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EFFECT OF SUCCINYLPHOSPHATIDYLCHOLINE ON PHOSPHATIDYLCHOLINE VESICLES

STRUCTURAL STUDIES BY GEL CHROMATOGRAPHY, ELECTRON MICROSCOPY AND NUCLEAR MAGNETIC RESONANCE

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Summary

The effect of an aqueous dispersion of succinylphosphatidylcholine on an aqueous suspension of phosphatidylcholine vesicles was studied by gel chromatography, freeze-fracture electron microscopy and proton nuclear magnetic resonance with Mn^{2+} (broadening paramagnetic reagent). Total phospholipid concentrations were in the range 10—20 mM.

Succinylphosphatidylcholine is in micellar form and behaves as a detergent. The structures obtained depend on the molar percentage of succinylphosphatidylcholine.

Above a succinylphosphatidylcholine molar percentage of 60%, mixed micelles are formed, assumed to be essentially spherical.

Below a succinylphosphatidylcholine molar percentage of 30%, principally mixed vesicles are observed, with an external diameter of 215—240 Å, and an almost constant internal volume.

Between 30 and 60% of succinylphosphatidylcholine, a mixture of these structures is obtained; rod-shaped profiles are also observed in electron microscopy, which may correspond to sections of leaky vesicles or to a new kind of cylindrical micelle.

Introduction

The structure and permeability of biological and model membranes are modified by various lipids such as lysophosphatidylcholines [1—4] or synthetic phosphatidylcholines [3].

In the same way, lipid peroxidation also increases the permeability of liposomes [5]. As previously reported in our laboratory [6], peroxidative attack of unsaturated phosphatidylcholines promotes the formation of dicarboxylic phospholipids, structurally-related to 1-acyl-2-succinyl-*sn*-glycero-3-phosphorylcholine (succinylphosphatidylcholine) [7] (Fig. 1) or to 1-acyl-2-glutaryl-*sn*-glycero-3-phosphorylcholine (glutarylphosphatidylcholine), the synthesis of which has been described elsewhere [8]. These compounds display lytic properties against both red blood cells [9,10] and multilamellar liposomes [11].

Furthermore, succinylphosphatidylcholine can play an important role in the hydrolysis of phosphatidylcholine by *Crotalus adamanteus* venom phospholipase A_2: whereas it is a rather poor substrate, it can behave either as an inhibitor or as an activator, depending on the molar fraction of succinylphosphatidylcholine (Dang, Q.Q., Ha Dang, T.B.T. and Douste-Blazy, L., unpublished results). As interfacial enzyme kinetics are highly dependent on the physical state of the substrate [12,13], we assumed that this reversal of activity was due to a structural change of the substrate induced by succinylphosphatidylcholine.

In the present study, the nature of this structural change induced in a lipid bilayer by succinylphosphatidylcholine has been investigated using phosphatidylcholine vesicles. The shape, size and composition of the resulting structures have been studied by gel filtration, freeze-fracture electron microscopy and NMR, as well as by turbidity measurements.

Materials and Methods

1. Materials

Pure egg phosphatidylcholine was prepared according to Hanahan et al. [14]. Succinylphosphatidylcholine was synthetized (Dang, Q.Q., Ha Dang, T.D.T. and Douste-Blazy, L., unpublished results) as previously described for glutarylphosphatidylcholine [8] followed by decomplexation by ion-exchange resins [15] and preparative thin-layer chromatography on silicagel with Merck plates and $CHCl_3/CH_3OH/6$ M NH_4OH, 70 : 30 : 5 (v/v/v), as a solvent. In some experiments, [^{14}C]choline-labelled phosphatidylcholine from rat liver [16] (a gift from Mr. C. Vieu) and [^{14}C]choline-labelled succinylphosphatidylcholine were also used.

