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Skeletonized Hybrid Liposomes

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SUMMARY

Giant hybrid liposomes of polymerizable lipid and cholesterol were prepared by the hydration method and were polymerized by UV irradiation. The obtained giant liposomes were freeze-dried and washed with chloroform to skeletonize the frame structure of the polymerized membrane. The skeletonized liposomes were directly confirmed by scanning electron microscopy. The single-layered and fully spherical liposome frame were easily confirmed by the stereo-pair of the obtained micrographs.

INTRODUCTION

Lots of polymerizable lipids have hitherto been synthesized and tens of studies on the characteristics of polymerized liposomes have been published vigorously in this decade. We have been studying the preparation and basic characteristics of these polymeric liposomes[1,2]. The hybrid liposome systems containing more than two kinds of lipids or lipid analgues have also been investigated[3-5]. A phase separation phenomenon on the hybrid liposomes is one of most important subject to be developed and be regulated for the design of several functional liposome systems[4]. The micro-phase separation can be induced on the liposome containing both polymerizable and non-polymerizable components by their different affinity[5]. To confirm these phase separation, TEM, DSC and fluorescence depolarization have been applied, and the structural characteristics of the lipids in the hybrid liposome system were revealed to be one of very effective factor to induce the phase separation[5].

In the present paper, the unpolymerized components were washed out by chloroform from the polymerized hybrid liposome and the resulting skeletonized structure was directly analyzed by scanning electron microscopy.

EXPERIMENTAL

Materials

1-[9(p-Vinylbenzoyl)nonanoyl]-2-O-octadecyl-rac-glycero-3-phosphocholine (I) was synthesized according to the method as reported previously[1]. Purity of monomer I was confirmed by TLC analysis just before use.

Cholesterol, standard for chromatography, and sodium choleate were purchased from Sigma and were used without further purification.

Chloroform dried over calcium chloride was distilled and immediately used.

Methods

Preparation of Giant Liposomes[3,6]

I or cholesterol was dissolved individually in chloroform to prepare 1 mM solution. 200 µl of the mixed solution of I and cholesterol with suita-

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ble composition was slowly evaporated in moderately rotated sample tube to prepare thin film of the lipid mixture on the inner wall of the tube. 10 ml of N_2 bubbled pure water containing 0.02 wt% sodium azide was slowly added to sufficiently dried sample tube. A slightly turbid solution was obtained after incubation of this at 50°C for 24-48 hr.

Polymerization of the Giant Liposomes

The giant liposomes suspension was irradiated with UV light under N_2 atmosphere for 4 hr. The degree of polymerization was confirmed by UV spectroscopy.

Skeletonization of the Polymerized Liposomes

A) Few drops of polymerized hybrid liposome suspension were poured on Millipore membrane filter (VCWP-025). Freeze-dried liposomes were washed with chloroform, and the filtrate was slowly discarded by aspirator.

B) Polymerized hybrid liposome suspension (10 ml) was mixed with 1 ml of 22 wt% sodium choleate aqueous solution. The mixture was then dialyzed against water only containing 0.02 wt% sodium azide for a few days.

3-dimensional SEM Measurements

The skeletonized liposomes, collected on Millipore filter and dried in vacuo, were Au-sputtered with suitable thickness. Samples were directly observed by scanning electron microscope (JEOL, JSM-T20). Suitable images were selected and several pictures of the same image with the same magnification were taken by changing observation angle for 4-5°C.

RESULTS AND DISCUSSION

Preparation of giant liposomes has already reported by Ringsdorf et al[6]. Liposomes with radii of 1-100 μm were easily seen by optical microscopy[3,6]. A mixture of monomer I and cholesterol formed lots of giant liposomes by the same manner as previously reported. Polymerization of monomer I in the liposomes can be confirmed by the spectral change at 270 nm[1]. However there was no quantitative data here, the polymerization rate of monomer I in hybrid liposomes was independent of mixing ratio (I / cholesterol) at 50°C. This strongly suggested that monomer I and cholesterol showed phase separation in the membrane at 50°C. Fluorescence depolarization measurement also suggested the phase separation of cholesterol at temperature higher than 25°C.[7] The polymerization kinetics of the hybrid liposomes will be discussed elsewhere. Regardless of the phase separation behavior of monomer I / cholesterol hybrid liposomes, cholesterol should associate after polymerization of I. Because cholesterol molecules were expelled from the polymerized I domain.

A membrane frame structure of polymerized I was easily obtained by washing these polymerized hybrid liposomes with chloroform. Cholesterol was easily soluble in chloroform but polymerized I was not. The polymerized hybrid liposomes containing 25mol% cholesterol were freeze-dried and skeletonized by chloroform treatment. Typical stereo-pair of SEM picture of the skeletonized liposome was shown in Fig. 1. Lots of channels with radii of 500-5000 Å were seen in it. Ratio of the sum of channel area seen and that of frame area remained was about 1/3 which was equal to the composition of cholesterol and monomer I in feed. This strongly suggested that the assembled (phase-separated) cholesterol molecules were washed out by chloroform treatment.

