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Rapid Report

Potential usage of thermosensitive liposomes for macromolecule delivery

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Abstract

Since transient swelling during phase transition of liposomes with hyper-osmotic internal aqueous phase may cause permeation of large molecules through the lipid bilayer, this type of liposomes can be useful for delivering macromolecules. In fact, thermosensitive liposomes containing an internal solution with osmotic pressure 2-fold higher than the physiologic level and sized through 200-nm pore, released encapsulated macromolecules such as dextran (M_r 144000) effectively when they were incubated at 40–42°C. These liposomes were stable in the presence of serum compared to the liposomes with internal osmotic pressure more than 3-fold higher than the physiologic level.

Key words: Liposome; Thermosensitivity; Drug delivery system

Liposomes have been used successfully as carriers for drugs and biologicals in vivo. They have been shown to enhance the efficacy of encapsulated drugs, to prolong the circulation time, and to reduce the side effects [1–7]. Targeting of liposomes to the desirable site has been also achieved to some extent, especially targeting to the reticuloendothelial system (RES), or targeting to tumor tissues using some kind of RES-avoiding, long circulating, liposomes [8–10]. Liposomes are endocytosed by monophagocytic cells when trapped in RES, while they tend to reside in interstitial spaces when the target is not the RES. Therefore, the release of drugs from liposomes at the target site has been attempted by using target-sensitive liposomes [11,12] and thermosensitive liposomes [13,14]. RES-avoiding type of thermosensitive liposomes using GM1 ganglioside are also effective for delivering drugs to non-RES tissues [15].

Thermosensitive liposomes utilizing phase transition of the lipid bilayer are reported to release their internal aqueous contents effectively upon hyperthermic treatment when the osmotic pressure of the internal aqueous fluid is higher than that of the outside solution [16,17]. We previously described that the large molecules such as dextran (M_r 75000) could permeate through the lipid bilayer extensively during phase transition when liposomes prepared in 0.3 M glucose were incubated in saline [18]. This phenomenon was thought to be observed due to hyper-osmotic pressure generated by a fast influx of solutes from the outside of the liposomes. Therefore, hyperthermic release of macromolecules from hyper-osmotic liposomes is expected. In this paper, we describe the release of macromolecules from thermosensitive liposomes. The characteristics of the thermosensitive liposomes were also examined.

Dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) were gifted from Nippon Fine Chemical. Calcein, fluorescein isothiocyanate-dextran-10 (FD-10, M_r 9400), fluorescein isothiocyanate-dextran-150 (FD-150, M_r 144000), and dicetyl phosphate (DCP) were purchased from Sigma. Reduced Triton X-100 was obtained from Aldrich. Fetal bovine serum (FBS) was purchased from Hazleton.

Preparation of liposomes was as follows: DPPC (9 μmol), DSPC (1 μmol), and DCP (1 μmol), dissolved in $CHCl_3$ were dried under reduced pressure and stored in vacuo for at least 1 h. Liposomes were

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DSPC, distearolyphosphatidylcholine; DCP, dicetyl phosphate; FD-10, fluorescein isothiocyanate-dextran, average M_r 9400; FD-150, fluorescein isothiocyanate-dextran, average M_r 144000.

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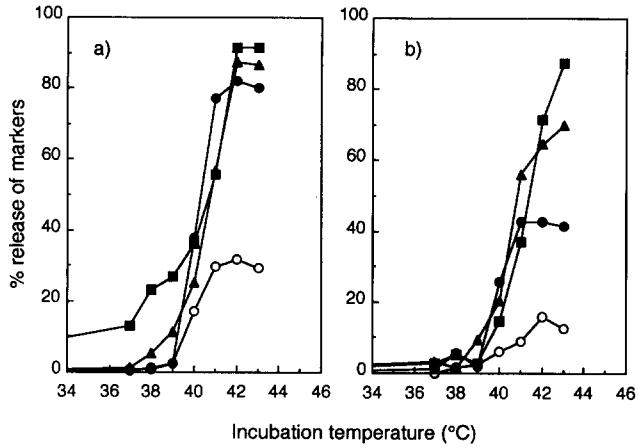