2. Preparation of phospholipid dispersions

(a) Phosphatidylcholine vesicles. These were prepared by sonication followed by ultracentrifugation essentially according to the method of Huang [17] modified by Barenholz et al. [18]. The suspending medium (pH 7.5) contained 0.1 M NaCl, 0.01 M boric acid and 0.02% (w/v) sodium azide. Phosphatidylcholine in $CHCl_3$ solution (usually 50 or 100 μmol) was evaporated to dryness, first in a rotavapor at 50°C, then under a vacuum air pump for 1 h. The residue was dispersed in 5 ml of the specified buffer by manual shaking in a 50°C water bath, the suspension was bubbled with nitrogen then sonicated intermittently under nitrogen in an ice bath, using an MSE sonicator with a 9-mm diameter tip (20 kHz, 8 μm amplitude), until sonication produced no further clearing of the homogeneous opalescent dispersion: 20 times (1 min sonication plus 1 min

pause) were sufficient. The dispersion was then ultracentrifuged for 1.5 h at 160 000 × *g* in a Beckman L5-65 ultracentrifuge with a 50 TI rotor.

The clear supernatant contains the vesicle preparation. Thin-layer chromatography (see Methods, 7) showed no alteration of phosphatidylcholine by ultrasonication. The preparation could be stored under nitrogen at room temperature to be used within 1 week. NaN_3 prevented bacterial alteration of phospholipids during storage, and of Sephadex and Sepharose in gel filtration.

(b) Succinylphosphatidylcholine aqueous dispersion. This was obtained by evaporation to dryness of a $CHCl_3$ solution of succinylphosphatidylcholine and further dissolution in an appropriate volume of the above-mentioned buffer, in order to obtain the same concentration as for the phosphatidylcholine vesicles; 1 or 2 min of manual shaking at 50°C were sufficient to produce a clear solution.

(c) Mixed dispersions of succinylphosphatidylcholine with phosphatidylcholine. These were obtained by mixing appropriate volumes of the two previous aqueous dispersions, shaking in a 50°C water bath, stirring for 1 min (Vortex) and leaving to equilibrate at room temperature for 1 h. The total phospholipid concentration was kept constant in each experiment (usually between 10 and 20 mM).

3. Analytical gel chromatography

(a) Gel chromatography on Sepharose 4B. The method was essentially derived from that of Huang [17]. A column of Sepharose 4B (Pharmacia) 1.6 × 20 cm was equilibrated at room temperature, at a water pressure of 20 cm, with the following elution solution: 0.1 M NaCl, 0.01 N H_3BO_3 (pH 7.5), 0.02% NaN_3. 1 ml of the phospholipid dispersion was applied to the column.

The flow rate was 1.25 ml/min and fractions of 0.9 ml were collected. The molar percentage of succinylphosphatidylcholine, 100 SPC/(PC + SPC) in the eluates was determined by thin-layer chromatography and liquid scintillation (see section 7).

(b) Gel chromatography on Sephadex G-50: sucrose entrapment. Determinations of sucrose entrapment were performed in order to evaluate the size of the internal compartment of the particles as suggested by Kornberg and McConnell [19]. Gel chromatography on Sephadex was adapted from the method of Johnson and Bangham [20], using an isotonic eluant as described by Kinsky et al. [21].

The phosphatidylcholine vesicle suspension was prepared as described in 2(a), except that the suspension medium was: 0.01 M H_3BO_3 (pH 7.5), 0.02% NaN_3, 0.3 M sucrose and 0.5 μCi/ml of [6,6′(*n*)-^{3}H]sucrose, Amersham.

The succinylphosphatidylcholine dispersion was prepared as described in 2(b), the mixed phospholipid dispersion as in 2(c), using also this new suspension medium.

The column containing 3 g of Sephadex G-50 (Pharmacia), 1.6 × 20 cm, was equilibrated at room temperature by gravity with the following elution solution: isotonic buffer 0.01 M H_3BO_3 (pH 7.5), 0.075 M NaCl, 0.075 M KCl, 0.02% NaN_3. Fractions of 1.6 ml (45 s) were collected.

The fractions were tested for their phosphorus content using the Dittmer-Lester reagent [22] and the amount of entrapped sucrose by liquid scintillation in picofluor vials with a Packard Tricarb 3390.

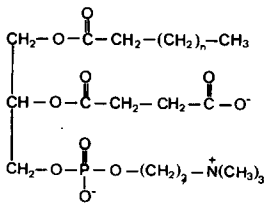

Fig. 1. Molecular structure of succinylphosphatidylcholine (or 1-acyl-2-succinyl-*sn*-glycero-3-phosphorylcholine).

