# Reversible disc-to-vesicle transition of melittin-DPPC complexes triggered by the phospholipid acyl chain melting

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Freeze-fracture electron microscopy, quasi-elastic light scattering and $^{31}P$ solid-state NMR have been utilized in order to follow morphological changes of melittin-dipalmitoylphosphatidylcholine complexes as a function of temperature. For lipid-to-protein molar ratios greater than 10 and up to 30, a reversible transition occurs, from discoidal structures of diameter ~ 100 to 400 Å, at low temperature, to vesicular structures of diameter ~ 3000–6000 Å, at high temperature. This transition appears to be triggered by the phospholipid acyl chain melting. Higher concentrations of melittin (e.g. lipid-to-protein ratio of 5.2) prevent this morphological transition, i.e. small complexes of ~ 100–250 Å diameter remain stable throughout the temperature range.

*Melittin* *Phospholipid membrane* *Disc-to-vesicle transition* *Chain melting* *NMR* *Freeze-fracture* *Quasi-elastic light scattering*

## 1. INTRODUCTION

Interaction of amphipathic $\alpha$-helices with lipids is a key problem in the determination of the structure and dynamics of membrane assembly. Melittin is an amphipathic peptide whose main property is direct membrane lysis [1]. Like other 'membrane-invading' peptides it reorganizes membrane lipids and stabilizes various kinds of structures [2]. When dispersed in water DPPC is known to form spontaneously well-characterized bilayers which undergo a gel-to-fluid phase transition at 41°C. Here we report on the supramolecular structure of melittin-DPPC systems and show how the physical state of the lipid chains may be crucial in determining the overall structure of the complexes.

*Abbreviations:* DPPC, dipalmitoylphosphatidylcholine; QELS, quasi-elastic light scattering; Ri, lipid to protein molar ratio

## 2. MATERIALS AND METHODS

Melittin was purchased from Bachem (Switzerland) and dissolved in buffers (pH 7.5), 1 mM EDTA, either phosphate (20 mM) or Tris-HCl (20 mM, 100 mM NaCl, for NMR experiments). Due to the concentrations used melittin was always tetrameric [3]. DPPC was obtained from Sigma (USA) and dispersed in the same buffers. Unless otherwise mentioned samples were initially incubated at 50°C for 30 min and allowed to stabilize at a given temperature, for 20 min, prior to performing experiments. QELS measurements were carried out by means of a krypton ion laser source ($\lambda$ = 647 nm, Spectra Physics). The correlation function of the scattered light was recorded at 90° and fast clipped on a 100-channel correlator (ATNE, Paris) with a time resolution of 10 ns. NMR was performed on a Bruker WH 270 spectrometer implemented to perform solid-state $^{31}P$ measurements at 109.35 MHz (Dufourc, E.J. and