Before skeletonization, there were a lot of giant hybrid liposomes with radius larger than 10 μm, these larger liposomes were not seen after washing out. Probably lots of those were destroyed by rapid freeze-dry process. Those very large skeletonized liposomes should be prepared by milder preparation method.

Ringsdorf and his co-workers have already reported the polymerized

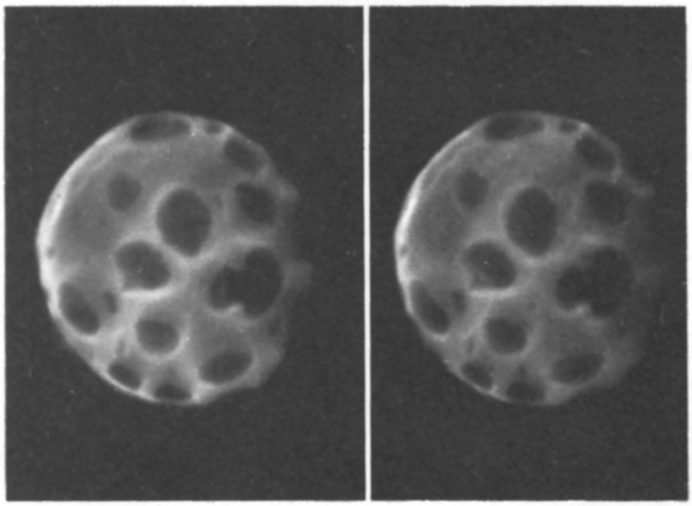

Fig. 1 Stereo pair of SEM pictures of skeletonized liposome. (skeletonized by method (A))

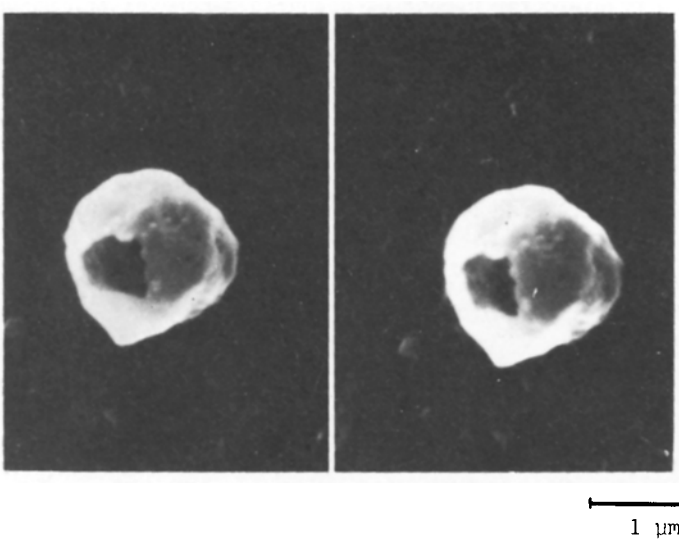

Fig. 2 Stereo pair of SEM pictures of skeletonized liposomes with fewer channels (skeletonized by method (B))

liposomes with several holes in the liposomes called "uncorked liposomes"[8]. Their unique idea to uncork the polymerized liposomes was the cleavage of disulfide linkage of two lysolipids incorporated in the liposomes. They have also suggested the possibility of these corked liposomes as chemical amplifier. In our system, it is quite convenient to skeletonize the polymerized liposomes and one need not prepare specific molecule as "cork".

Skeletonization of hybrid liposomes can also be performed by surfactant treatment such as sodium choleate in an aqueous medium. This method is one of potent technique to prepare skeletonized liposome containing water-soluble macromolecules such as proteins, polymeric drugs etc. in their inner aqueous phase just like birds in a cage. Skeletonization of liposomes can therefore be carried out in an aqueous solution without organic solvent treatment. The polymeric liposomes containing 10 mol% cholesterol were skeletonized by the dialysis with sodium choleate for 48 hr. One of these liposomes was shown in Fig. 2. The 3-dimensional image of the skeletonized liposome can also be seen from these stereo pair of SEM pictures. Two large channels (radius of about 2000 Å) were seen in it.

It is quite interesting and charming subject to control the number and size of channels. The portion of total surface area of the channels per liposome ($\Sigma Sc / S$) is the function of the mole fraction (f) of unpolymerizable component and surface area per molecule (A). S is the surface area of liposome before skeletonization.

$$\frac{\Sigma Sc}{S} = f \cdot \frac{Ac}{Ap} \qquad (1)$$

where c and p mean the unpolymerizable (to form channel) and polymerizable component, respectively. At the present time, we do not have strict knowledges to regulate the number of channels. However so, it is quite interesting that the liposomes, skeletonized by dialysis with surface active detergents, have relatively fewer channels as typically seen in Fig. 2. This suggested that there were some possibilities to control the number of channels per liposomes. Details on this will be published elsewhere.

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