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THE DISTRIBUTION OF CHOLESTEROL IN BILAYERS OF PHOSPHATIDYLCHOLINES AS VISUALIZED BY FREEZE FRACTURING

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SUMMARY

The crystallization behaviour of bilayers of synthetic phosphatidylcholines in the presence of cholesterol was investigated by freeze fracturing.

1. Below the lipid-phase transition, cholesterol is randomly distributed over the lateral plane of a bilayer of the single species dimyristoylphosphatidylcholine, as far as can be detected within the lateral resolution of freeze fracturing.

2. In an equimolar mixture of dilauroyl- and dimyristoylphosphatidylcholine, which shows cocrystallization, cholesterol is distributed randomly in the bilayers below the phase-transition temperature.

3. In an equimolar mixture of dipalmitoyl and 1-palmitoyl-2-oleoylphosphatidylcholine, which shows monotectic behaviour, cholesterol interacts preferentially with the liquid crystalline species 1-palmitoyl-2-oleoylphosphatidylcholine when the other component dipalmitoylphosphatidylcholine passes from the liquid crystalline to the gel state upon cooling the mixture.

INTRODUCTION

The effect of cholesterol upon the phase transition(s) occurring in various mixtures of synthetic phosphatidylcholines was recently investigated by differential-scanning calorimetry [1]. It was concluded that cholesterol does not show a preferential interaction with one of the phosphatidylcholine species in a mixture that shows cocrystallization. However, in phosphatidylcholine mixtures that show monotectic behaviour, cholesterol interacts preferentially with the phosphatidylcholine species with the lowest transition temperature.

Freeze-fracture electron microscopy has demonstrated that liposomes prepared from synthetic phosphatidylcholines have smooth fracture faces when quenched from above the transition temperature, whereas below that temperature characteristic band patterns were observed for each phosphatidylcholine species [2]. It was also shown that, because of this characteristic crystallization behaviour of synthetic phosphatidylcholines, one can visualize a phase separation in phosphatidylcholine

mixtures [3]. In an attempt to visualize the distribution of cholesterol in phosphatidylcholine bilayers the crystallization behaviour of (i) a single phosphatidylcholine species, (ii) phosphatidylcholine mixtures that show cocrystallization, and (iii) mixtures that show phase separation, was investigated in the presence of low concentrations of cholesterol.

MATERIALS AND METHODS

1,2-Dilauroyl-*sn*-glycero-3-phosphorylcholine ↓ (12 : 0/12 : 0-phosphatidylcholine); 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (14 : 0/14 : 0-phosphatidylcholine); 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (16 : 0/16 : 0-phosphatidylcholine) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine (16 : 0/18 : 1-phosphatidylcholine) were synthesized as described before [4]. Cholesterol (Fluka AG, Buchs, Switzerland) was recrystallized twice from ethanol. All lipids were chromatographically pure.

The samples were made as described before [1]. After 1 h equilibration at the desired temperature, the samples were quenched and fractured in a Denton Freeze etch machine at −196 °C as described before [3]. The replicas were examined with a Siemens Elmiskop IA.

RESULTS AND DISCUSSION

The influence of cholesterol on the crystallization behaviour of one phosphatidylcholine species

It was recently suggested [5–7] that in bilayers of phosphatidylcholine and cholesterol a phase separation occurs at low cholesterol concentrations in that clusters of cholesterol–phosphatidylcholine complexes are present next to free phosphatidylcholine. If this lateral phase separation is comparable with that demonstrated in a mixture of phosphatidylcholines that shows monotectic behaviour this should be able to be visualized with freeze fracturing. On fracture faces of phosphatidylcholine–cholesterol mixtures above 20 mole % cholesterol, smooth fracture faces are seen on quenching from below the transition temperature. One should therefore expect, in the case of partial phase separation, these smooth areas adjacent to areas with the bandpattern characteristic for the solid phosphatidylcholine species. We have therefore quenched mixtures of dimyristoylphosphatidylcholine and cholesterol from below the phase transition temperature.

Fig. 1A shows pure dimyristoylphosphatidylcholine liposomes with the typical bandpattern (structure of the so-called $p\beta'$ phase [8]) with lateral periodicities of 233 and 117 Å as reported earlier [11]. At 10 mole % (Fig. 1B) and 15 mole % cholesterol (Fig. 1C) there is still a typical dimyristoylphosphatidylcholine bandpattern with similar periodicities, although less pronounced, probably indicating a decrease in the amplitude of the wave. At 25 mole % the band pattern cannot be seen, Fig. 1 D. It is still possible that a wave is present, but not demonstrable as the amplitude is below the resolution of freeze fracturing.

However, since at 10 and 15 mole % of cholesterol a homogeneous bandpattern is visible all over the fracture plane of the bilayer, it can be concluded that within the lateral resolution of freeze fracturing the bilayers are completely homogeneous. In an

Fig. 1a, b, c and d. Fracture faces of dimyristoylphosphatidylcholine, containing 0 mole %, 10 mole %, 15 mole % and 25 mole % cholesterol respectively, quenched from 5 °C. About 60 000 ×.

idealized type of object (a sharp step) the resolution of platinum carbon shadowing at the best is in the order of 90 Å [9]. No indication of clusters of cholesterolphosphatidylcholine complexes with patches of free phosphatidylcholine, as was detected by other techniques [5–7] was found.