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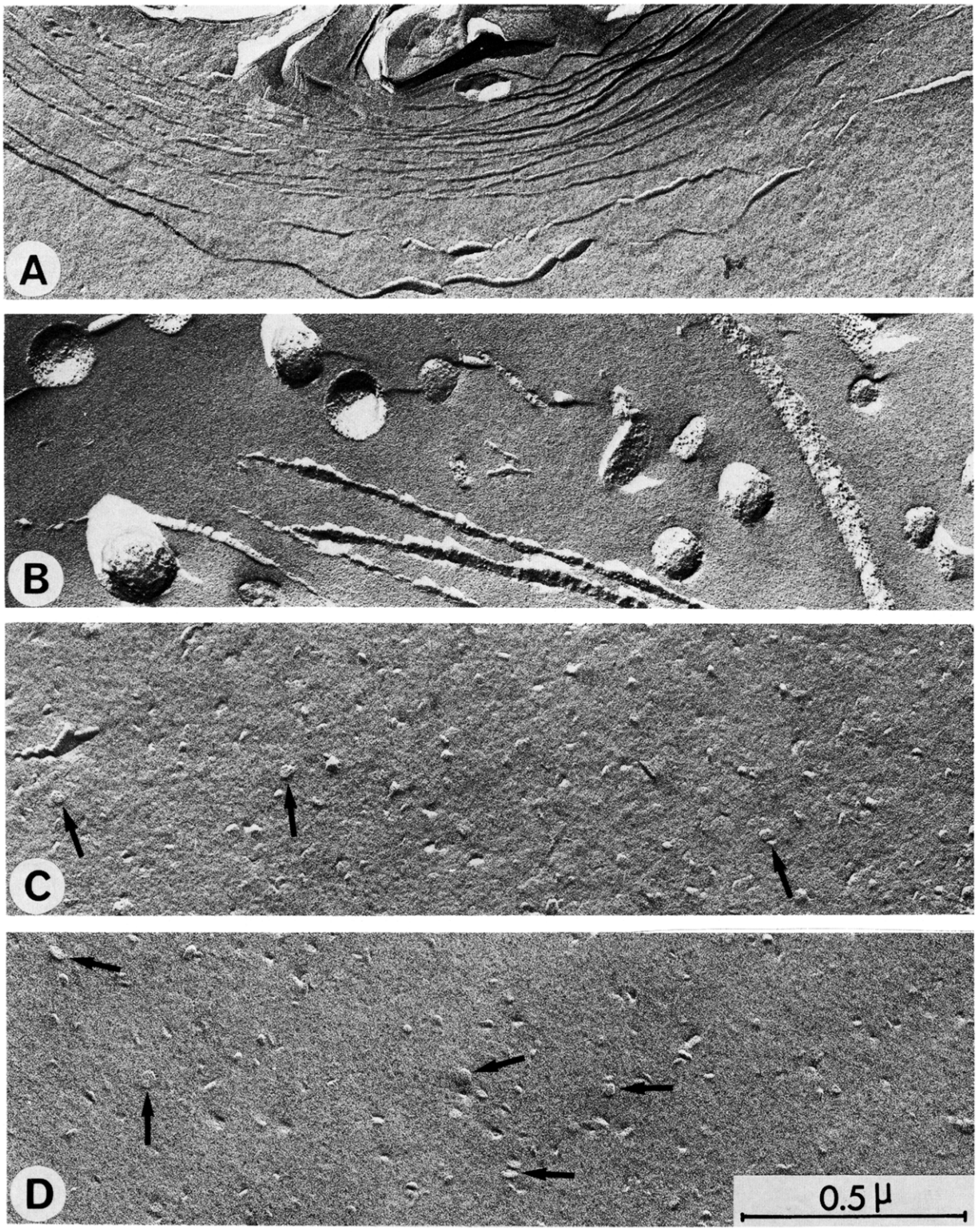
A
B
C
D
0.5 μ

Fig.1. Freeze-fracture electron micrographs of melittin-DPPC mixtures. (A–C) Ri = 30, (D) Ri = 15. Freeze-fracture has been performed from 20°C (A), 50°C (B) and 20°C after incubation at 50°C (C,D). Arrows show some of the discoidal structures (see text).

Clin, B., unpublished). NMR data were recorded by means of the Hahn-echo pulse sequence [4]. Freeze-fracture and replication were performed using a Balzers BAF 301 apparatus equipped with an electron gun for platinum-carbon shadowing. Replicas were examined in a Phillips 301 electron microscope.

## 3. RESULTS

### 3.1. *Electron microscopy*

Freeze-fracture electron microscopy was used to investigate the overall morphological changes induced by melittin on DPPC multilayers. When melittin is added to DPPC dispersions (Ri = 30) at 20°C, i.e. below the order-disorder transition temperature of pure lipids (fig.1A), no major changes can be detected as compared with replicas obtained with pure lipids [5]. However, when the same system is heated through the gel-to-fluid phase transition of lipids and frozen from 50°C a drastic change occurs (fig.1B). Most of the lipids, originally detected as flat multilayers, are converted into large unilamellar vesicles whose diameter is about ~4000 Å, although some lipid multilayers are still visible. When the same system is cooled to 20°C (fig.1C), totally different pictures are observed: isolated lamellae and small discoidal objects with an average diameter of 280 Å and a thickness compatible with that of a single bilayer are formed. When increasing the amount of melittin in the system (Ri = 15) very similar features are observed after incubation at 50°C and cooling to 20°C: freeze-fracture images show only discoidal objects (fig.1D).