Fig. 1. Temperature-dependent marker release from liposomes with hyper-osmotic internal aqueous phase. Liposomes prepared in 0.15 M (0.3 osM, ○), 0.3 M (0.6 osM, ●), 0.45 M (0.9 osM, ▲), or 0.6 M (1.2 osM, ■) sodium gluconate containing trace of calcein (a) or FD-10 (b) were incubated in PBS (0.3 osM) for 10 min at indicated temperature. Released markers were determined as described in text. The points are means of duplicate determinations. Similar results were obtained in three separate experiments.

produced by hydration of the thin lipid film with 1 ml of various concentrations of sodium gluconate containing fluorophore (2.5 mM calcein, 330 μM FD-10, or 50 μM FD-150) and extruded through a polycarbonate membrane filter with 200-nm pore size (Nucleopore) after three cycles of freeze-thawing using liquid nitrogen. The liposomal solutions were diluted with saline, and untrapped fluorophore was removed by centrifugation at 100 000 × g for 5 min using a Beckman TL100. Since the density of the internal aqueous phase of the liposomes was higher than that of the external solution, all liposomes were easily sedimented by centrifugation. After the liposomes were washed twice with saline, the liposomal pellet was resuspended in 10 ml of saline. Fluorescence intensity and phosphorus content were determined before extrusion of the liposomes and after washing of the liposomes to calculate the encapsulation efficiency, which was revealed to be constant in liposomes with hyper-osmotic internal up to 1.2 osM. Liposomal size distribution was determined by dynamic light scattering (Phobol, ELS-800).

To determine the temperature-dependent fluorophore efflux from liposomes, liposomes (0.1 mM as PC) were incubated in 400 μl of phosphate-buffered saline (PBS, pH 7.0) for 10 min at various temperatures and centrifuged at 100 000 × g for 5 min using a Beckman TL100 after cooling to 4°C. An aliquot of 200 μl was collected from the supernatant of each sample and the fluorescent intensity was determined using a Corona MTP-100F (excitation, 490 nm; emission, 520 nm). 100% release was determined by the treatment of liposomes with 1% reduced Triton X-100 at 70°C for 10 min.

Fig. 1 shows the temperature-dependent release of calcein and FD-10 from liposomes with various internal osmotic pressures. Marker release was observed at 40–42°C, and the release was fully enhanced when the liposomal internal osmotic pressure was twice, namely 0.6 osM, or more higher than the external osmotic pressure. These data are consistent with those previously reported, where cisplatin (CDDP) was used as a marker [16]. In particular, the figure shows the release of FD-10, with average molecular weight 9400, from these liposomes as well as the release of calcein (M_r 622).

Next, we determined the stability of liposomes in PBS and in 50% serum. Although liposomes, containing 0.6, 0.9, and 1.2 osM internal solutions, were quite stable in PBS for at least 2 months at 4°C, and calcein efflux from these liposomes was less than 10%, their stability decreased in serum at 37°C, especially when the internal osmotic pressure was more than three times the external osmotic pressure (Fig. 2). Therefore, liposomes with 0.6 osM internal solution might be practically useful as a carrier of macromolecules in vivo.

When liposomes are administered into the bloodstream, the biodistribution is affected by the liposomal size, and small liposomes with a diameter of less than 200 nm tend to remain in the bloodstream. Therefore, we examined the thermosensitivity of liposomes with various sizes (Table 1). As previously reported [16], liposomes of more than 200 nm in diameter showed a remarkable thermosensitivity while all three kinds of liposomes showed temperature-dependent release of calcein. All of the other experiments presented here were performed using 200-nm-sized liposomes.

