Effect of Freeze-Thawing and Polyethylene Glycol (PEG) Lipid on Fusion and Fission of Phospholipid Vesicles

Supaporn SRIWONGSITANONT and Masaharu UENO*

Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University; 2630 Sugitani, Toyama 930–0194, Japan. Received September 18, 2003; accepted March 9, 2004

The effect of freeze-thawing on the size of egg yolk phosphatidylcholine (EggPC) vesicles in the presence of 0—40 mol% distearoylphosphatidylethanolamine-polyethylene glycol 2000 (DSPE-PEG) was studied. Mean diameters of the vesicles fell into a range of 80—150 nm after 10 times freeze-thawing in spite of their original size. In the process of freeze-thawing, two opponent events, one is fission and the other is fusion, occurred at the same time. DSPE-PEG accelerated the fusion event.

Key words phosphatidylcholine; vesicle; freeze-thawing; polyethylene glycol

Freeze-thawing technique have been used in the process of liposome preparation to accelerate hydration of lamellar phase of phospholipid aggregates. It was known that repeating freeze-thawing changes liposome size; in a case reduces their size and in another case increases it.[1,2)]

Recently, polyethylene glycol (PEG)-grafted liposomes have been widely used for drug delivery system with their long circulation in blood because PEG on liposome surface prevents the capture by reticuloendothelial system.[3)] However, there is little report describing the effect of freeze-thawing on the size of PEG-grafted liposomes.[4)] We started systematic study to clear the effect of repeating freeze-thawing on the size change of liposomes, especially, PEG-grafted liposomes.

Liposomes were prepared by octylglucoside-removal method.[5)] Average liposome size and size distribution were evaluated by Quasi Elastic Light Scattering (QELS) and gel exclusion chromatogram. As shown in Fig. 1, without freeze thawing, the sizes of liposomes ranged from 50 to 250 nm depending on distearoylphosphatidylethanolamine (DSPE)-PEG content. The average diameter of liposomes composed of phospholipid alone was about 250 nm. The diameter of liposomes containing DSPE-PEG decreased their size. Over 10 mol% of DSPE-PEG, vesicles and micelles coexisted, but about 90% was in vesicle form in the condition of containing 20 mol% of DSPE-PEG (data not shown). Repeating freeze-thawing reduced size of liposomes with an original diameter over 130 nm. On the contrary small liposomes increased their size. After 10 times of freeze-thawing, average size of liposomes became ranging from 80 to 150 nm in spite of their original size. These tendencies were observed for liposomes prepared by another method, such as, extrusion method using nuclear pore filter with different pore size (data not shown).

In Fig. 2, gel exclusion chromatography on Sephacryl S-1000 was shown. In the absence of PEG lipid (Fig. 2a), liposome size became small with repeating freeze-thawing. In the presence of 1 mol% of PEG lipid, the main peak of the elution profile moved to small size side as repeating freeze-thawing. But new peak appeared at a elution volume of near 7 ml at 35 times freeze-thawing. Appearance of new peak became more prominent with increasing PEG lipid. At 20 mol% of PEG lipid, in which the original size of the liposomes was about 60 nm in diameter, new peak at a elution volume of near 7 ml was observed clearly at 5 times freeze-thawing. The size of new peak rages from 200 to 400 nm.

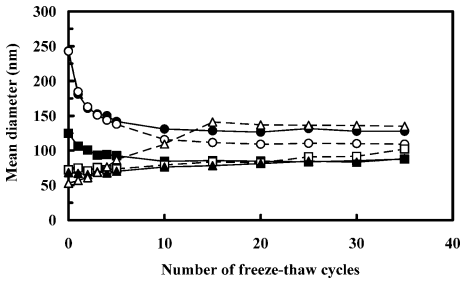

Fig. 1. Effect of Number of Freeze-Thawing Cycles on the Mean Diameter of EggPC/DSPE-PEG2000 Liposomes Prepared by Detergent-Removal Method

DSPE-PEG2000 concentration (mol%): ●, 0; ○, 1; ■, 5; □, 10; ▲, 20; △, 30.

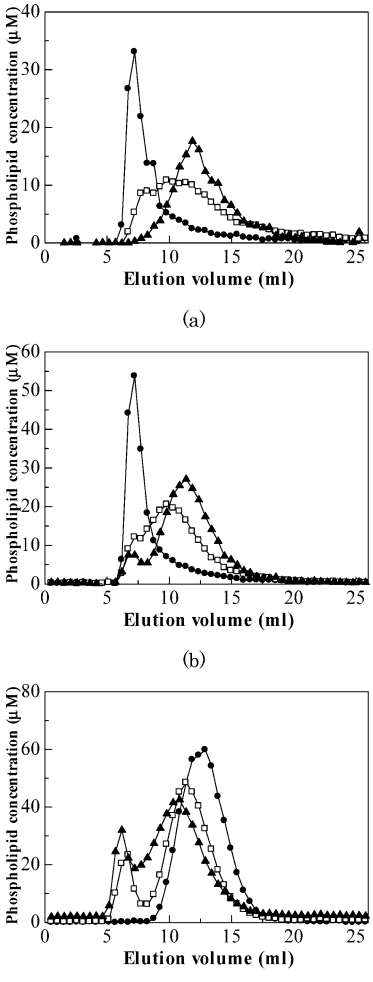

Fig. 2. Gel Exclusion Chromatography of EggPC Liposomes Containing DSPE-PEG2000 of 0% (a), 1% (b), 20% (c) before Freeze-Thawing (●), after Freeze-Thawing of 5 Times (□), and after Freeze-Thawing of 35 Times (▲)