### 3.2. *Quasi-elastic light scattering*

The average hydrodynamic radius of DPPC-melittin complexes was estimated by QELS as a function of temperature. At 50°C, i.e. corresponding to fig.1B, large objects of diameters 4000–6000 Å are detected (fig.2, Ri = 10.4 and 20.8). The sizes remain almost constant down to 41°C and after a dramatic decrease in size in a narrow temperature domain, the diameters range, at 22°C, from 100 to 250 Å, depending on the amount of melittin. In the transition domain, the autocorrelation functions were not monoexponential indicating heterogeneity of the system, i.e. coexistence within the sample of small and large objects. At very low lipid-to-protein ratio (fig.2, Ri = 5.2) the transition in size, formerly observed at about $T_c$, is no longer detected, although a slight decrease in size for the small structures is still observed between 28 and 32°C (fig.2). Finally, one has to emphasize that the lipid-melittin mixture must be brought once above $T_c$ in order to obtain reversible size and shape changes upon successive heating and cooling scans [6]. In other words, at 50°C for Ri $\geqslant$ 10, QELS detects an initial decrease

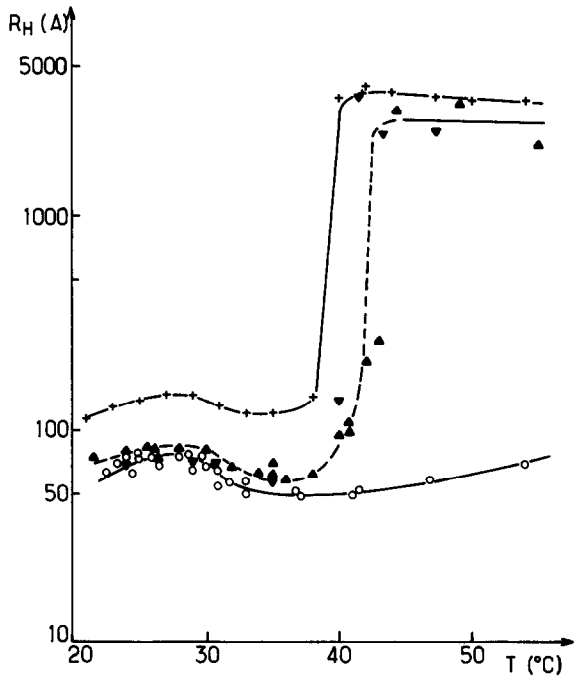


Fig.2. Temperature variation of the hydrodynamic radius, $R_h$, of DPPC-melittin systems as determined by QELS. (+) Ri = 20.8, heating scan, [melittin] = 0.26 mM. (▲, ▼) Ri = 10.4, heating and cooling scans, respectively; [melittin] = 2.6 mM for 22°C < $T$ < 38°C and [melittin] = 0.26 mM for $T$ > 38°C. (○) Ri = 5.2, heating scan, [melittin] = 2.6 mM.

in size from ~2 $\mu$m to 4000–6000 Å. This is compatible with the vesicularization process demonstrated by electron microscopy (fig.1A,B).

### 3.3. *$^{31}P$ solid-state NMR*

Fig.3 reports the temperature dependence of the second moment, $M_2$, for spectra of DPPC-melittin complexes (Ri = 15). Below 34°C, a narrow, single NMR line is observed, whereas above 42°C, broad $^{31}$P-NMR powder patterns are detected (see insets). Burnell et al. [7] have shown that the $^{31}$P-NMR line shape is very sensitive to the size of the vesicles: for usual solution viscosities and lateral diffusion constants in lipid bilayers, vesicle diameters lower than 1000 Å lead to symmetric, narrow, single $^{31}$P-NMR lines (reflecting isotropic tumbling of the small structures) whereas vesicle diameters greater than 4000–5000 Å lead to asymmetric, broad, so-called $^{31}$P-NMR powder patterns (characteristic of phospholipid axially symmetric reorientation in the membrane). As shown in fig.3, second moments may be used to quantify the change in shape and width of these NMR spectra and have been calculated by defining the zero in frequency at the position of the isotropic chemical shift. For temperatures between 34 and 42°C one observes

