25% ammonia in water (85 : 15, v/v). Since also the total tryptophan fluorescence was recovered by this reversal of the formylation reaction, we can conclude that the N-formylation is specific and does not lead to irreversible damage of the chemically labile tryptophans. The peptide was incorporated into DOPC (prepared according to Ref. 28) by hydrating a mixed film in either 100 mM NaCl, 10 mM Tris-HCl (pH 7.0), or in H_2O (as a 50%, w/w, solution) [29]. Both procedures yielded identical results. The structure of the peptide-lipid recombinants were investigated by ^{31}P-NMR [12,20], small-angle X-ray diffraction [22] and freeze-fracture electron microscopy [22].

Figs. 1A and B show that the incorporation of 1 gramicidin per 10 DOPC molecules results in a change in ^{31}P-NMR spectrum from a typical 'bilayer' to a mixed 'bilayer/H_{II}' lineshape [12], indicating H_{II} phase formation for a large part of the lipids, whereas incorporation of an identical amount of the N-formyl gramicidin does not affect the overall ^{31}P-NMR lineshape (Fig. 1C). Also, X-ray diffraction and freeze-fracture electron microscopy experiments (data not shown) showed that the H_{II} phase-specific ($1/\sqrt{3}$) reflection and striated freeze-fracture morphology, induced by

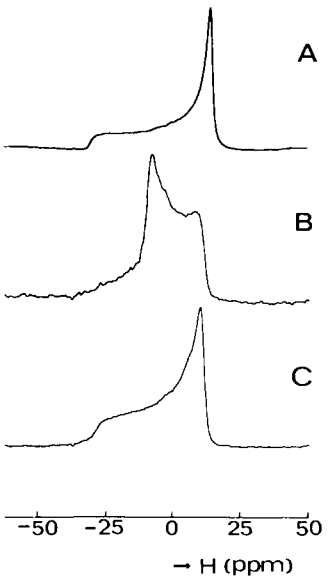

Fig. 1. 81.0 MHz ^{31}P-NMR spectra of aqueous dispersions of DOPC (A), DOPC/gramicidin (10:1, mol/mol) (B) and DOPC/*N*-formylgramicidin (10 : 1, mol/mol) (C). Spectra were recorded as described in Ref. 22. The 0 ppm position corresponds to isotropically moving DOPC molecules as present in sonicated vesicles. The amount of H_{II} phase present in sample (B) was approx. 50% as determined by computer subtraction methods, in agreement with previous data [20,21,30]. The slightly different lineshape of spectrum C as compared to spectrum A, most likely originates from an increase in motional freedom of the lipid headgroup due to the presence of the N-formylated gramicidin.

the incorporation of gramicidin in DOPC [20], was completely absent for the N-formyl derivative. The H_{II} phase-inducing activity of the peptide could be regained by deformylation of the formylated tryptophan residues. That N-formylated gramicidin does incorporate into the lipid was demonstrated by density centrifugation in $^2H_2O/H_2O$ mixtures. Whereas DOPC liposomes float at 20% ^2H_2O, centrifugation of a N-formylated gramicidin/DOPC (1 : 10, molar ratio) sample resulted in quantitative pelleting of the lipid with the peptide.

Thus, it can be concluded that the tryptophans are essential for the lipid structure-modulating activity of the peptide. Preliminary experiments with gramicidin analogs in which either the 9- or 11-tryptophan was replaced by a phenylalanine showed a large reduction in H_{II} phase formation by the peptide in model membranes. There are several possible molecular interpretations for this

effect. Since lateral gramicidin self-association can occur in model membranes and appears to be highly important for H_{II} phase formation in DOPC systems [30], we favor the idea that intermolecular tryptophan-stacking interactions cause gramicidin molecules to segregate into tubular structures such as found in the hexagonal H_{II} phase.

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