*Biochimica et Biophysica Acta*, 311 (1973) 651–654


## BBA Report

BBA 71173

# Spray–freezing of liposomes

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(Received May 29th, 1973)

SUMMARY

In freeze–etch studies it was found that liposomes of some lecithins exhibited wrinkled structures on fracture faces, when quenched from above the transition temperature. The formation of this artifact can be prevented by spray–freezing the liposome suspension.

Freeze–etching has been proven to be a valuable technique for the structural analysis of temperature-dependent lipid–water systems[1]. Recently Verkleij *et al.*[2] demonstrated that liposomes of many lecithins display smooth fracture faces when quenched from above the transition temperature. However, when the material was quenched from below this temperature, the liposomes showed regular band patterns on fracture faces. These were ascribed to a crystalline structure in which the bilayers are undulated. Other lecithins such as dipalmitoyllecithin with a rather high transition temperature (41 °C), when quenched from above that temperature display irregular, wrinkled structures instead of the expected smooth fracture faces. Such structures were notably found by Pinto da Silva[3]. It occurred to us that these surface structures do not correspond to the real lipid configuration in the liposomes above the transition temperature, but have to be considered as an artifact resulting from an insufficient quenching speed in the transition-temperature region. To overcome this problem we made use of the spray–freezing method as developed by Bachmann and Schmitt[4,5]. By this technique extremely high cooling rates can be obtained (> 10.000 °C/s) and segregation phenomena were reduced to a minimum in glycerol–water systems, solutions of macromolecules and dispersions of small particles[5]. No obvious differences could be observed between cell membranes which were spray–frozen and those that were quenched in the usual way[6]. In this communication we show that by spray–freezing of

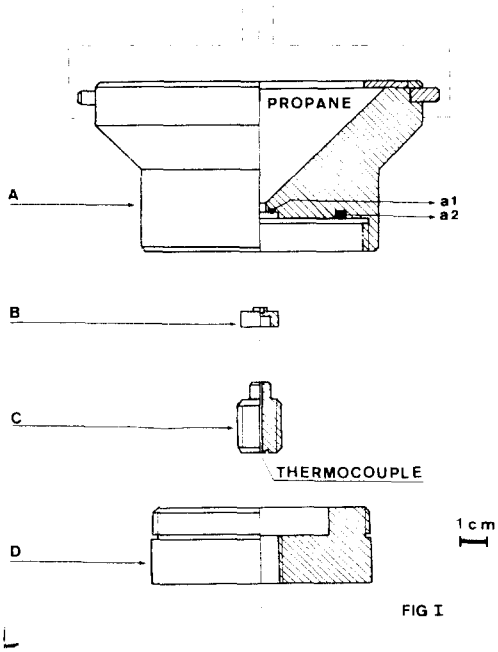


Fig.1. Adapted version of a spray–freeze device. A, funnel; B, specimen holder; C, fixing screw; D, heat sink; a1, vacuum-O ring; a2, O ring to prevent corroding of funnel and heat sink.

liposomes the generation of the wrinkled structure on fracture faces can be prevented.

1% (w/v) lipid dispersions in 5% glycerol were prepared as described before[2]. 0.05 ml of the sample was transferred to a Hamilton syringe of 0.1 ml. The syringe was provided with a 70 $\mu$m diaphragm as suggested by Bachmann *et al.*[7] or the spray piece of a commercial vaporizer. Then the syringe was equilibrated at the desired temperature. After equilibration for 30 min the dispersion was sprayed into liquid propane at $-180$ °C contained in a funnel at the apex of which the specimen holder was directly attached (Fig. 1). The spray droplets sedimented within 15 min in the specimen holder. Most of the propane was sucked off and the remainder was vacuum evaporated at a temperature of between $-120$ and $-100$ °C. The funnel was unscrewed from the specimen holder, which was transferred to a plateau kept at $-85$ °C. Then the specimen holder was further filled with precooled *n*-butyl benzene and a small hollow, open cylinder was placed on top of it. The so completed specimen holder, containing a sludge of frozen droplets and *n*-butyl-benzene at $-85$ °C was placed in liquid $N_2$ and then transferred to a Denton freeze–etch machine. The specimen was fractured at $-100$ °C, etched for 1 min and subsequently Pt/C shadowed. The replica was stripped

