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RAMAN SPECTROSCOPIC INVESTIGATION OF THE INTERACTION OF GRAMICIDIN A WITH DIPALMITOYL PHOSPHATIDYLCHOLINE LIPOSOMES

E. WEIDEKAMM[a], E. BAMBERG[a], D. BRDICZKA[a], G. WILDERMUTH[a], F. MACCO[b], W. LEHMANN[b] and R. WEBER[b]

Universität Konstanz, [a]Fachbereich Biologie and [b]Fachbereich Physik, D-775 Konstanz (G.F.R.)

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Summary

The interaction of gramicidin A with dipalmitoyl phosphatidylcholine liposomes is investigated by Laser-Raman spectroscopy. As revealed by the methylene C-H stretching mode the phase transition of the hydrocarbon chains near 40°C is eliminated in the presence of gramicidin A. Liposomes prepared from a mixture of lecithin and cholesterol seem to be unaffected by gramicidin A and show only the normal broadened phase transition.

Gramicidin A is a linear pentadecapeptide which has been isolated from *Bacillus brevis*. If it is introduced into natural membranes or into artificial lipid bilayers the membranes become permeable for monovalent cations. There is good evidence that gramicidin A forms transmembrane channels in lipid membranes which consist of a helical dimer. The peptide carbonyl groups line the central hole axis of this helix, whereas the hydrophobic residues are located at the exterior [1–5].

In this paper we use gramicidin A incorporated into liposomes as a model system for the interaction of a hydrophobic polypeptide with the lipid core.

The study of lipid-protein interactions in biomembranes as well as lipid-lipid interactions is sometimes complicated by the introduction of reporter groups which may also act as perturbants [11]. Raman spectroscopy avoids these problems and the study of lipid phase transitions in a variety of both natural and model membranes provided useful insight into the molecular structure and interactions of these systems [6–10].

Gel-liquid crystalline transitions as well as environmental perturbations are reflected by intensity changes of certain vibrational transitions occurring

in the spectral region 2800 –3000 cm^{-1} (C-H stretching vibrations) and in the 1050–1150 cm^{-1} region (C-C stretching modes and PO_2^- stretching vibrations [13]. In order to investigate the structural alterations within the hydrocarbon skeleton of dipalmitoyl phosphatidylcholine liposomes (Fluka, puriss.) due to gramicidin A (Nutritional Biochem. Corp., Ohio), we monitored the temperature dependent intensity changes of the 2800–3000 cm^{-1} region. Spiker and Levin [13] using model molecules assigned the Raman frequencies in this region to methyl and methylene C-H transitions:

2962 cm^{-1} :	$-(CH_3)$	C-H Asym. stretch
2936 cm^{-1} :	$-(CH_3)$	C-H Sym. stretch
2883 cm^{-1} :	$-(CH_2)$	C-H Asym. stretch
2847 cm^{-1} :	$-(CH_2)$	C-H Sym. stretch

Fig. 1 shows the Raman spectra of dipalmitoyl phosphatidylcholine/gramicidin A liposomes (molar ratio 150:1) in the 2800–3000 cm^{-1} region at different temperatures. (Tryptophan fluorescence was used to determine the gramicidin A concentrations. Samples were dissolved in methanol and referred to a