4. Electron microscopy

Phosphatidylcholine-succinylphosphatidylcholine samples were mixed with equal volumes of a buffer containing 60% glycerol, and were incubated for 2 h. Small droplets of the preparation in 3-mm gold cups were rapidly frozen in liquid Freon 22 (—160°C). The specimens were freeze-fractured at —115°C and shadowed with platinum-carbon in a Balzer's BAF 300 freeze-etch apparatus (Balzer High Vacuum Cor.). The Pt-C replicas were floated off the cups with distilled water, cleaned with methanol, picked up on electron microscope grids and examined in a Philips EM 301 electron microscope.

The average diameter of the vesicles was calculated from the micrographs by Weibel's method [23] for spherical particles at 75 000 final magnification, using an optical micrometer.

5. NMR

Proton NMR spectra were recorded at 90 MHz (Bruker WH 90). Phospholipid preparations were made as in section 2, but using ^2H_2O instead of H_2O. The temperature of the sample in the spectrometer was 30°C. Spectra were recorded without or with 1 mM $MnCl_2$ [24,25].

6. Measurement of turbidity

The turbidities of mixed dispersions of phosphatidylcholine and succinylphosphatidylcholine were measured by recording absorbance at 450 nm in cuvettes of 1-cm light path.

7. Analytical methods and critical micellar concentration

Phospholipid concentrations were measured by phosphorus determination [26]. The molar composition of the eluates was determined by separation of the components by thin-layer chromatography on silica gel F 254 (Merck) using $CHCl_3/CH_3OH$/6 M NH_4OH (70 : 30 : 5, v/v) as a solvent, by phosphorus determination of the scraped spots.

In control experiments, mixtures of ^{14}C-labelled phosphatidylcholine or succinylphosphatidylcholine of known specific radioactivity (about 0.1 Ci/mol) were used, and the molar ratio of these compounds in the eluates was measured by thin-layer chromatography and scintillation counting: eluate samples (100 μl) were dried in vacuo, redissolved in $CHCl_3/CH_3OH$ (1 : 1) and chromatographed as before; phosphatidylcholine and succinylphosphatidylcholine spots were collected in Instafluor vials. Countings were performed in a Packard

Tricarb 3390 liquid scintillation spectrometer with automatic quenching correction.

The critical micellar concentration of succinylphosphatidylcholine was measured according to Bonsen et al. [27] in the buffer solution described in section 2 (Methods).

Results

1. Gel chromatography on Sepharose 4B: verification of homogeneity, size and composition of the particles

The elution diagrams obtained are shown in Fig. 2. A phosphatidylcholine dispersion prepared by ultrasonication without any centrifugation gives the dotted curve of Fig. 2A. It looked like the elution diagram previously reported by Huang [17]; it shown a small peak corresponding to a turbid suspension of multilamellar liposomes, eluted at the void volume V_0, and another peak eluted at $V_{e1} = 1.67\ V_0$, corresponding to monolamellar liposomes (vesicles).

A phosphatidylcholine suspension, prepared by ultrasonication and centrifugation according to Barenholz et al. [18] (Methods, 2(a)), gave a single peak of vesicles at the same elution volume V_{e1} (solid line in Fig. 2A).

A succinylphosphatidylcholine dispersion, prepared as in Methods 2(b), gave also a unique peak corresponding to an elution volume $V_{e2} = 2.1\ V_0$ (Fig. 2D) greater than that of the vesicles V_{e1}. V_{e2} is found close to the elution volume of lysophosphatidylcholine, which was found to be in a spherical micelle state [28,29] (diagram not shown here). This suggests that succinylphosphatidylcholine might also be in a micellar state.

At high molar percentages of succinylphosphatidylcholines (60—100%), one single peak was also obtained at about the same elution volume, V_e, as for succinylphosphatidylcholine (Fig. 2C, $V_e = 2.1\ V_0$), suggesting a micellar state. Thin-layer chromatography of the eluates, with phosphorus determination of the spots, gave the molar percentage of succinylphosphatidylcholine (stars on the eluation diagram Fig. 2C). This confirms that there were mixtures of phosphatidylcholine and succinylphosphatidylcholine, and that a quite good homogeneity was reached.