The influence of cholesterol on the crystallization behaviour of a mixture of phosphatidylcholine that shows cocrystallization

It is known from differential scanning calorimetric experiments that phosphatidylcholine mixtures of two species that differ by only two carbon atoms have only one thermotropic peak [10]. When cholesterol was added, there was no change on the transition temperature but only a decrease in the energy content of the transition, indicating a phosphatidylcholine–cholesterol interaction [1]. From these data it was concluded that there was no specific interaction with one of the phosphatidylcholine species.

For this reason equimolar mixtures of dilauroyl and dimyristoylphosphatidylcholine were quenched from below the phase transition temperature. Without cholesterol this mixture has a typical and new homogeneous corrugated band pattern with a periodicity of 180 Å (Fig. 2A)(see also ref. 3). When 10 mole % of cholesterol is added to the equimolar mixture, a homogeneous band pattern with a similar but less pronounced periodicity (as with the single species dimyristoylphosphatidylcholine) is still visible when quenched from below the phase transition temperature (Fig. 2B). No morphological features suggesting an interaction between cholesterol and only one of the phosphatidylcholine species in the mixture were seen in that. No

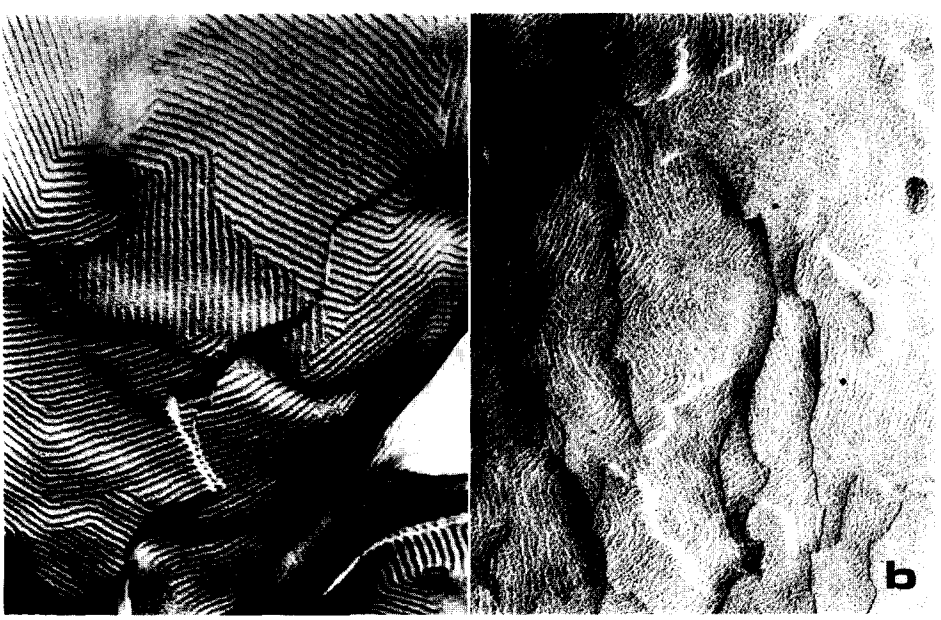

Fig. 2a and b. Fracture faces of an equimolar mixture of dilauroyl- and dimyristoylphosphatidylcholine, containing 0 and 10 mole % of cholesterol respectively, quenched from −10 °C. About 70 000 ×.

smooth areas of the cholesterol phosphatidylcholine complexes next to areas demonstrating a characteristic phosphatidylcholine band pattern, were present. This observation confirms the conclusion drawn from calorimetric data [1] that cholesterol

