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Stabilization of small unilamellar phospholipid vesicles during spray-drying

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In the presence of 10% (0.3 M) sucrose in the aqueous medium, small unilamellar phospholipid vesicels are preserved during freeze-drying and spray-drying. Moreover, the bilayer integrity and permeability barrier are maintained during these processes.

Recently unilamellar lipid vesicles have become important in several research areas dealing with membrane-mediated processes such as membrane fusion, interfacial catalysis, energy conduction and conversion, drug delivery and targeting. There is good hope that this kind of research will eventually lead to industrial applications of unilamellar lipid vesicles. In any practical application the questions of long-term storage and related to it vesicle and bilayer stability are important. It is well-known that aqueous dispersions of small unilamellar lipid vesicles (SUV) are thermodynamically unstable. For instance, SUV made of zwitterionic phosphatidylcholines tend to aggregate and/or fuse to large multilamellar lipid particles at room temperature. Furthermore, they undergo chemical degradation with time. The process of fusion of SUV is greatly accelerated when SUV are subjected to freeze-thawing or dehydration. It was shown that SUV of egg phosphatidylcholine revert to large multilamellar structures upon freezing and thawing [1]. SUV are therefore an ideal system to test the stabilizing effect of various additives. Certain organisms, such as nematodes, were reported to survive dehydration in the presence of trehalose [2]. The stabilizing effect of sugars on sarcoplasmic reticulum subjected to freeze-drying and rehydration, and on microsomes and egg phosphatidylcholine SUV subjected to freeze-thawing were also noted before [1–3]. Here we report that SUV made of phospholipids are stabilized in the presence of sucrose: their average size and size distribution as well as the bilayer integrity are preserved during dehydration-rehydration cycles as effected by spray-drying and redispersing the dry powder. Gel filtration of sonicated phospholipid dispersions was carried out on calibrated Sepharose 4B columns. The elution profiles shown in Fig. 1A and B were obtained from sonicated phospholipid dispersions (1-palmitoyl-2-oleoyl-*sn*-phosphatidylcholine (POPC)/dioleoyl-*sn*-phosphatidylserine monosodium salt (DOPS), mole ratio 7 : 3; phospholipids were a gift of Ciba-Geigy, Basel) in buffer containing 10% (0.3 M) sucrose before and after

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Abbreviations: SUV, small unilamellar vesicles; egg PC, egg phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-phosphatidylcholine; DOPS, monosodium salt of dioleoyl-*sn*-phosphatidylserine; ESR, electron spin resonance; CAT 16, 4-(*N*,*N*-dimethyl-*N*-hexadecyl)ammonium-2,2,6,6-tetramethylpiperidine-1-oxyl, iodide.

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spray-drying the phospholipid dispersion in a Büchi spray-drying apparatus, respectively. The elution patterns are similar but not superimposable as evident from the difference profile in Fig. 1C. The main peak of SUV (Fig. 1A and B) is eluted at $V_e = 18.5$ ml corresponding to a Stokes radius of 13.5 nm. The difference profile indicates that the spray-drying-rehydration cycle leads to some (10%) aggregation and/or fusion of SUV. Similar results to those in Figs. 1A and B were obtained when phospholipid dispersions containing sucrose were subjected to a freeze-drying-rehydration cycle (data not shown). However, subjecting the same POPC/DOPS dispersions to spray-drying-rehydration in the absence of sugar led to aggregation/fusion of the phospholipid SUV. After this treatment, 97% of the phospholipid was pelleted by centrifugation at $12000 \times g$ for 5 min as compared to 2% before treatment. The results obtained by gel filtration were confirmed by dynamic light-scattering (data not shown).

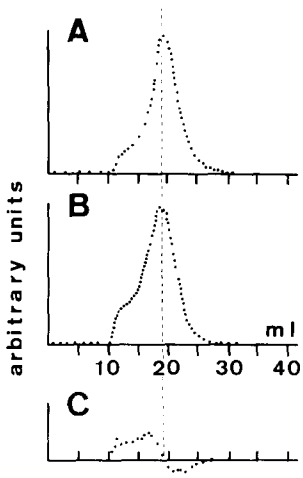

Fig. 1. Gel filtration profiles obtained with a calibrated Sepharose 4B column (46–0.9 cm). For details see Refs. 4 and 5. Sonicated phospholipid dispersions were made as described previously [1]. This method of preparation gave SUV with sucrose being present both outside and inside the vesicles. The column was equilibrated with 0.01 M sodium phosphate buffer (pH 7) containing 10% (0.3 M) sucrose and 0.05% NaN_3. The phospholipid (POPC/DOPS, mole ratio 7:3) was suspended in the same buffer (50 mg/ml) and sonicated. After sonication the sample was diluted 5-times and 0.5 ml were applied to the Sepharose 4B column (A). Another 45 ml of this dispersion were spray-dried in a Büchi spray-drying apparatus (Büchi Laboratory-Techniques, Flawil, Switzerland). The inlet temperature was 140°C, the outlet temperature 67°C. 50–75% of the dried phospholipid was recovered and redispersed in the appropriate amount of H_2O to give the same phospholipid concentration as in the original dispersion. TLC analysis of the phospholipid dispersion before and after spray-drying indicated that phospholipid degradation was insignificant. 0.5 ml of the spray-dried and redispersed sample were chromatographed in B. C is the difference profile obtained by subtracting A from B.