At low molar percentages of succinylphosphatidylcholine (0—30%), essentially one peak was obtained at about the same elution volume as for phosphatidylcholine vesicles (Fig. 2B, $V_e = 1.59\ V_0$), this suggests the presence of particles of similar size, probably vesicles. Small shoulders observed at elution volume V_0 and $2.1\ V_0$ suggest that small percentages of multilamellar liposomes and micelles should also be present. Thin-layer chromatography confirms the presence of a mixture of phosphatidylcholine and succinylphosphatidylcholine.

At mean values of the molar percentage of succinylphosphatidylcholine (30—60%), apparently two peaks were obtained at elution volumes close to those of phosphatidylcholine microvesicles (approx. $1.6\ V_0$) and succinylphosphatidylcholine micelles (approx. $2.1\ V_0$), respectively (not shown here). This suggests structures of size similar to that of the two previous ones, probably mixed vesicles and mixed micelles.

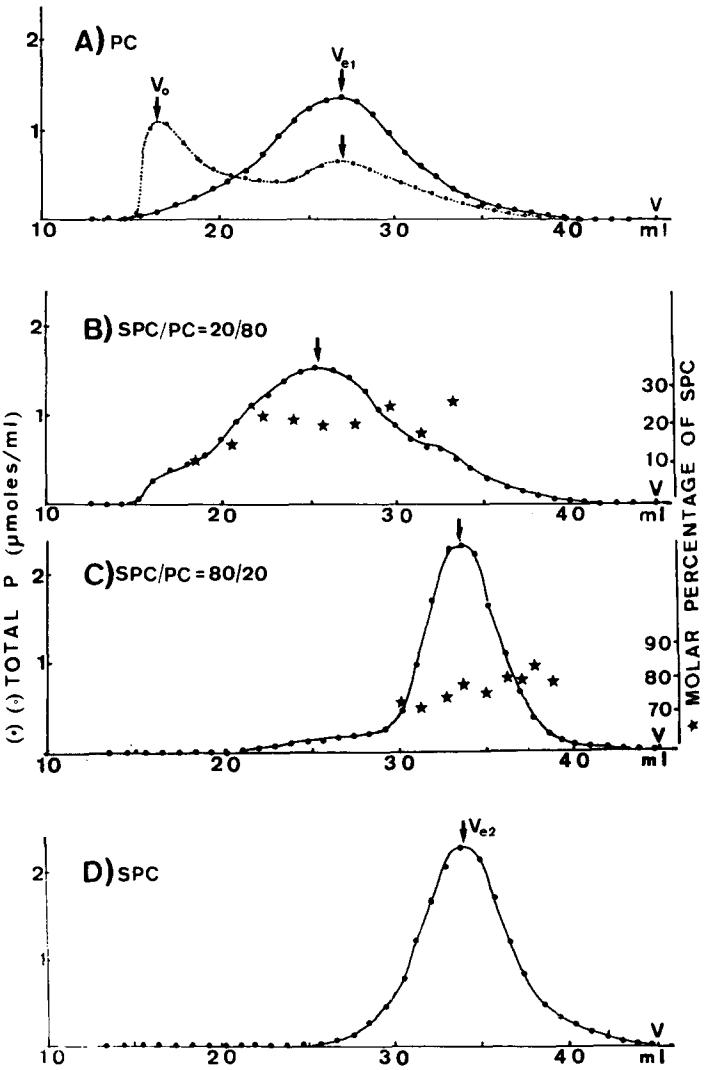

Fig. 2. Elution diagrams of phosphatidylcholine (PC)-succinylphosphatidylcholine (SPC) dispersion in gel chromatography on Sepharose 4B. For procedure, see text. Applied sample: 1 ml (20 μmol lipid phosphorus). (A) dotted line, ultrasonicated phosphatidylcholine dispersion; solid line, ultrasonicated and ultracentrifugated phosphatidylcholine dispersion (vesicles) (B) A mixture of succinylphosphatidylcholine and of phosphatidylcholine vesicle suspension at a final molar ratio of 20/80. (C) same as in B except that the molar ratio was 80/20. (D) succinylphosphatidylcholine dispersion alone.