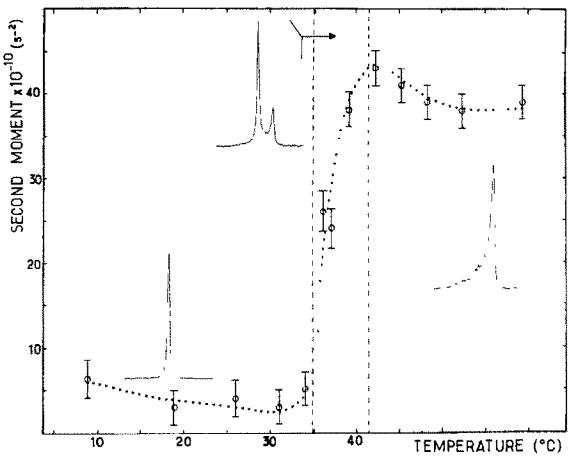


Fig.3. Temperature dependence of the second moment $M_2$ of $^{31}$P-NMR spectra of DPPC in the presence of melittin; Ri = 18. Insets represent typical spectra for corresponding temperature ranges. Experimental parameters: $\pi/2$ pulse length, 8 $\mu$s; delay between echo pulses, 60 $\mu$s; quadrature detection on a spectral width of 50 kHz; recycle time, 3–4 s; 500–2000 transients for each spectrum; proton broadband decoupling.

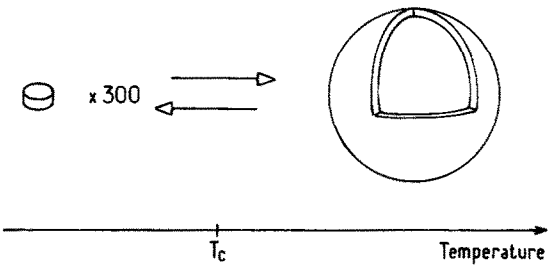


Fig.4. Schematic representation of the reversible disc-to-vesicle transition occurring at about $T_c$, for DPPC-melittin complexes (see text).

narrow lines superimposed on powder patterns (middle inset), indicating that the rate of exchange of phospholipids between the two structures is less than the frequency difference between the two NMR features, i.e. ~3–4 kHz. Therefore, in this temperature range the second moment represents a weighted average of $M_2$ originating from each subspectrum. This indicates that small and large objects coexist, on the millisecond time scale, and that the amount of each species varies continuously with temperature, between 34 and 42°C. As in QELS experiments the observed effects are reversible.

## 4. DISCUSSION

Three physical techniques show that melittin reorganizes the lipid assembly. Above $T_c$, and for low to intermediate amounts of protein vs lipids (Ri > 10), the DPPC multilayers are transformed into unilamellar vesicles (cf. fig.1A and B). Lowering the temperature below $T_c$ leads to the formation of very small discoidal structures (fig.1C,D). As observed by QELS and NMR this effect is reversible and the transition occurs at around $T_c$, i.e. when acyl chains melt. It is interesting to note that this size transition starts at temperatures below $T_c$, although, at Ri = 18–20, the main effect in size is observed between 37 and 41°C. This agrees with previous fluorescence and Raman data on the thermotropism of DPPC in the presence of melittin [6]. The change in the physical state of lipids (gel-to-fluid) therefore appears to trigger this morphological transition. Interestingly, the system must be incubated once above $T_c$, in the lipid fluid phase in order to produce such behavior. Above

$T_c$, at low lipid-to-protein ratio (e.g. Ri = 5) the large vesicles are no longer detected; instead, small objects of diameter ~120 Å are observed. In such melittin-rich systems the small decrease in size of the discs, centered at about 30°C as detected by QELS, only remains and may be related to fluorescence experiments [6] which report a broad transition starting at 30°C. This size change could also be correlated with the increase in binding of melittin at this temperature [3,5]. According to the size of the discs and their composition [5], one may calculate that several hundred discs merge, when lipid chains melt, into a single vesicle (fig.4). Conversely, the freezing of lipid chains induces the fragmentation of a large vesicle into a plethora of small discs. It is worth mentioning that vesicle-to-disc changes on varying the amount of amphiphiles to lipids have been reported [8,9], but this is the first time, to our knowledge, that a temperature-triggered reversible disc-to-vesicle transition has been demonstrated.

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