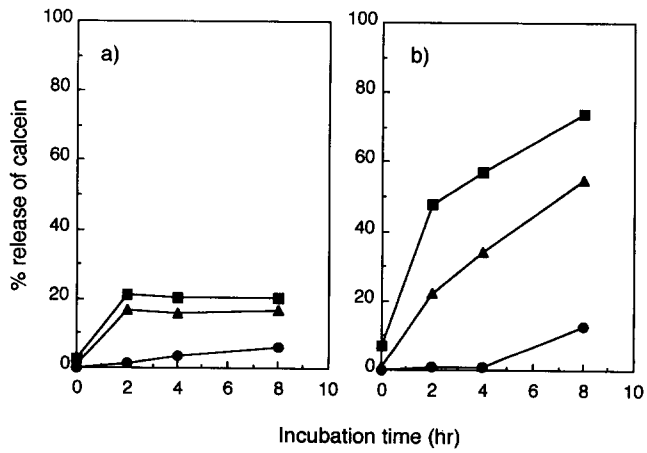

Fig. 2. Calcein release from liposomes with hyper-osmotic internal aqueous phase. Liposomes prepared in 0.3 M (0.6 osM, ●), 0.45 M (0.9 osM, ▲), or 0.6 M (1.2 osM, ■) sodium gluconate containing trace of calcein were incubated in PBS (a) or in 50% serum at 37°C for indicated time. Released calcein was determined as described in text. The points are means of duplicate determinations. Similar results were obtained in two separate experiments.

Table 1
Temperature dependent relese of calcein from various sizes of liposomes with hyper-osmotic internal aqueous phase

Extruded membrane Pore size (nm)	Liposomal size (nm)	% release of calcein		
		37°C	39°C	41°C
100	123±14	0	0	35.7
200	209±17	1.3	2.0	71.9
400	386±47	0	0.8	73.0

Liposomes with various sizes prepared in 0.3 M (0.6 osM) sodium gluconate and trace of calcein were incubated in PBS for 10 min at indicated temperature. Liposomal size and calcein release were determined as described in text. The results are expressed as the mean of duplicate determinations.

Table 2 summarizes the thermosensitive release of macromolecules when the osmotic pressure of the liposomal aqueous phase is twice as high as that of outer phase. Even FD-150 with average molecular weight 144000 was effectively released from the liposomes. Since a number of serum proteins are known to bind liposomes in vivo, the temperature sensitivity of these liposomes was examined in the presence of serum. As shown in the table, temperature-dependent release of FD-10, and FD-150 from the liposomes with 0.6 osM internal was also observed in the presence of 50% serum. In this experiment, liposomes were incubated for 10 min since osmotic burst of liposomes at phase transition occurred within 5 min [18]. This fast release of contents from the liposomes is favorable for the actual usage of thermosensitive liposomes for drug delivery.

In conclusion, we prepared thermosensitive liposomes with a hyper-osmotic internal aqueous phase to release macromolecules, according to the observation that colloid osmotic burst of liposomes at phase transition releases macromolecules from the liposomes. In fact, these liposomes actually released macromolecules temperature-dependently and effectively. Since many attempts have been made to deliver macromolecules such as cytokines by liposomal formulations, thermosensitive-liposomes which release internal macromolecules at the desired site by local heating might be useful for delivering macromolecules to the site.

Table 2
Temperature dependent release of macromolecules from liposomes with hyper-osmotic internal aqueous phase

Markers	Average M_r	Incubated in	% release of dextran		
			37°C	39°C	41°C
FD-10	9400	PBS	1.1	2.2	42.7
		50% FBS	3.3	4.1	29.6
FD-150	144000	PBS	0	1.3	40.2
		50% FBS	0	3.7	38.3

Liposomes prepared in 0.3 M (0.6 osM) sodium gluconate containing trace of FD-10 or FD-150 were incubated in PBS (0.3 osM) or in PBS containing 50% FBS for 10 min at indicated temperature. The release of markers was determined as described in text. The results are expressed as the mean of duplicate determinations.

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