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The reversible aggregation-disaggregation and fusion of phosphatidylcholine liposomes with and without polyethylene glycol

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SUMMARY

Aggregation-disaggregation phenomena were observed with light microscopy as functions of the liposome dispersion temperature and the concentration of liposomes which were made of dipalmitoyl phosphatidylcholine in distilled water. Colonies formed by aggregation of the liposomes remaining below the pretransition temperature (about 34°C) disaggregated at the pretransition temperature within 2–10 mg/ml of dipalmitoyl phosphatidylcholine upon heating. Within 15–30 mg/ml, the colonies have disaggregated at the maintransition temperature (42°C). These phenomena were reversible with respect to temperature. Furthermore, the aggregation and fusion of liposomes have been observed using polyethylene glycols. The abilities for aggregation and fusion, which became stronger with increasing molecular weight or concentration of the polyethylene glycols, were qualitatively observed

Key words: dipalmitoyl phosphatidylcholine, liposome, aggregation, fusion, polyethylene glycol.

INTRODUCTION

There have been some reports on fusion and aggregation of phospholipid liposomes.^{1,2)} These authors^{1,2)} reported that liposomes, which were less than 100 nm in diameter, irreversibly aggregated at temperatures below the pretransition temperature (T_p : about 34°C³⁾) or below the maintransition temperature (T_c : 42°C³⁾). Wong et al.⁴⁾ reported that phosphatidylcholine (PC) liposomes, which were 25 nm in diameter, fused within a few days or a few weeks at

temperatures below T_c . However, the aggregation could not be precisely distinguished from the fusion. Also, although the fusion of liposomes by adding PEGs has been studied,^{5–11)} the molecular weights of the PEGs were mainly more than 4000, and the distinction between aggregation and fusion was also not precise.

The cell fusion technique using polyethylene glycols (PEGs) has been established and applied in various bioscience fields. However, the mechanism of cell fusion has not been fully explained. As a first step in studying the mechanism, we

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have investigated fusion and aggregation using multilamellar liposomes made of dipalmitoyl phosphatidylcholine (DPPC) and PEGs. On the basis that an irreversible aggregation was fusion, the distinction between fusion and aggregation was observed by light microscopy with changing temperatures. Furthermore, effects of PEGs on the fusion and aggregation were investigated with PEGs of various molecular weights and concentrations.

MATERIALS AND METHODS

Preparation of DPPC liposomes and addition of PEG solution

Dipalmitoyl phosphatidylcholine was purchased from Sigma Chemical Co. and used without further purification. Polyethylene glycols (300, 400, 600, 1000, 1500, 2000, 3000) were purchased from Wako Pure Chemical Industries Ltd. Other PEGs (PEG 4000, 6000, 10000, 20000, 50000) were purchased from Merck. All PEGs were of reagent grade.

For preparation of the liposomes, fresh distilled water was added to DPPC in a test tube. After incubating the tube for more than 15 min at 50°C, the tube was sonicated for 1 min above the T_c for dispersion of the liposomes. The dispersion was kept below the T_p for more than 15 min and then used in the experiments. The liposomes were less than 5 μm in diameter. Solutions of PEGs were prepared and added to the dispersion immediately before measurement. For removing PEGs, the dispersion was centrifuged for 5 min at 1000 rpm, and then distilled water was added to the precipitation. This operation was repeated several times.

Measurements

The liposome dispersion was placed in the hollow of a slide glass with a cover glass. The liposomes were observed using an optical microscope (BH-2, Olympus Co.). A thermo-control system, which controlled the temperature of the dispersion, consisted of two thermomodules (CP1.4-71-061, Melcor Co.) for cooling and heating, and a program controller (JP-1231, Chino Co.) with a power supply (GP035-10, Takasago Co.). The cooling and heating scanning speed was set at 1.0°C/min, and the temperature of the

dispersion was controlled with a precision of $\pm 0.2^\circ\text{C}$. A video recording system (C-2400, Hamamatsu Photonics Co.) was used to permanently record the results. These systems were controlled by a microcomputer (PC-8801, NEC).

RESULTS

Reversible aggregation-disaggregation of DPPC liposomes

Colonies formed by the aggregation of liposomes were observed at temperatures below the T_p within 1-40 mg/ml of DPPC (Fig. 1(a)). Three phenomena on aggregation and disaggregation were observed with changing DPPC concentrations upon heating and cooling.

At concentrations below 10 mg/ml of DPPC, upon heating from a temperature below the T_p to that above the T_c , the colonies were suddenly disrupted and disaggregated to individual liposomes in the neighborhood of the T_p (Fig. 1(b) and (c)). The disaggregated liposomes moved violently at temperatures above the T_c . The liposomes aggregated and returned to the state of colonies when temperature dropped below the T_p . This reversible aggregation-disaggregation phenomenon was briefly called the A/D/D phenomenon from the three states of the aggregation at temperatures below the T_p , the disaggregation between the T_p - T_c and the disaggregation above the T_c (Fig. 2).

Within 15-30 mg/ml of DPPC, liposomes remained in the state of aggregation and formed colonies in spite of the temperature being above the T_p . The colonies began to sway at a temperature close to the T_p and suddenly disaggregated at the T_c (A/A/D phenomenon). This phenomenon was also reversible. The A/D/D phenomenon was observed again when the concentration was decreased below 10 mg/ml of DPPC by adding distilled water.

