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Heat-specific drug release of large unilamellar vesicle as hyperthermia-mediated targeting delivery

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Summary

The heat-specific 6-CF or CDDP release characteristics and liposomal properties of thermosensitive LUVs which were prepared with DPPC and DSPC have been demonstrated in comparison to a thermosensitive SUV. The entrapped amount of 6-CF or CDDP per lipid in the LUV was about 6 times as high as that in the SUV. The LUV was stable in long-term storage (more than 97% latency at room temperature after 6 months). Unlike the LUV, the SUV was unstable. The LUV showed very sharp release-rate increase between 40 °C and 41 °C. The amount released at 42 °C was about 80%. The release occurred explosively in a short time (a few seconds). Unlike the LUV, the SUV showed only a small release rate increase. The optimum lipid composition of the LUV for HT-mediated drug release was found to be DPPC/DSPC = 9/1 (w/w). Heat-specific drug release from the LUV and the drug permeability of the LUV at the phase transition temperature depended on the ratio of the osmotic pressure of the internal aqueous fluid to the osmotic pressure of the liposomal suspension fluid (release test media). These results indicate that the LUV is more favourable than the SUV for thermosensitive delivery with respect to drug encapsulation capacity, liposome stability and drug release and that the osmotic pressure of the internal aqueous space should be 1.5 or more times as high as the physiological osmotic pressure for heat-specific drug release.

Introduction

In recent years, considerable attention was given to liposomes as a carrier for drug delivery at a targeted site in a specific tissue. However, few reports have shown the possibility. Thermosensitive liposomes which are designed for local drug-release in response to local hyperthermia (HT) can achieve drug tumor targeting. The approach was first proposed by Yatvin et al. (1978) and Weinstein et al. (1979).

Generally, the lamellar structure of liposome membrane at the gel-to-liquid-crystalline transition phase is somewhat loose and porous. Therefore, when a liposome is heated at the phase-transition temperature, it is supposed to release its content. The thermosensitive liposome facilitates this characteristic. The liposome is prepared with a lipid composition so as to exhibit the gel-to-

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liquid-crystalline phase transition at HT temperatures (40–45 °C) (Yatvin et al., 1978; Weinstein et al., 1979; Weinstein et al., 1980; Yatvin et al., 1981; Magin and Weinstein, 1982; Weinstein, 1984).

The heat-specific drug release, however, depends not only on such a lipid composition but also on the liposomal type and preparation method. Small unilamellar vesicle (SUV) does not release a drug satisfactorily (Yatvin et al., 1981). This may be disadvantageous for the local drug release. SUV has also disadvantages in drug-encapsulating efficiency and stability (Szoka and Papahadjopoulos, 1980). Large unilamellar vesicle (LUV) is generally thought to exhibit higher drug-encapsulating efficiency and higher stability than SUV. Magin and Niesman (1984), Maynard et al. (1985), and Bassett et al. (1986) reported that LUVs released an encapsulated drug more rapidly at HT temperatures. Therefore, LUV may be more favourable for drug release in response to local HT. However, there has been little information on the drug release mechanism.

In the present paper, we report on the heat-specific drug release characteristics and other physicochemical properties of a thermosensitive LUV which encapsulates carboxyfluorescein (6-CF) or cisplatin (CDDP). The purpose is to find an optimum formulation and to elucidate the mechanism of the heat-specific drug release.

Materials and Methods

Liposome preparation

Dipalmitoylphosphatidylcholine (DPPC, Nippon Fine Chemical), distearoylphosphatidylcholine (DSPC, Nippon Fine Chemical), 6-CF (Sigma), and CDDP (Aldrich) were used for SUV and LUV preparations. LUVs were prepared by reverse-phase evaporation method (Szoka and Papahadjopoulos, 1978). To get a lipid composition with the phase transition temperature near HT temperatures, we preferably used the mixture of DPPC and DSPC. A CDDP solution of 1000 μg/ml (sodium chloride solution) and a 6-CF solution of 50 μmol/ml (phosphate buffer, pH 7) were prepared for the aqueous phase. The osmotic pressure of the aqueous phase was adjusted so that the osmotic pressure of the internal aqueous fluid in the obtained liposome was 1.5 or more times higher than the physiological osmotic pressure. Six hundred mg of the lipid mixture was dissolved in 200 ml of isopropyl-ether-chloroform mixture [1 : 1 (v/v)]. The lipid solution was mixed with 30 ml of the 6-CF solution or the CDDP solution in a 500 ml round-bottom glass flask by a mixer (Polytron, Kinematica). The obtained w/o emulsion was homogenized with a sonicator (Ohtake). Then, the emulsion was transferred to a 1000 ml round-bottom glass flask, and the organic solvent in the emulsion was evaporated gradually by a rotary evaporator at 60 °C to form a LUV suspension. The free CDDP or the free 6-CF in the LUV suspension was removed by dialysis against saline at room temperature (20–25 °C) for 2 days, using a dialyzing tube (Spectrapor, molecular weight 8000 cut-off, Spectrum Medical). The medium (2000 ml) was changed at least 5 times during the dialysis. A SUV liposome containing CDDP was prepared according to a reported method (Papahadjopoulos et al., 1974). Five ml of the aqueous drug solution was added to 400 mg of a dry lipid film which was prepared in a round flask, and mixed with a vortex mixer (Thermolyne, Sybron). The consequent multilamellar vesicle (MLV) was sonicated to form a SUV suspension. The free CDDP in the suspension was removed by the same technique as above.