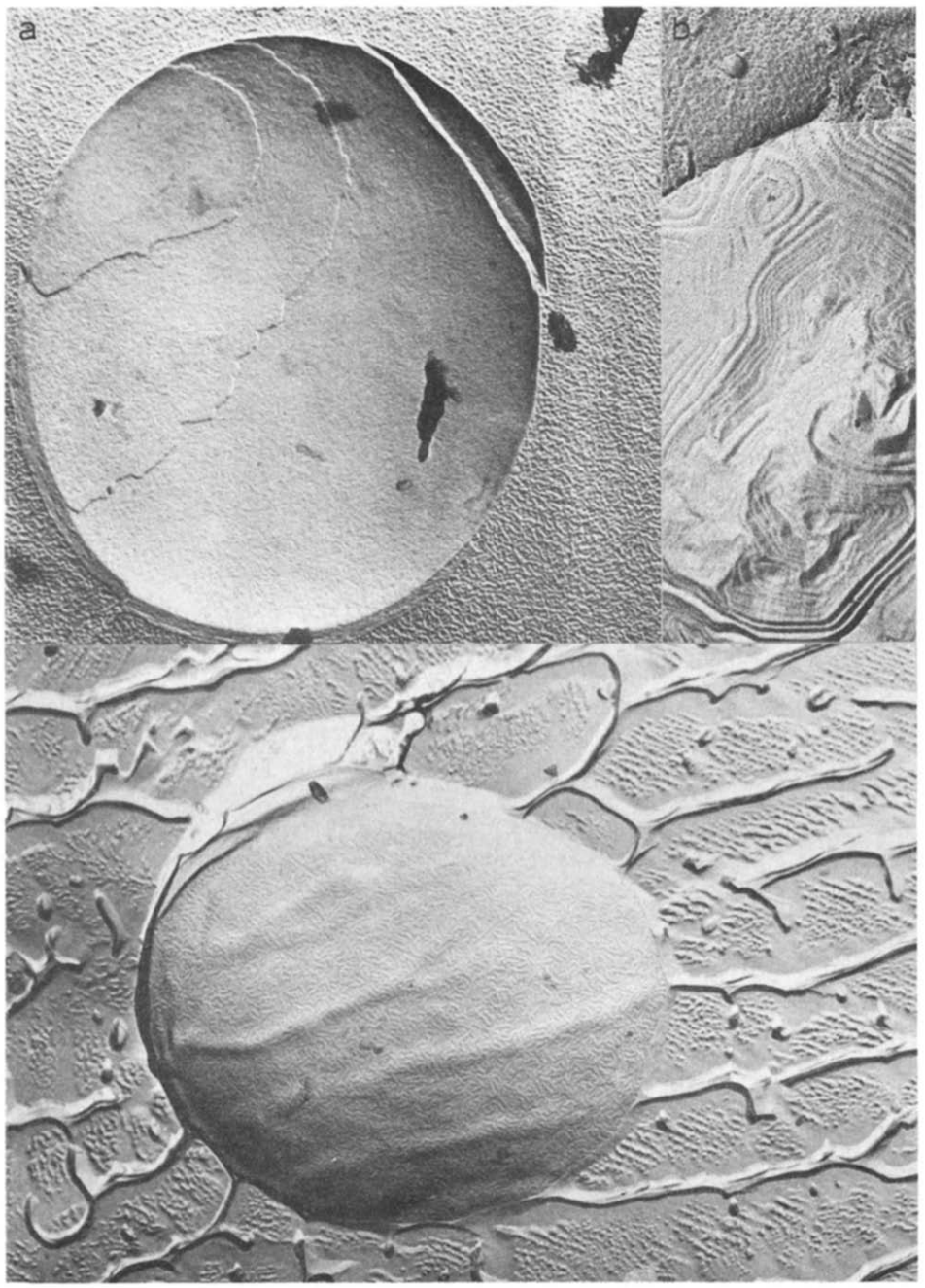


Fig. 2. a, This micrograph is characteristic for a dimyristoyl- or a dipalmitoyllecithin dispersion in 5% glycerol spray–frozen from 60 °C (*i.e.* 40 °C respectively 20 °C above the transition temperature). Note the small ice–glycerol compartments. b, Fracture face of a dipalmitoyllecithin dispersion in 5% glycerol spray–frozen from below its transition. The fractured material of a and b has been exposed ("etched") to the vacuum at −100 °C during 2 min. Magnification 40 000 ×.

Fig. 3. Fracture face of a dimyristoyllecithin dispersion in 5% glycerol quenched from 60 °C in the usual way. Note the large ice–glycerol compartments. The fractured material has been exposed ("etched") to the vacuum at −100 °C during 2 min. Magnification about 40 000 ×.

off at an interface of water and petrol ether (b.p. 40–60 °C), cleaned in pure acetone and stretched on distilled water.

In this way liposomes of dipalmitoyllecithin or dimyristoyllecithin were sprayed from 60 °C and further prepared. They display fracture faces that are completely smooth. The ice compartments surrounding the liposomes in a spray droplet are very small (50–150 Å) (Fig. 2a). Liposomes spray–frozen from below the transition temperature again show the typical crystalline structure which is found upon quenching in the usual way (Fig. 2b).

When, however, liposomes are quenched from 60 °C in the conventional way, that is with low quenching speed, wrinkled structures are found on the fracture faces.

In that case liposomes are embedded in areas showing large ice compartments with a diameter from 0.2 to 1 μm or even larger (Fig. 3). In addition to the wrinkled structure, we frequently found large rilles on liposomes which seemed to be continuous with the walls of large ice compartments. Our results show that these wrinkled structures are artifacts generated by the quenching procedure. This artifact is prevented by spray–freezing the material. With this technique the liposomes display smooth fracture faces as can be expected for lipid structures quenched from above the transition temperature[2].

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