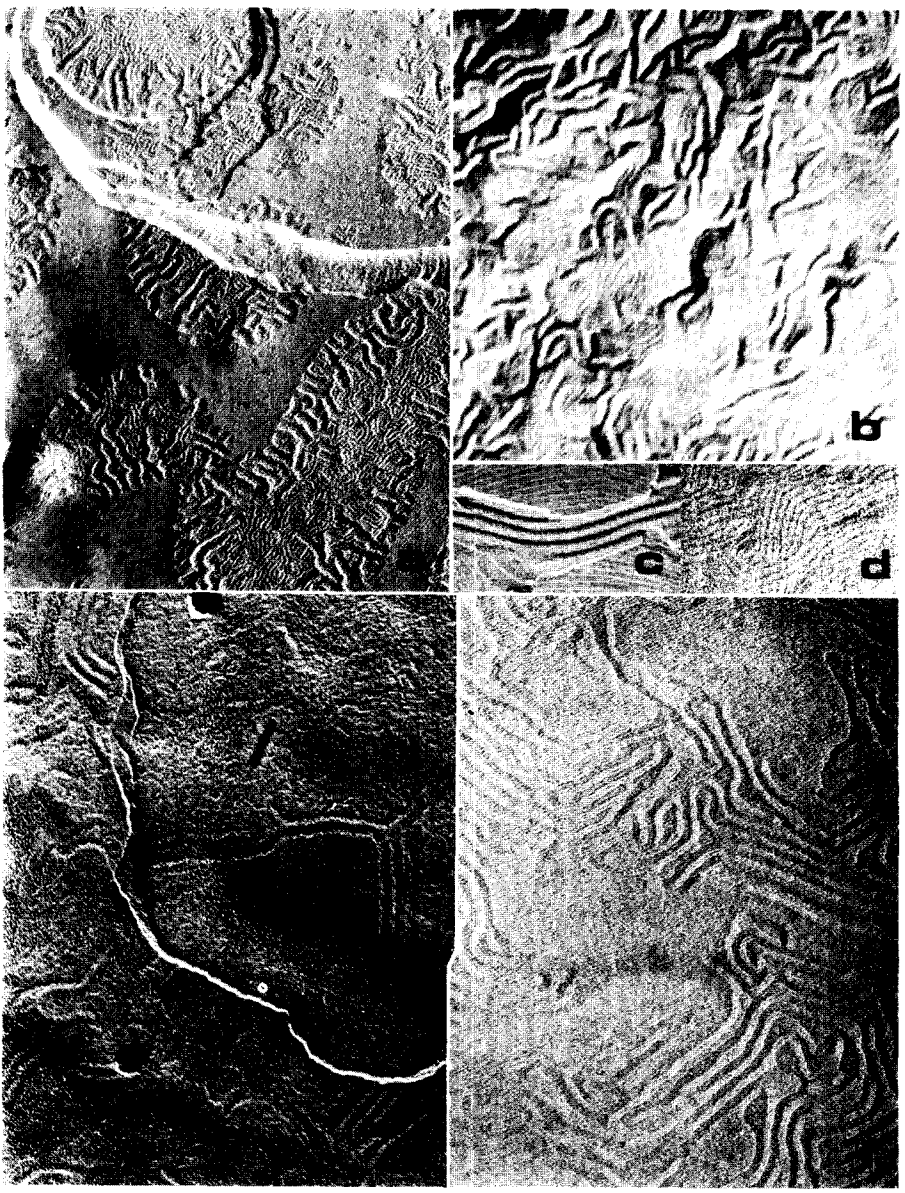

Fig. 3a and b. Fracture faces of an equimolar mixture of dipalmitoyl- and 1-palmitoyl-2-oleoyl-phosphatidylcholine quenched from at 23 and −10 °C respectively. c and d. Fracture faces of dipalmitoylphosphatidylcholine and 1-palmitoyl-2-oleoylphosphatidylcholine respectively quenched from 23 and −10 °C respectively. e and f. Fracture faces of an equimolar mixture of dipalmitoyl- and 1-palmitoyl-2-oleoyol-phosphatidylcholine containing 10 mole % of cholesterol quenched from 23 and −10 °C respectively. All about 60 000 ×.

has no preference for one of the phosphatidylcholine species in a mixture that shows cocrystallization.

The influence of cholesterol on the crystallization behaviour of a mixture of phosphatidylcholines that shows monotectic behaviour

In equimolar mixtures of two phosphatidylcholine species, that show monotectic behaviour, it was concluded that cholesterol preferentially interacted with the phosphatidylcholine species with the lowest transition temperature upon cooling the mixture. To see how cholesterol influences the crystallization behaviour of such a mixture, an equimolar mixture of dipalmitoyl and 1-palmitoyl-2-oleoylphosphatidylcholine was chosen, as the phase separation of this mixture can unambiguously be demonstrated by freeze fracturing (Fig. 3A and 3B, see also ref. 3). When the mixture was quenched from 23 °C (i.e. between the two thermotropic peaks [3]) one observes discrete regions with a band pattern, predominantly consisting of dipalmitoylphosphatidylcholine, suspended in the liquid crystalline 1-palmitoyl-2-oleoylphosphatidylcholine. When the mixture was quenched from −10 °C, where both species are in the gel state, the individual band pattern of both species is observed. The pattern of the separate species in the gel state are also shown in Fig. 3C and 3D.

When 10 mole % of cholesterol is added to this mixture, corrugated areas next to smooth areas are present on the fracture faces at 23 °C, Fig. 3E. It is likely that these bands originate from solid dipalmitoylphosphatidylcholine. Below both thermotropic peaks (−10 °C, Fig. 3F) the typical band pattern of 1-palmitoyl-2-oleoylphosphatidylcholine is no longer present, but areas with band patterns characteristic of dipalmitoylphosphatidylcholine and smooth areas representing the 1-palmitoyl-2-oleoyl-phosphatidylcholine–cholesterol fraction are seen.

Thus, in agreement with the differential scanning calorimetric data [1], it is suggested that cholesterol interacts with the lowest melting species upon cooling the mixture. Similar results were found with the equimolar mixture dipalmitoyl- and dilauroylphosphatidylcholine.

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