2. Gel chromatography on Sephadex G-50: sucrose entrapment in phospholipid particles

Phosphatidylcholine vesicle suspension, succinylphosphatidylcholine dispersion and mixed dispersions were chromatographed on Sephadex as described in Methods section 3(b). The phospholipid particles and the labelled sucrose entrapped within, were eluted at the void volume, while the free sucrose was eluted long afterwards [1].

A phosphatidylcholine vesicle suspension showed a significant amount of entrapped sucrose (Fig. 3) confirming the presence of an internal compartment. But a succinylphosphatidylcholine dispersion showed no entrapped sucrose, suggesting structures with no internal compartment, such as micelles.

For mixed dispersions, the amount of entrapped sucrose versus the molar percentage of succinylphosphatidylcholine is given in Fig. 3. Results are expressed as μmol of sucrose per μmol of phosphatidylcholine (and not per μmol of total phospholipid) so as to best compare the total internal volume of the particles (vesicles), before and after addition of succinylphosphatidylcholine.

Above a succinylphosphatidylcholine molar percentage of 60%, no significant amount of sucrose was entrapped, suggesting structures with no internal volume: micelles or leaky vesicles.

Below a succinylphosphatidylcholine molar percentage of 30%, the amount of entrapped sucrose was about the same as for pure phosphatidylcholine vesicles, suggesting intact (not leaky) vesicles.

At intermediate values (30—60%) of succinylphosphatidylcholine, the amount of trapped sucrose dramatically decreased, suggesting transformation of intact vesicles into structures devoid of internal volume: micelles or leaky vesicles.

3. Electron microscopy: shape and size of the particles

Freeze-fracture electron microscopy showed that a merely ultrasonicated phosphatidylcholine suspension was made of a mixture of multilamellar and monolamellar (vesicular) liposomes (not shown here). But ultracentrifuged preparation as described in Methods section 2(a) consisted of monolamellar lipo-

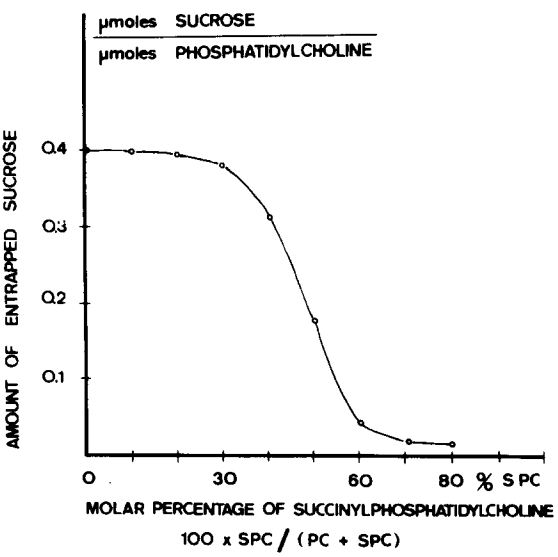

Fig. 3. Amounts of entrapped sucrose as a function of succinylphosphatidylcholine molar percentage. Entrapped and free sucrose were separated by gel filtration through Sephadex G-50.

somes (vesicles) only (Fig. 4A). However, a succinylphosphatidylcholine preparation gave no visible particle.

With mixed phosphatidylcholine-succinylphosphatidylcholine dispersions, below a succinylphosphatidylcholine molar percentage of 30%, the vesicles still remained (Fig. 4B); above a molar percentage of 60%, almost all the vesicles disappeared, probably with formation of mixed micelles (Fig. 4D). Between these two limits (30—60%), there appeared, in addition to vesicles, another kind of particle, the profiles of which were rod shaped, with a diameter of about 70—80 Å (Fig. 4C).

Up to a succinylphosphatidylcholine molar percentage of 30%, the vesicles could be considered as spherical. The average diameter of the vesicles, calculated according to Weiber [23] was 210 ± 25 Å for pure phosphatidylcholine, and increased slightly to 240 ± 25 Å when the succinylphosphatidylcholine molar percentage increased from 0 to 30%.