The gel filtration results were also confirmed by freeze-fracture electron microscopy. Comparing the electron micrographs of freeze-fractured preparations in Fig. 2, the stabilizing and preservative effect of sucrose upon negatively charged dispersions of POPC/DOPS (mole ratio 7:3) during spray-drying is obvious. Sonicated mixed dispersions of POPC/DOPS consisted of SUV of a diameter of 18–70 nm, both in the absence and presence of 10% (0.3 m) sucrose (Fig. 2A and B). Subjecting these dispersions to spray-drying and redissolving the dry residue in H_2O had little effect on the average vesicle size and size distribution provided sucrose was present in the buffer (Fig. 2D). In contrast, this treatment in the absence of sugar led to vesicle aggregation and fusion (Fig. 2C). Large unilamellar vesicles are visible (diameter ≈ 0.2–2 μm) which contain smaller unilamellar vesicles entrapped in their aqueous cavity. The electron micrograph (Fig. 2C) is very similar to that obtained with unsonicated POPS/DOPS (mole ratio 7:3) dispersions.

The main conclusion that we can draw from the results presented so far is that, in the presence of sucrose, the average size and size distribution of SUV are not significantly affected by spray-drying-rehydration.

Another question of considerable practical interest is whether or not the integrity and in turn the permeability barrier of the phospholipid bilayer is affected by dehydration/rehydration cycles. In order to shed light on this question the spin label 4-(*N*,*N*-dimethyl-*N*-hexadecyl)ammonium-2,2,6,6-tetramethylpiperidine-1-oxyl, iodide (CAT 16) was incorporated in the phospholipid bilayer. The free radical of this spin label is in the polar group region of the bilayer and, hence, located at the outer and inner vesicle surface. Addition of sodium ascorbate (30 mM) at 0°C, that was shown to be impermeable at this temperature, reduced the spin label located on the

