RAPID PAPER

Stearylamine Changes the Liposomal Shape from MLVs to LUVs

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Edited by M. Iwahashi, Kitasato Univ., and accepted January 17, 2005 (received for review December 17, 2004)

Abstract: The shape of mixed dipalmitoylphosphatidylcholine (DPPC)/stearylamine (SA) liposomes prepared by the Bangham method was examined using freeze-fracture electron microscopy. The shape of the mixed DPPC/SA liposomes ($X_{SA} = 0.05$) was not that of multilamellar vesicles (MLVs) but large unilamellar vesicles (LUVs) with a diameter of approximately 1.0 μm . These facts overthrow the preconceived idea that the shape of the DPPC/SA liposomes prepared by the Bangham method is that of MLVs. The trapping efficiency of the mixed DPPC/SA liposomes ($X_{SA} = 0.05$) was ten times greater than that of the DPPC liposomes, since the DPPC liposomes were MLVs. The data of the DSC peak for the DPPC/SA liposomes also indicated that the shape of the DPPC/SA liposomes is that of LUVs. We emphasize that the shape of the DPPC liposomes changes from that of MLVs to LUVs by the mixing of a small amount of SA into the DPPC liposomes.

Key words: stearylamine, large unilamellar vesicle, freeze-fracture electron microscopy, mixed liposome, trapping efficiency

1 Introduction

Liposomes are drug carriers that are both biodegradable and of minimal toxicity, and liposomal drug delivery systems have been widely researched from the purpose of targeting drugs to specific cells (1). Large unilamellar vesicles (LUVs) are useful as drug carriers due to their high trapping efficiency. Many techniques, such as the organic solvent injection method (2), the reverse phase evaporation (RPE) method (3) and the supercritical reverse phase evaporation (scRPE) method

(4) have been reported for the preparation of LUVs. On the other hand, the Bangham method (5) is a simplified procedure, and is convenient for the preparation of liposomes. Liposomes prepared by the Bangham method (5) are generally multilamellar vesicles (MLVs). We found using an inverted microscope with a transmitted light differential interference contrast attachment that the dipalmitoylphosphatidylglycerol liposomes (6) and the mixed dipalmitoylphosphatidylcholine (DPPC)/ceramide 3 liposomes (7) prepared by the Bangham method are MLVs, which were observed to

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have an onion-like structure with an inner aqueous phase. We also found using freeze-fracture electron microscopy that the shape of the DPPC liposomes (8) prepared by the Bangham method was that of MLVs. Furthermore, we found that the shape of the mixed DPPC/dipalmitoyldimethylammonium bromide (DPAB) liposomes (8) prepared by the Bangham method is that of LUVs. DPAB is a cationic amphiphilic substance with dialkyl chains, and DPAB can form pure DPAB vesicles. The shape of the DPAB vesicles was that of MLVs (8). Furthermore, we confirmed using freeze-fracture electron microscopy that the shape of the DPPC liposomes was changed from that of MLVs to LUVs by the mixing of a small amount of DPAB into the DPPC liposomes (8). On the other hand, stearylamine (SA) is also a cationic substance, and SA has commonly been used to prepare positively charged liposomes. The mixed DPPC/SA liposomes have been widely used to study drug delivery systems. Many investigators (9-14) have been under the impression that the shape of the mixed DPPC/SA liposomes prepared by the Bangham method is that of MLVs. However, they (9-14) have never actually confirmed the liposomal shape. Therefore, the liposomal shape, MLVs, written in their reports (9-14) may be doubtful. Schwarz and Jaehnig (15) reported that the shape of the mixed DPPC/egg-phosphatidylglycerol (PG) (9:1) liposomes prepared by the Bangham method was that of MLVs, LUVs and small unilamellar vesicles (SUVs). However, nobody has observed the shape of the mixed DPPC/SA liposomes prepared by the Bangham method. It is interesting to confirm whether the shape of the mixed DPPC/SA liposomes is that of MLVs or LUVs, since the shape of the mixed DPPC/DPAB liposomes was that of LUVs (8). In addition, it is important to make clear the liposomal shape for the experimental systems of drug carriers and/or models of the biological membrane.

In this study, we observed the shape of the mixed DPPC/SA liposomes using freeze-fracture electron microscopy. A new finding was obtained, which overthrows the preconceived idea that the shape of the DPPC/SA liposomes prepared by the Bangham method is that of MLVs. So we promptly report the result in this paper.

2 Experimental

2.1 Materials

Stearylamine (SA) was purchased from Sigma Co. Cholesterol (Chol) and D-glucose were purchased from Wako Pure Chemical Ind., Ltd. L- α -Dipalmitoylphosphatidylcholine (DPPC) was supplied by NOF Co. Water for injection (Ohtsuka Pharmaceutical Co.) was used as the solvent.