At concentrations above 35 mg/ml of DPPC, the colonies of liposomes remained even beyond the T_c and under violent vibration (A/A/A phenomenon). When the DPPC concentration was decreased to 15-30 mg/ml or below 10 mg/ml by adding distilled water, the A/A/D phenomenon or the A/D/D phenomenon appeared again, respectively.

Fig.1. Light micrographs of DPPC liposomes at 5 mg/ml in distilled water upon heating.

(a) colonies of the liposomes at 25.0°C, (b) beginning of disaggregation at 34.1°C, (c) liposomes after disaggregation at 36.0°C (bar: 10 μ m).

Fig.2. The aggregation-disaggregation temperature of DPPC liposomes versus the concentration of DPPC upon heating and cooling.

These phenomena shown in Fig.2 were independent of the heating or cooling scanning speed between 0.1 and 10.0°C/min and were repeatable anytime with the same liposome suspension. Fusions of liposomes have never been observed between 5–70°C.

Reversible aggregation-disaggregation and fusion with PEGs

The liposome dispersion at around 1 w/w% PEG 4000 displayed the A/D/D phenomenon as in the case without PEG 4000 (Fig.3). The A/A/D phenomenon was observed in 1–2 w/w % but the A/D/D phenomenon was recovered by removing PEG 4000. Although the A/A/A phenomenon was observed in 2–10 w/w% of PEG 4000, the A/A/D phenomenon reappeared in the concentration range of 1–2 w/w% and also the A/D/D phenomenon did at below 1 w/w % by removing the PEG 4000.

At concentrations above 10 w/w % PEG 4000, fusion of the liposomes was observed at the T_c with increasing temperature (Fig.3, Fig.4(a) and (b)). Clumps of DPPC which were formed by the fusion remained even below the T_p . Myelin figures^{12–14)} were sometimes formed from the clumps (Fig.4(b)).

Other PEGs whose molecular weights were smaller than that of PEG 4000 also caused the same phenomena at different concentrations (Fig.5). Using PEG 600, fusions of liposomes were observed at concentrations above 25 w/w%. The A/D/D phenomenon was observed below 2 w/w

Fig. 3. The disaggregation, aggregation and fusion of DPPC liposomes (5 mg/ml) versus the concentration of PEG 4000 upon heating.

Fig. 5. The concentration of PEGs for aggregation, disaggregation and fusion of DPPC liposomes (5 mg/ml) versus the molecular weights of PEGs upon heating.

The regions of (I) A/D/D phenomenon, (II) A/A/D phenomenon, (III) A/A/A phenomenon and (IV) fusion phenomenon. A and D represented aggregation and disaggregation at temperatures below the T_p , the T_p - T_c , above the T_c in turn, respectively. Symbols ●, ▲ and ■ were the measured maximum concentration caused the A/D/D, A/A/D and A/A/A phenomenon, respectively.

%, the A/A/D phenomenon was observed within 2-3 w/w% and the A/A/A phenomenon was observed within 3-25 w/w%.

DISCUSSION

The T_c and T_p are investigated by calorimetric studies,³⁾ the ESR method¹⁵⁾ and the observation of myelin figure formation.¹²⁾ Ogihara¹⁶⁾ considered that it was possible to determine the T_c and T_p with a microscopic observation of liposomes of phosphatidylcholines. We investigated the effects of DPPC concentrations on the phenomena of reversible aggregation-disaggregation and clarified the relationship between DPPC concentrations and the phenomena in this report. The microscopic observation of liposomes thus provides an easy, rapid and sensitive method for the determination of the T_c and T_p of phosphatidylcholines.

It is said that the surface potential of phosphatidylcholine (PC) liposomes is usually electrically neutral. Tatulian¹⁷⁾ reported that the surface potential of liposomes which were made of

Fig. 4. Light micrographs of the fusion of DPPC liposomes (5 mg/ml) by (a) 20 w/w% and (b) 12 w/w %PEG 4000 at the T_c upon heating.

arrow : the position of myelin figure, bar : 10 μ m.

dimyristoyl phosphatidylcholine (DMPC) became slightly negative at the T_c with increasing temperature at a low concentration of potassium salts. If the change in the surface potential is caused by structural exchange of a PC molecule at the T_c , it can not explain our results only by a potential change at the T_c , because there are two states at temperatures above the T_c ; one is an aggregation state in the A/A/A phenomenon and the other one is a disaggregation state in the A/D/D or the A/A/D phenomenon (Fig. 2).

It has been reported that liposomes which were less than 100 nm in diameter fused at temperatures below the T_p .^{1,2,4)} On the other hand, we have observed the aggregation of liposomes (less than 5 μ m in diameter) at temperatures below the T_p but have never observed the fusion at temperatures below the T_p , between the T_p - T_c or above the T_c . The small liposomes (diameters of 25 nm⁴⁾ and less than 100 nm^{1,2)} presumably contained many unilamellar liposomes, so that their liposomes may be easily fused. However, these authors^{1,2,4)} did not precisely distinguish fusion from aggregation.

A PEG concentration which was a boundary between the A/D/D phenomenon and A/A/D phenomenon and also a boundary between the A/A/D phenomenon and A/A/A phenomenon did not change with a PEG of more than 1000 in molecular weight but increased with decreasing molecular weight of the PEG which was less than 1000 (Fig. 5). The PEG concentration which caused fusion of DPPC liposomes became lower with PEGs of higher molecular weight (Fig. 5). Thus, it was confirmed that the abilities for aggregation and fusion were stronger with increasing molecular weight or concentration of PEG.

Finally, we propose that it will be important to pay attention to the distinction between fusion and aggregation when studying the mechanism of cell or membrane fusion.

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