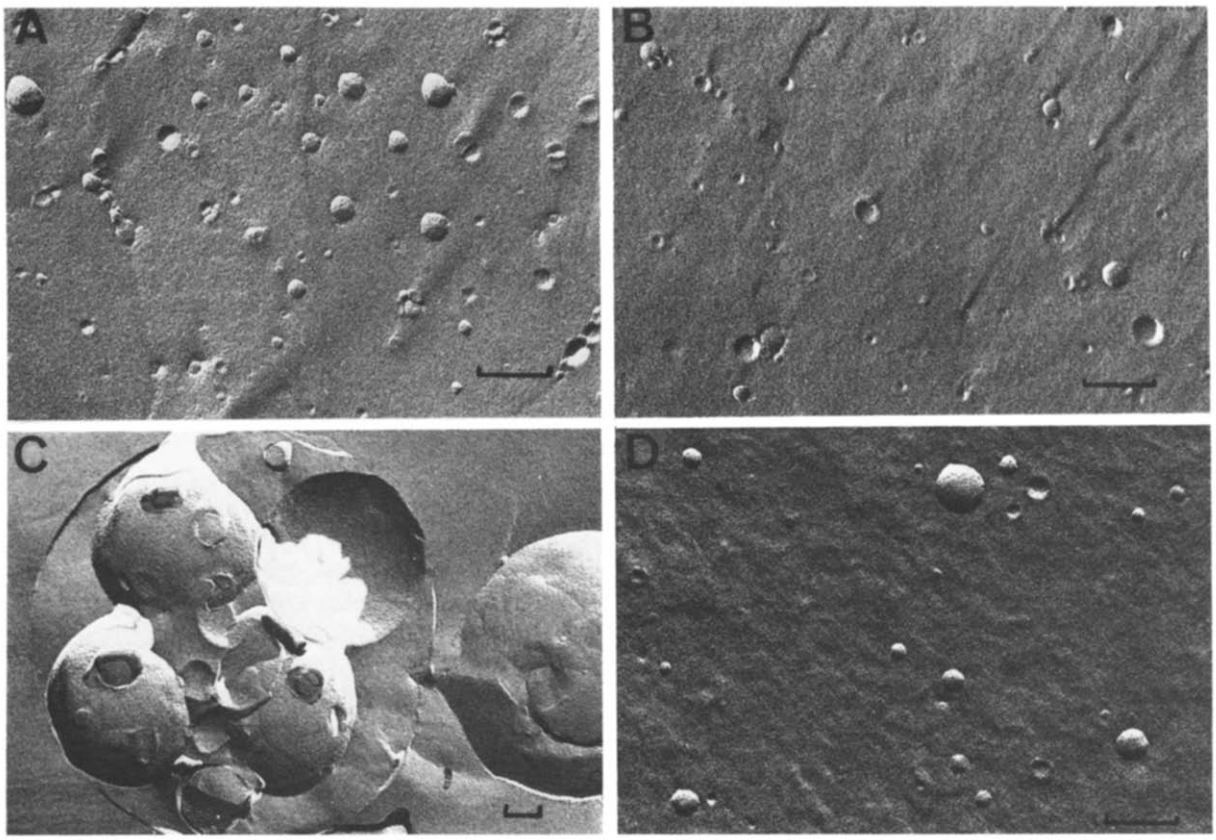

Fig. 2. Electron micrographs of sonicated mixed phospholipid dispersions consisting of POPS/DOPS (mole ratio 7:3). The phospholipds (10 mg/ml) were suspended in 0.01 M sodium phosphate buffer (pH 7) either without sucrose (A,C) or with 10% (0.3 M) sucrose (B,D) and sonicated [1]. In A and B, the phospholipid dispersions were jet-frozen and freeze-fractured, in C and D the phospholipid dispersions were subjected to spray-drying in the Büchi apparatus, the dry residue was redispersed in H_2O and then jet-frozen and freeze-fractured. The bars represent 100 nm.

outer membrane. This is evident from a reduction in ESR signal intensity with the remaining signal intensity arising from spin label present on the inner surface. Results are summarized in Table I indicating that, only in the presence of sucrose, bilayers of SUV are stabilized and impermeable to ascorbate during freeze-drying and spray-drying. In the presence of 10% sucrose similar values (30–35%) were obtained for the residual ESR signal before and after freeze-drying and spray-drying (Table I). In contrast the complete loss of the ESR signal intensity during these procedures in the absence of sucrose is evidence that the bilayer becomes permeable to ascorbate (Table I).

Sonicated POPC/DOPS vesicles (mole ratio 7:3) were also prepared in 0.01 M sodium phosphate buffer (pH 7) containing 10% sucrose (0.3 M), 0.1 M $K_3Fe(CN)_6$ and a trace-amount of ^{3}H-labeled raffinose. External $K_3Fe(CN)_6$ and raffinose were removed by gel filtration on Sephadex G-50 equilibrated with 0.01 M phosphate buffer (pH 7) containing 10% sucrose. The SUV eluted from the Sephadex G-50 column, which contain $K_3Fe(CN)_6$ and raffinose entrapped in their internal cavity, were spray-dried. The dry residue was redispersed in H_2O to give approximately the same concentration as in the original dispersion. This dispersion was rechromatographed on Sephadex G-50: 90% of the originally entrapped raffinose (determined by radioactivity measurement) and $K_3Fe(CN)_6$ (determined by measuring absorbance at 420 nm) remained en-

TABLE I

RESIDUAL ESR SIGNAL INTENSITY

Sonicated dispersions of phospholipids (10 mg/ml) labeled with CAT 16 (phospholipid/spin label = 100:1, mole ratio) were used. Dispersions were cooled to 0°C and ascorbate (30 mM) was added before freeze-drying. To dispersions subjected to spray-drying ascorbate was added after redispersing the dry residue in H_2O and cooling to 0°C (for details of the freeze-drying procedure see Ref. 6).

Phospholipid	ESR signal intensity (%)	Experimental conditions
POPC/DOPS (7:3)	31	0.01 M sodium phosphate (pH 7)
POPC/DOPS (7:3)	0	0.01 M sodium phosphate (pH 7) after freeze-drying
POPC/DOPS (7:3)	30-33	0.01 M sodium phosphate (pH 7) 10% sucrose
POPC/DOPS (7:3)	33	0.01 M sodium phosphate (pH 7) 10% sucrose after spray-drying

trapped in the vesicle cavity during spray-drying. The ESR and the entrapment experiments taken together thus indicate that in the presence of sucrose the bilayer integrity is preserved during the process of spray-drying.

Conclusions. (1) In the presence of 10% (0.3 M) sucrose, SUV made of negatively charged phospholipids (POPC/DOPS, mole ratio 7:3) are preserved during dehydration-rehydration cycles as effected by freeze-drying or spray-drying and resuspending the dry residue. In the absence of sugar these processes lead to aggregation and fusion of SUV. The final fusion product appears to be the large unilamellar vesicle (diameter ≈ 0.2–2 μm) containing smaller unilamellar vesicles entrapped in its aqueous cavity. This is apparently different from the fusion of SUV made of egg PC which leads to large multilamellar particles [1].

(2) In the presence of sucrose the bilayer integrity of SUV is preserved during dehydration-rehydration. Without sucrose this treatment disrupts the bilayer leading to exchange and equilibration of contents between internal and external compartments.

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References

1 Strauss, G. and Hauser, H. (1986) Proc. Natl. Acad. Sci. USA 83, 2422–2426
2 Crowe, J.H. and Crowe, L.M. (1982) Cryobiology 19, 317–328.
3 Crowe, J.H. and Crowe, L.M. (1984) in Biological Membranes (Chapman, D., ed.), Vol. 5, p. 57, Academic Press, New York
4 Schurtenberger, P. and Hauser, H. (1984) Biochim. Biophys. Acta 778, 470–480
5 Nozaki, Y., Lasic, D.D., Tanford, C. and Reynolds, J.A. (1982) Science 217, 366–367
6 Strauss, G., Schurtenberger, P. and Hauser, H. (1986) Biochim. Biophys. Acta 858, 169–180