Novel Preparation of Asymmetric Liposomes with Inner and Outer Layer of Different Materials

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Asymmetric liposomes whose inner and outer layer with different materials, which are very useful in liposome and membrane research, have been prepared layer by layer from reverse micelles. In general, the diameters of the liposomes ranged from 30 nm to 100 nm. Fluorescein quenching experiment showed that asymmetric liposomes with inner layer being lipid and outer single-chain $[(bpy)_2Ru(diazafluorenone)(CH_2)_{15}CH_3](PF_6)_2$ had been success-fully prepared. The half-life of flip-flop of bilayer is estimated for 17 days. The method present is of significance in liposome and membrane study.

Liposomes have been widely used as model membranes, as capsules for agents in assays and drug delivery.[1-3] Unfortunately, liposomes in traditional methods are prepared in a mixed solution containing lipids and the chemical agents, so the construction of bilayer is in a random way. Recently, there is increasing interest in preparation of liposomes from reverse micelles because it provided a controllable method to prepare liposomes.[4,5] In this paper, we reported utilizing this technique to prepare asymmetric liposomes with inner layer being lipid and outer another amphiphilic ligand, which is of great significance in liposome and membrane research.

A typical preparation contains 30 μmol egg yolk phosphatidylcholine (EPC) and 30 μmol cholesterol in 10 ml of n-decane. 0.2 ml of entrapped aqueous solution was dropped into the above solution. The mixtures were then sonicated with a Model H66MC ultrasonic cleaner at room condition for several minutes until the aqueous phase disappears completely. 2 ml deionized water was put into a clean tube. Amphiphilic ligands $[(bpy)_2Ru(diazafluorenone)(CH_2)_{15}CH_3](PF_6)_2$ (DAFBR) (Figure 1) solved in n-decane solution (1 mg/ml), necessary for the continuous formation of a monolayer at the interface, was added to the water surface and formed monolayer at oil/water interface. The suspension of reverse micelles prepared above was drawn with a clean suction pipe, and then was slowly dropped into the oil phase in tube. The tube was centrifugated (6000 /min), and when the reverse micelles go into water phase from oil they would be coated by a second DAFBR layer to form asymmetric liposomes. After eliminating the oil phase, the asymmetric liposomes suspension was obtained.

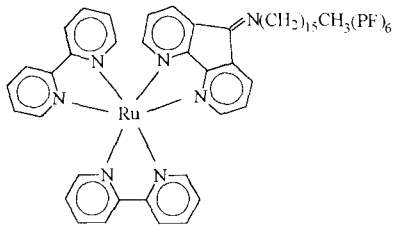

Fig.1 The structure of amphiphilic ligands $[(bpy)_2Ru(diazafluorenone)(CH_2)_{15}CH_3](PF_6)_2$ which can form monolayer and bilayer.

The fluorescent spectra of DAFBR in liposome suspension were taken to confirm the formation of asymmetric liposomes. 0.1 M Trihydroxyanthraquinone (THA) of Phosphate-buffered saline (PBS pH=7.4) solution was entrapped in asymmetric liposomes respectively. The fluorescent spectra (λ_{ex}=440 nm, λ_{em}=516 nm) of liposomes suspension were determined on RF-5000 after freeing nonencapsulated THA and incorporated DAFBR molecules on Sephadex G-100 column. The samples were then added 0.4 ml of 0.1 M THA aqueous solution and were detected the spectra. 0.1 M of THA of PBS solution was entrapped in asymmetric liposomes and the nonencapsulated THA was freed from suspensions. The fluorescent intensity of DAFBR during aging at room condition was utilized to determine the ratio of flip-flop of the asymmetric liposome bilayer.

Single-chain amphiphilic ligands can be used as bilayer forming agent,[6,7] because these compounds not only have the structural characteristic of common surfactants, but also have the chemical properties of normal ligands. We chose DAFBR as amphiphilic ligand not only because it can form monolayer and bilayer, but also it has characteristic fluorescent spectra from which the asymmetric liposomes can be demonstrated.

Figure 2 is the schematic drawings demonstrating the

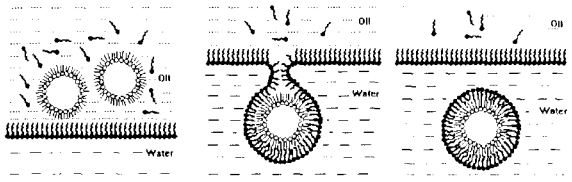

Fig.2 Formation of asymmetric liposome from a reverse micelle (schematic). The reverse micelle with an aqueous phase inside penetrating the interface is coated by the second DAFBR layer.

formation of an asymmetric liposome from an reverse micelle as described by Trauble et al.[8] Due to centrifugation, the reverse micelle goes down and the interaction of hydrophobic chains leads to the reverse micelle covering with the DAFBR monolayer at the oil/water interface. When the reverse micelle passes through the interface, a liposome is formed.