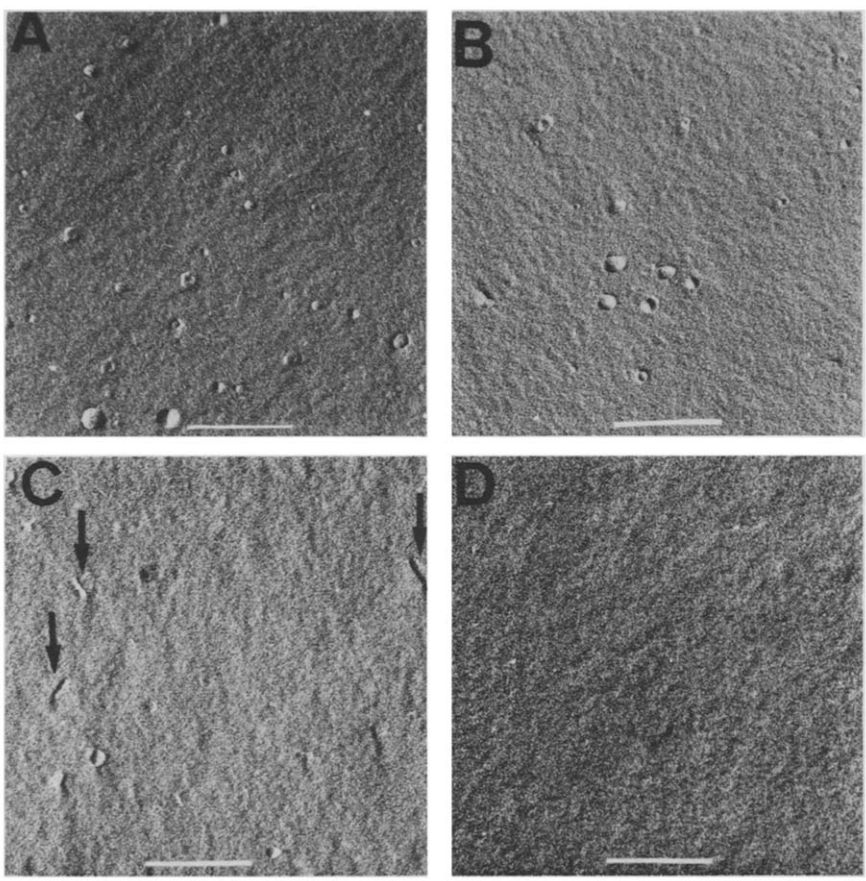

Fig. 4. Electron micrographs of phosphatidylcholine-succinyl phosphatidylcholine dispersions at various molar ratios SPC/PC: (A) 0/100; (B) 20/80; (C) 45/55; (D) 80/20. The mixed dispersion (total phospholipid concentration 12 mM) was added to an equal volume of suspension medium containing 60% glycerol, hand mixed, and incubated for 2 h. Bars represent 2000 Å final magnification. Arrows indicate rod shaped profiles.

4. NMR spectra

(a) Mn^{2+} broadening reagent/polar heads $N(CH_3)_3$: revelation of an internal compartment. Proton NMR spectra were recorded at various succinylphosphatidylcholine/phosphatidylcholine ratios without, and then with, addition of a low percentage of $MnCl_2$ (1 mM for about 20 mM phospholipid).

With phosphatidylcholine vesicle suspension, the area of the $N(CH_3)_3$ proton peak was reduced by Mn^{2+} to 33% of its value without Mn^{2+}. This means that 67% of the polar heads interacted with the Mn^{2+} and 33% did not, or 33% of the polar heads corresponds to an inner compartment [24,25]. The last result agrees with those of previous investigators, who reported a ratio between outer and inner polar heads of 1.8—2.2 for sonicated vesicles [30—32].

On the other hand, with succinylphosphatidylcholine dispersion, the area of the $N(CH_3)_3$ proton peak disappeared completely: Mn^{2+} interacted with all the polar heads. This suggests a micelle state or other structures without internal compartment.

Nearly the same was observed above a succinylphosphatidylcholine molar percentage of 60%: the $N(CH_3)_3$ peak disappeared almost completely (Fig. 5B) suggesting the formation of structures without internal compartment.

At low molar percentage of succinylphosphatidylcholine (below 30%) the reduction of the $N(CH_3)_3$ peak area was about the same as for the pure phosphatidylcholine, this area is reduced to about 31% by Mn^{2+} effect, suggesting essentially intact vesicles (Fig. 5A).