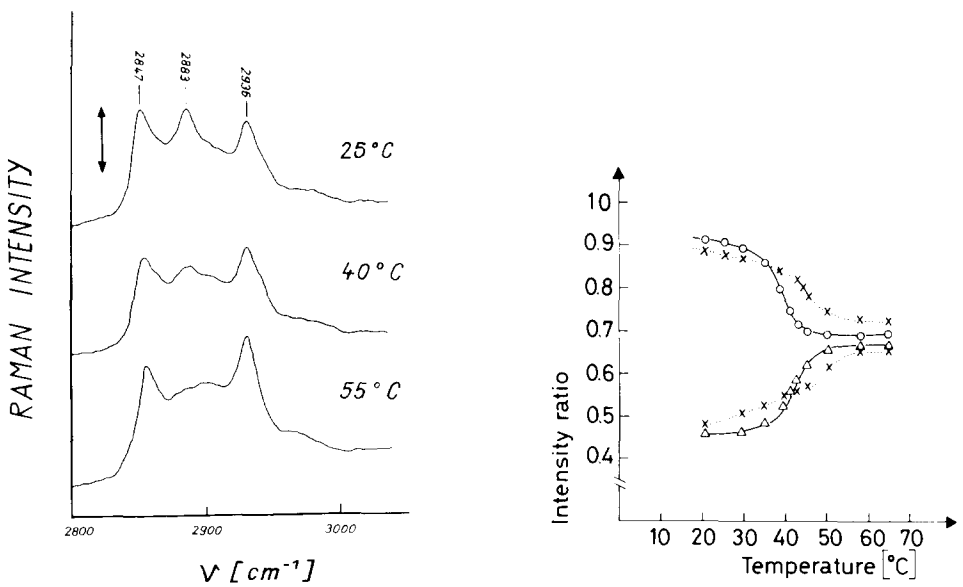

Fig. 1. Raman spectra of the C-H stretch region of dipalmitoyl phosphatidylcholine liposomes plus gramicidin A (molar ratio 150:1) at different temperatures. The spectra were recorded with incident power 250 mW, radiation was polarized perpendicular to the scattering plane, excitation at 4880 Å, scanning speed 0.5 cm^{-1}/s and spectral resolution 2 cm^{-1}. Dark count rate was less than 10 counts/s. The arrow indicates 1500 counts/s. Phospholipids and gramicidin A were dissolved in methanol, mixed in the given ratios, evaporated under nitrogen. Then 0.1 M KCl was added and the samples were sonicated for 15 min at room temperature. Free gramicidin A and lipid aggregates were removed by centrifugation (10 min at 15 000 × g) and by chromatography on Sephadex G-50. Samples containing 35—40 mg phospholipid/ml were transferred into quartz micro cuvettes (2 mm inner diameter) and placed in a thermostated cuvette holder.

Fig. 2. Temperature dependent intensity ratios of Raman C-H stretch peaks of dipalmitoyl phosphatidylcholine liposomes: ○—○, $I_{2883\ cm^{-1}}/I_{2847\ cm^{-1}}$; △—△, $I_{2936\ cm^{-1}}/I_{2847\ cm^{-1}}$; ×- - -×, peak ratios of dipalmitoyl phosphatidylcholine liposomes plus cholesterol (molar ratio 4:1). Upper curve $I_{2883\ cm^{-1}}/I_{2847\ cm^{-1}}$; lower curve $I_{2936\ cm^{-1}}/I_{2847\ cm^{-1}}$. Concentrations and technical data as in Fig. 1.

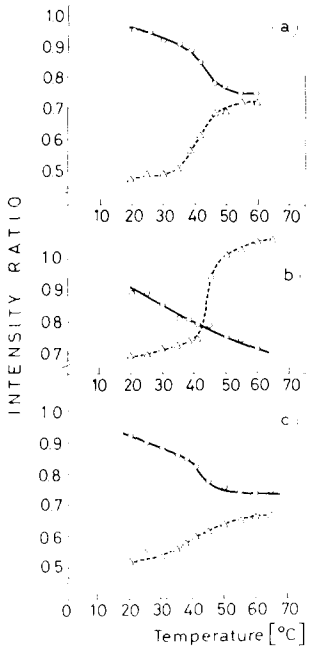

Fig. 3. Plots of C-H stretch intensity ratios vs. temperature: ○—○, $I_{2883\ cm^{-1}}/I_{2847\ cm^{-1}}$; △- - -△, $I_{2936\ cm^{-1}}/I_{2847\ cm^{-1}}$. (a) Dipalmitoyl phosphatidylcholine (liposomes) plus gramicidin A (molar ratio 650:1). (b) Dipalmitoyl phosphatidylcholine(liposomes) plus gramicidin A (molar ratio 150:1). (c) Dipalmitoyl phosphatidylcholine(liposomes) plus gramicidin A plus cholesterol (molar ratio 150:1:37.5). Concentrations and technical data as in Fig. 1.

standard solution of the peptide in methanol.) If temperatures are raised above T_m the relative intensity of the 2883 cm^{-1} band is reduced, while the intensity of the 2936 cm^{-1} band increases in comparison to the reference band at 2847 cm^{-1}. These intensity changes parallel the increasing number of *gauche* conformations in the hydrocarbon chains of the bilayer.

At the gramicidin concentrations used in our experiments CH_3 containing amino acids do not detectably contribute to the C-H stretch of the fatty acids at 2936 cm^{-1} [12]. Therefore the evaluation of the 2936 cm^{-1}/2847 cm^{-1} intensity ratios as a function of temperature should reflect the thermotropic lipid transitions for the CH_3-termini (i.e. the interior of the lipid bilayer), while the 2883 cm^{-1}/2847 cm^{-1} ratios should reflect the transitions of the CH_2-groups of the hydrocarbon chains.

In pure dipalmitoyl phosphatidylcholine liposomes this transition is centered near 40°C for both methylene and methyl groups (Fig. 2). Addition of gramicidin A to these liposomes (molar ratio 1:650) broadens the transitions for the methylene groups but does not affect the methyl intensity ratio 2936 cm^{-1}/2847 cm^{-1} (Fig. 3a). Increase of the gramicidin A concentration to a molar ratio of 1:150 (gramicidin A:dipalmitoyl phosphatidylcholine) completely eliminates the methylene group transition (Fig. 3b) while the methyl termini still exhibit a marked transition only slightly shifted to higher temperatures (42–43°C).