Figure 3 is the negative staining microscopes of asymmetric liposomes. The samples are prepared as follows: the lipid concentration was 3 mM; the mole ratio of EPC to cholesterol was 1:1; the time and intensity of sonication when preparing reverse micelles were 10 min, 80 W respectively. By measuring the diameters of liposomes on the image, we obtained the size distribution. In general, the diameters of the liposomes ranged in 30-100 nm. Figure 4 shows the cumulative amount of leaked fluorecein from asymmetric liposome as a function of the aging period, it leaks little fluorecein in 120 h.

THA is the fluorescence quenching agent of DAFBR. If

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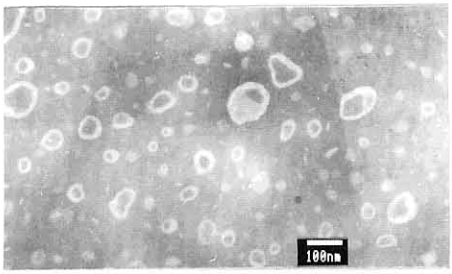

Fig.3 Negative staining electronmicrography of asymmetric liposome formed from reverse micelles.

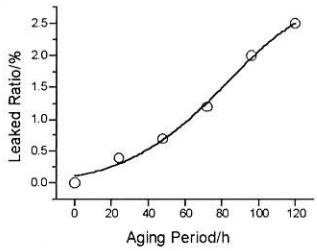

Fig.4 Percent cumulative amounts of fluorescein leaked out of asymmetric liposomes as a function of aging time.

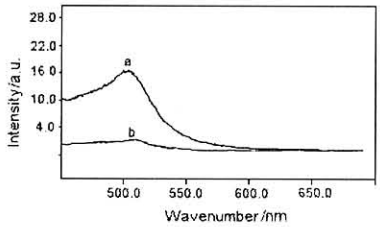

Fig.5 The fluorescence spectra of asymmetric liposome with inner layer being lipid and outer layer single-chain $[(bpy)_2Ru(diazafluorenone)(CH_2)_{15}CH_3](PF_6)_2$ before and after adding Trihydroxyanthraquinone. (a)Before, (b)After.

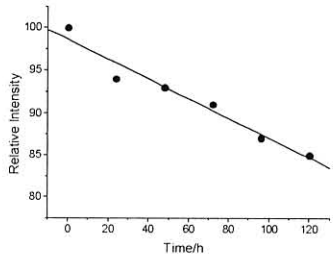

Fig.6 The relative fluorescent intensities of DAFBR in liposomes as a function of aging time show the ratio of the flip-flop of the asymmetric liposomal bilayer.

DAFBR is coated only on the outer layer of liposome results from the mechanism of the liposome formation, the fluorescence of DAFBR in liposome suspension can be observed strong even if THA is entrapped in liposome, and decreases remarkably when THA is added to the liposome suspension. Figure 5 is a typical fluorescent spectra of liposome with inner EPC, outer DAFBR and entrapment THA solution or PBS. Curve a is the spectrum of liposome suspension without adding THA and curve b is the one after adding THA. The fluorescence intensity is very stronger before than after adding THA, (about 50 times), which is consist with the theoretical analysis. The results collected show that asymmetric liposomes with different materials of bilayer respectively have been prepared.

Asymmetric liposomes is a useful model for studying flip-flop of liposomal bilayer. The asymmetric liposomes are prepared as above with inner layer being lipid, outer DAFBR and entrapment THA solution. The flip of DAFBR molecules from the outer to the inner layer will make the fluorescent intensity of DAFBR decrease because of the quenching of THA. Figure 6 shows the results of relative fluorescent intensities of liposome samples detecting every 24 h. The value at beginning is set 100% and the latter ones are calculated according to this value. Because the asymmetric liposome is stable enough as demonstrated above, the fluorescent intensity of DAFBR during aging is attributed to flip-flop of liposomal bilayer. Linear fit of these digits give a simple equation I=98.67-0.117t, where I stands for fluorescent intensity and t for time. From the above Eq, we can estimate the ratio of flip-flop of bilayer, that it requires about 17 days when the intensity decrease 50% of value at beginning.

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References and Notes

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