At a molar percentage of succinylphosphatidylcholine between 30% and 60%, the remaining $N(CH_3)_3$ peak area dropped sharply from 31% to nearly 0% of its value without Mn^{2+}. This suggests increased accessibility of Mn^{2+} to the remaining polar heads, i.e., transformation of intact vesicles into leaky vesicles or micelles.

(b) Line-width of the hydrophobic chain $(CH_2)_n$ peaks. Without Mn^{2+}, the proton NMR spectra of the various succinylphosphatidylcholine/phosphatidylcholine dispersions showed:

— relatively wide $(CH_2)_n$ peaks at low percentages of succinylphosphatidylcholine (0—30%), the line-widths at mid height were quite similar for pure phosphatidylcholine (18 Hz) and 20% molar succinylphosphatidylcholine (18 Hz).

— much narrower $(CH_2)_n$ peaks for high percentages of succinylphosphatidylcholine (60—100%), the line-widths at mid height were 4 Hz for pure succinylphosphatidylcholine and 7 Hz for 80% molar succinylphosphatidylcholine.

5. Critical micellar concentration

The critical micellar concentration of succinylphosphatidylcholine in the suspension medium mentioned in Methods section 2, was 0.1 mM, similar to that of lysophosphatidylcholine [28].

In the range of concentration tested (10—20 mM) succinylphosphatidylcholine must be in the micellar form and very little in the monomeric form.

Under the same conditions, phosphatidylcholine showed an experimental 'critical micellar concentration' of about 0.01 mM.

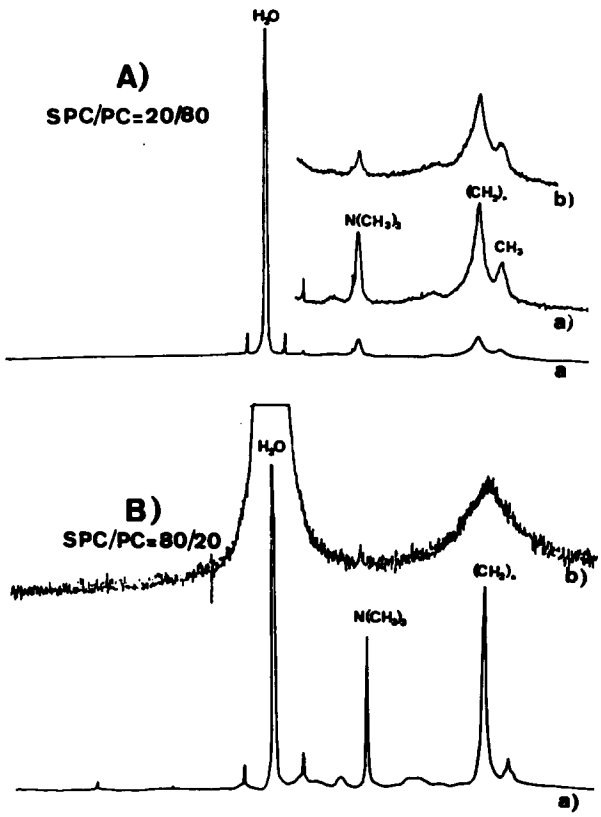

Fig. 5. Proton NMR spectrum in ^2H_2O of succinylphosphatidylcholine-phosphatidylcholine dispersions, at molar ratios SPC/PC. (A) 20/80, (B) 80/20, (total phospholipid concentration = 10 mM). (a) without $MnCl_2$; (b) with $MnCl_2$.

6. Turbidity measurements: effect of incubation time and molar percentage of succinylphosphatidylcholine

A succinylphosphatidylcholine dispersion (Methods section 2(b)) was added to a phosphatidylcholine vesicle suspension (Methods section 2(a)) mixed as described in Methods section 2(c), and left at room temperature.

Above a succinylphosphatidylcholine molar percentage of 60%, the absorbance at 450 nm decreased immediately after mixing to a value close to that of succinylphosphatidylcholine dispersion ($A \cong 0.05$), Below this succinylphosphatidylcholine molar percentage, the curve absorbance versus succinylphosphatidylcholine molar percentage was stabilized after about 1 h for at least 4 h. The absorbance below 30% molar percentage of succinylphosphatidylcholine was then close to that of pure phosphatidylcholine vesicle suspension ($A = 0.20$—0.26).