* To whom correspondence should be addressed. e-mail: mueno@ms.toyama-mpu.ac.jp

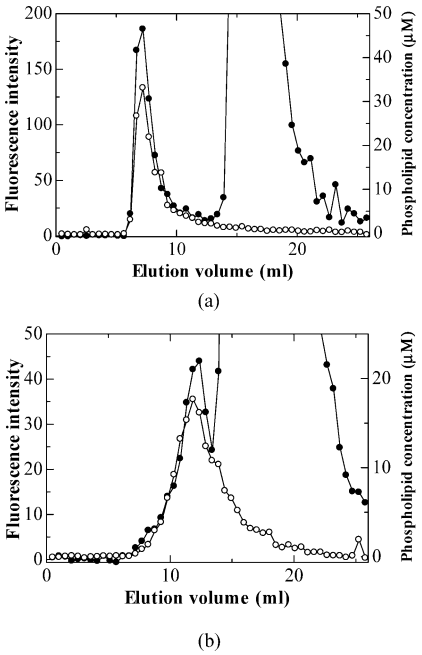

Fig. 3. Gel Exclusion Chromatograpy of EggPC Vesicles in the Absence of PEG Lipid before Freeze-Thawing (a) and after 35 Cycles of Freeze-Thawing (b)

○, phospholipid concentration; ●, fluorescence intensity.

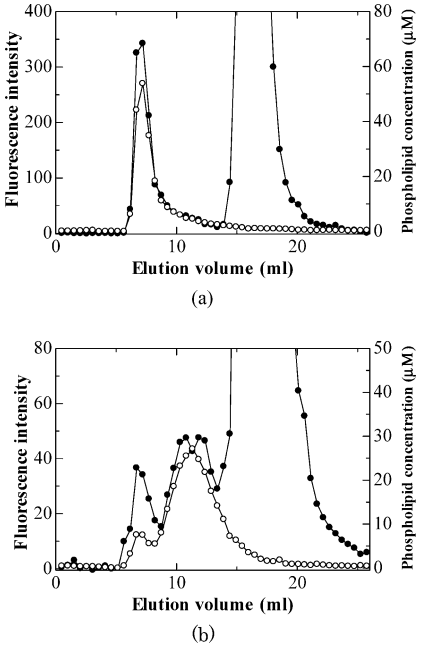

Fig. 4. Gel Exclusion Chromatography of EggPC Vesicles Containing 1 mol% of DSPE-PEG2000 before Freeze-Thawing (a) and after 35 Cycles of Freeze-Thawing (b)

○, phospholipid concentration; ●, fluorescence intensity.

Next liposomes were prepared in calcein-containing buffer solution. In Fig. 3, elution profile of liposomes without PEG lipid was shown. In Fig. 3a, the elution profile of liposomes before freeze-thawing was shown. The calcein peak near 8 ml of elution volume was in accordance with that of phospholipid, indicating that calcein was entrapped into liposomes. The large peak near 15—20 ml of elution volume is assigned to free calcein. After 35 times of freeze-thawing, as shown in Fig. 3b, both peaks of liposomes and calcein moved to small size side, indicating small size liposome formed by fission, in which calcein was entrapped.

In Fig. 4, elution profile of liposomes containing 1 mol% of PEG lipid was shown. In the absence of freeze-thawing, elution patterns of phospholipid and calcein (Fig. 4a) were similar to those of liposomes not containing PEG lipid (Fig. 3a). After 35 times of freeze-thawing, in contrast to elution pattern of liposomes not containing PEG lipid (Fig. 3b), new peaks both of phospholipid and calcein appeared at 8 ml of elution volume (Fig. 4b). The size of vesicles in new peak was about 300 nm according to QELS, which was larger than that of original liposomes. The calcein amount per unit of phospholipid on a peak at 8 ml of elution volume was near that of oliginal liposomes (Fig. 4a), which were not undergone freeze-thawing, suggesting that new particles are large and uni- or oligo-lamellar liposomes.

In conclusion, mean diameters of the liposomes, which had originally large size, decreased with increasing in the number of freeze-thawing cycles. On the contrary, mean diameters of the liposomes, which had originally small size, increased with increase in the number of freeze-thawing cycles. After freeze-thawing over 10 times, the liposome mean diameters fell into a range from 80 to 150 nm in spite of original size.

Gel exclusion chromatography showed that in the process of freeze-thawing, two opponent events, one is fission and the other is fusion, occurred at the same time. DSPE-PEG 2000 seemed to accelerate fusion event.

References

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