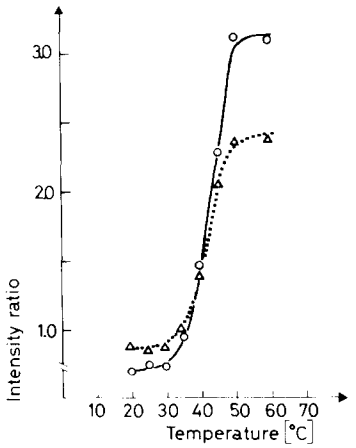

Fig. 4. Plot of C-C stretch intensity ratios $I_{1089\ cm^{-1}}/I_{1128\ cm^{-1}}$ vs. temperature. ○—○, dipalmitoyl phosphatidylcholine liposomes; △- - -△, dipalmitoyl phosphatidylcholine (liposomes) plus gramicidin A (molar ratio 150:1). Concentration and technical data as in Fig. 1.

Plotting of the intensity ratio 1089 cm^{-1} /1129 cm^{-1} vs. temperature (Fig. 4) shows that the addition of high gramicidin A concentrations to the dipalmitoyl phosphatidylcholine liposomes (molar ratio 1:150) greatly broadens the gel-liquid crystal transition similar to the effect of cholesterol described by Lippert and Peticolas [8].

Our findings are supported by differential scanning calorimetric experiments. The main endothermic transition (T_c) of dipalmitoyl phosphatidylcholine liposomes which occurs at 42°C is not shifted upon the addition of gramicidin A. However, the total enthalpy of the transition is drastically reduced from ΔH=8.6 ±0.4 kcal/mol in dipalmitoyl phosphatidylcholine liposomes to ΔH=4.1±0.3 kcal/mol when gramicidin A is added in a molar ratio of 1:150 indicating a loss of cooperativity. The transition temperature (T_c) of the liposomes was determined with a Differential Scanning Calorimeter (Perkin-Elmer DSC-2) using a scanning rate of 10°C/min and a sensitivity scale of 1 mcal/s for full-scale response. The enthalpy of the transition was calculated from the area under each peak and the amount of phosphate in the sample.

A similar reduction of the main transition endotherm in dipalmitoyl phosphatidylcholine/gramicidin A- mixtures (molar ratio <20:1) has been reported by Chapman et al. [18]. As described in refs. 8 and 9 addition of 25% cholesterol to dipalmitoyl phosphatidylcholine liposomes broadens but does not eliminate the phase transition visualized by Laser-Raman spectroscopy. Lippert and Peticolas [8] suggest that the addition of cholesterol decreases the interaction between adjacent hydrocarbon side chains, causing a broad non-cooperative gel-liquid crystal transition.

Incorporation of high concentrations of gramicidin A into lecithin/cholesterol liposomes (Fig. 3c) does not abolish the "CH_2 -transition" and cannot reconstitute the transition for the methyl termini as found in the absence of cholesterol (Fig. 3b), pointing to a blockade of the gramicidin A sites by cholesterol.

As reflected by the temperature dependent intensity ratio 1089 cm^{-1} / 1129 cm^{-1} (C-C stretch) the addition of gramicidin A to dipalmitoyl phosphatidylcholine liposomes fluidizes the lipid multilayers below the transition temperature, while they show more rigidity above this point. This effect is even more expressed if one follows the temperature characteristics of the intensity ratio 2883 cm^{-1} /2847 cm^{-1} (C-H stretch). At a molar ratio gramicidin A to phospholipid of 1:150 the phase transition is practically eliminated. This loss of cooperativity within the phospholipid layer is probably due to a long-range organization imposed by the polypeptide on the phospholipids through apolar interactions between hydrophobic amino acid side chains and the hydrocarbon chains of the lipids.

In contrast to this the intensity ratio 2936 cm^{-1} /2847 cm^{-1} is not altered upon the addition of gramicidin A. As the 2936 cm^{-1} band is assigned to the hydrocarbon CH_3 symmetric stretching mode one could speculate that the interior of the phospholipid bilayers is not affected by the incorporation of gramicidin A. However, this seems not to be compatible with the concept of lipid phase transitions and a transmembrane peptide channel, all the more as confusion regarding the methylene and methyl assignements exists in the literature [14 -17]. Therefore, we assume that the 2936 cm^{-1} band might not only reflect the C-H stretch vibration of the CH_3 -group but also contributions from the CH_2 -group at 2883 cm^{-1}, which increase with temperature. Addition of the intensity ratios 2883 cm^{-1} / 2847 cm^{-1} plus 2936 cm^{-1} /2847 cm^{-1} in Figs. 2 and 3 provides roughly constant values and support this assumption. In addition the intensity of the 2936 cm^{-1} band has been shown to increase with an increased degree of the polar environment of the hydrocarbon chains [14] and should reflect the increased incorporation of H_2O molecules into the bilayers at higher temperatures. However, this water incorporation parallels the acyl chain disorder and should also be reduced by the addition of gramicidin A and affect the transition of the CH_3 -group.

Studies with dipalmitoyl phosphatidylcholine with deuterated methyl groups should clarify these points as the position of the C^2H_3 -group is clearly distinct from that of the CH_2 -group in the Raman spectrum.

Acknowledgements

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