Discussion

The previous experiments suggest the following:

1. Pure succinylphosphatidylcholine dispersion is in a micellar state. This can

be explained by its chemical structure [13]. Like lysophosphatidylcholine, it has a single hydrophobic chain [28,29]. Its polar head displays one negative net charge resulting from three charged groups (Fig. 1). So it behaves as an anionic detergent and as a kind of wedge molecule [33,4].

2. When a succinylphosphatidylcholine dispersion acts on a suspension of phosphatidylcholine vesicles at constant total phospholipid concentration, the structures obtained depend essentially on the relative percentages of these two phospholipids.

(a) Up to a molar percentage of succinylphosphatidylcholine of 30%, the vesicles remain mostly intact. This is evidenced by electron microscopy, sucrose entrapment, sepharose gel chromatography and NMR, which all show similar properties to those of pure phosphatidylcholine vesicles, the external diameter being about 215—240 Å. Furthermore, incorporation of succinylphosphatidylcholine into the vesicles is verified by the thin-layer chromatography analysis.

A similar stability of the vesicle structure, with an external product incorporation up to 20—30%, has been previously observed, when phosphatidylcholine vesicles incorporated lysophosphatidylcholine [34], cholesterol [35] or an aminooxide detergent [36], or when sphingomyelin liposomes incorporated Triton X-100 [37].

(b) Above a molar percentage of succinylphosphatidylcholine of 60%, the negligible sucrose entrapment and the disappearance, from the NMR spectra, of the $N(CH_3)_3$ signals by addition of Mn^{2+}, showing the accessibility of all the polar heads to this ion, would suggest the production of either micelles of leaky vesicles. However, as no structure is visualized in electron microscopy, and since the phospholipids aggregates are eluted from sepharose at the same volume as succinylphosphatidylcholine micelles, one must rule out the hypothesis of leaky vesicle formation, in favour to spherical micelles. One could expect a micellar diameter of the order of 70 Å, as previously reported for lysophosphatidylcholine micelles [38].

Similar transformation of liposomes into micelles by the action of detergents has been reported with different systems: phosphatidylcholine + lysophosphatidylcholine [34], phosphatidylcholine + Triton X-100 [39,40] and phosphatidylcholine + sodium cholate [41,42].

The narrowing of the $(CH_2)_n$ signals observed during the transformation of vesicles into micelles indicates a greater mobility of this group. This may be due to a more rapid Brownian rotation of the particle as its diameter become smaller, or to a looser packing of the acyl chains in the micelles where they are radially oriented whereas they remain almost parallel to their neighbours in the vesicles, or even to a combination of both effects [43].

(c) At intermediary values of molar percentage of succinylphosphatidylcholine (30—60%), a mixture of different structures is observed: Gel chromatography on Sepharose 4B with two apparent peaks suggests a mixture of vesicles (intact or leaky) and spherical micelles. The sucrose entrapment drops significantly to nearly zero, suggesting the formation of leaky structures (leaky vesicles) or structures with zero internal volume (micelles). Electron microscopy shows in this range two kinds of profiles 'round' or 'rod'. This first one corresponds to intact vesicles. But rods may be sections of cylindrical micelles as well as sections of leaky discoid vesicles.

The diameter of our rod profiles were 70—80 Å. The diameter of cylindrical micelles reported for phosphatidylcholine + lysophosphatidylcholine + cholesterol was 40 Å [38], phosphatidylcholine + lysophosphatidylcholine was 60 Å [44]. In those studies, only neutral lipids were used. In our experiments, a charged phospholipid was incorporated in the particles. The repulsion between the negative polar heads could increase somewhat the diameter of cylindrical micelles. So this hypothesis cannot be entirely ruled out. But discoid structures have been also reported by Inoue et al. [44] for phosphatidylcholine/lysophosphatidylcholine (1 : 1) mixtures. Rod-shaped profiles may also be sections of discoid structures, the thickness of phospholipid bilayer being usually estimated to be 40—50 Å [1,17,19]. It is not yet possible with our experimental results to choose between these two hypotheses.

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