2.2 Preparation of Cationic Liposomes

Mixed DPPC/SA liposomes with and without Chol were prepared according to the procedure by the Bangham method (5); DPPC and SA were dissolved in chloroform in a test tube; the solvent was then removed by blowing nitrogen gas into the test tube, and the residual solvent was further dried overnight at room temperature in a desiccator under vacuum; a 4 ml portion of water was added to this lipid film and warmed (60°C) above the phase transition temperature of DPPC for 10 min; the test tube was then shaken vigorously on a vortex mixer, and mixed DPPC/SA liposomes were obtained.

2.3 Freeze-Fracture Electron Microscopy

Liposome sample was frozen immediately in liquid propane. The frozen sample was fractured in a freeze-replica apparatus (FR-7000A, Hitachi Science Co.) at -150°C. Replica was made by platinum-carbon shadowing followed by platinum shadowing. It was cleaned by acetone and distilled water, and then picked up on 150 mesh copper grids and observed by an electron microscope (JEM-1200EX, Japan Electron Co.). The outline was shown in our previous paper (16).

2.4 Measurement of Phase Transition Temperature of Liposomal Bilayer Membranes

The phase transition temperature between gel and liquid crystalline states of liposomal bilayer membranes was measured with a differential scanning calorimeter (DSC 8230, Rigaku Denki Co.). Liposome solution was put in a sampling vessel, which is made of stainless steel, and then the vessel was sealed. The measurement conditions were 1 K/min for the scanning rate, 20~80°C for the scanning range and 0.1 m cal/s for the sensitivity. The total lipid concentration was 20 mM.

Fig. 1 Freeze-Fracture Electron Micrographs of the Mixed DPPC/SA (9.5:0.5) Liposomes and the Mixed DPPC/Chol/SA (9.5:5:0.5) Liposomes.

2·5 Measurement of Trapping Efficiency of Liposomes

Glucose was trapped into the liposomes. Briefly, glucose was dissolved in water, liposomes were prepared using the glucose solution instead of water as described in section 2·2. The concentrations of liposomes and glucose were 5 and 0.2 mM, respectively, under consideration of osmotic pressure. The liposome dispersion was dialyzed against 0.1 M NaCl solution using a cellulose tube (Viskase Companies, Inc.) to remove the nonencapsulated glucose remaining in the dispersion medium. The temperature was kept at 5°C during the dialysis. The liposomes inside the tube were then destroyed by addition of ethanol. The amount of glucose in the solution was determined by the mutarotase GOD method (17) using a spectrophotometer (MPS-2000, Shimadzu Co.).

3 Results and Discussion

3·1 Freeze-Fracture Electron Micrographs of the Mixed DPPC/SA and the Mixed DPPC/Chol/SA Liposomes

The electron micrograph of the DPPC/SA liposomes is shown in **Fig. 1**.

The shape of the mixed DPPC/SA liposomes ($X_{SA} = 0.05$) was that of LUVs with a diameter of approximately 1.0 μm . Furthermore, the shape of the DPPC/Chol/SA (9.5:5:0.5) liposomes was also that of LUVs, while the shape of the DPPC/Chol (10:5) liposomes without SA was that of MLVs.

3·2 Phase Transition Temperature of Liposomal Bilayer Membranes

The phase transition temperatures of the DPPC liposomes and the mixed DPPC/SA liposomes from the gel to the liquid crystalline states were measured by DSC, and the results are shown in **Fig. 2**.

DPPC liposomes showed a sharp endothermic peak. The endothermic peak shifted to a higher temperature and broadened as the mole fraction of SA increased. It is known that the DSC peak of LUVs is broad compared with that of MLVs (18). The DSC data shown in **Fig. 2** indicate that the shape of the DPPC/SA liposomes is that of LUVs.

DSC data for the mixed DPPC/Chol/SA liposomes were not obtained, because Chol interferes the measurement.

3·3 Trapping Efficiency of the Mixed DPPC/SA Liposomes

The trapping efficiencies of the liposomes were measured using an aqueous-space marker, glucose. The

Fig. 2 DSC Curves for the Mixed DPPC/SA Liposomes ($X_{SA}=0 \sim 0.1$). Total lipid concentration: 20 mM.

trapping efficiency of the mixed DPPC/SA liposomes ($X_{SA} = 0.05$) was ten times greater than that of the DPPC liposomes since the DPPC liposomes were MLVs, and the value was nearly equal to that of the mixed DPPC/DPAB liposomes ($X_{DPAB} = 0.05$) (8), whose liposomal shape was that of LUVs. These results indicate that the shape of the mixed DPPC/SA liposomes is indeed that of LUVs.

Many investigators have been under the impression that the shape of the mixed DPPC/SA liposomes with and without Chol prepared by the Bangham method is that of MLVs, although they have never observed the liposomal shape. Our findings overthrow this preconceived idea. We emphasize that the shape of the DPPC/SA liposomes prepared by the Bangham method is that of LUVs and that the shape of the DPPC liposomes changes from that of MLVs to LUVs by the mixing of a small amount of SA into the DPPC liposomes.

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