Liposomal 6-CF and CDDP content

The liposomally-entrapped amount of 6-CF or CDDP was determined by measuring the free and the total amount of 6-CF or CDDP in the liposomal suspension. The free 6-CF or CDDP was separated from the liposomal suspension by a filter (Centrisart, molecular weight cut-off 20000, Sartorius). The 6-CF concentration was assayed by fluorescence spectrometry (fluorescence spectrometer, F-3000, Hitachi). The excitation and emission wave-lengths were 494 and 515 nm, respectively. The CDDP concentration was assayed by atomic absorption spectroscopy (flameless, F7000, Hitachi) (Irie et al., 1983) or HPLC (Bannister et al., 1979). In the HPLC assay, the sample was mixed with an equal volume of 10%

sodium diethyldithiocarbamate (DDTC, Wako Pure Chemical) and stood at room temperature for 30 min to form platinum DDTC adduct. The adduct was extracted with *n*-hexane and applied for HPLC (column, Zorbax CN; eluent, heptane/isopropylalcohol = 8/1 (v/v); flow rate, 1 ml/min; detector, UV 254 nm).

Differential scanning calorimetry

Phase transition temperatures of hydrated lipid mixtures and liposomal suspensions were determined by differential scanning calorimetry (SSC 5000, Seiko). The hydrated lipid mixtures were prepared by sonicating the mixtures of DPPC and DSPC in saline. The liposomal suspensions were prepared by suspending the LUV (DPPC/DSPC = 9/1, w/w) in sodium chloride solutions of different concentrations. The sample volume for the assay was 15 μl. The heating rate was 2°C/min.

Mean size and size distribution of liposome

The mean size or size distribution of liposomes was determined by three different methods; electron micrography, light scattering and filter extrusion. For the electron micrography, a SUV (DPPC/DSPC = 9/1, w/w) and a LUV (DPPC/DSPC = 9/1, w/w), both of which did not contain the drug, were diluted 40-fold with saline. Carbon- and collosion-coated grid (400-mesh) was floated onto the surface of a drop of each liposome. After removing excess fluid, a drop of 2% ammonium molybdate (pH 7.4) was placed on the grid for negative staining. The grid was allowed to air dry, and then observed in a JEM-1200EX (JEOL) at 120 kV. For the light scattering, a submicron analyzer (N4 submicron particle analyzer, Coulter Electronics) was used. The liposomes were diluted with saline 1 : 100 for the assay. The normalized size distribution was obtained by size-distribution processor (SDP) analysis. For the filter extrusion, filters of different pore sizes (Acrodiscs, Gelman; 0.45, 1.2 and 5.0 μm, respectively) were used. The liposomes were diluted with saline 1 : 20 and the liposomal suspensions (2 ml) were extruded from the filter. The percentage of the liposome passing through the filter without disruption was evaluated by measuring the concentration of the liposomally-entrapped drug in the extruded fluid.

Osmotic pressure of internal aqueous fluid in liposome

The osmotic pressure of the internal aqueous fluid in a liposome was measured by directly applying 3 ml of the liposome obtained before dialysis (pre-dialysis liposome) to an osmometer (Osmette A, Amuco Corp.). A preliminary study indicated that the osmotic pressure of such a sample was equal to that of the external aqueous fluid. Also, the osmotic pressure of the internal aqueous fluid did not change after dialysis for removing the free drug. Therefore, the osmotic pressure of the internal aqueous fluid in the liposome was assumed to be the osmotic pressure of such a liposomal sample before the dialysis.

In vitro drug release

The rate of release of 6-CF from a thermosensitive LUV as a function of heating time was examined by passing the liposome through a heated thin polyethylene tube (PE50, Intramedic). The tube consisted of two parts. The first part (about 20 cm) was immersed in a water bath maintained at 37°C for pre-heating the liposome, and the second part was immersed in another water bath maintained at various temperatures for heating the liposome. The length of the second part was varied from 1 to 5 cm in order to change the sample resident time in the heated tube. The liposome was diluted 10-fold with saline and was passed through the tube at 0.42 ml/min. It was ascertained that the time required for the sample to reach the water bath temperature in the heated tube was less than 1 s. This was achieved by measuring the temperature of the sample flowing out of the heated tube. The sample flowing out was pooled in a plastic tube. The release rate of 6-CF was determined as a function of the time required for the sample to pass through the heated tubing.

Temperature release-rate profiles of 6-CF and CDDP liposomes were determined by incubating the liposomes at various temperatures as follows. The liposomes were diluted 10-fold with saline. Two ml of each sample was placed in a Centrisart

tube and incubated for 15 min in a water bath (BT-21, Yamato) maintained at constant temperatures (variation was less than 0.1° C). The released 6-CF or CDDP was separated from the liposomal suspension and assayed by the same method as in the liposomal drug-content assay.

Liposomal-membrane permeability

Membrane permeabilities of a 6-CF thermosensitive LUV at the gel phase, the gel-to-liquid-crystalline transition phase and the liquid-crystalline phase were evaluated by examining the release of 6-CF from the liposome at temperatures of the three phases. Preliminary temperature release-rate test suggested that when the liposome was heated above its phase transition temperature, it released 6-CF before reaching the test temperature. To avoid this, the pre-dialysis liposome was used for the test sample. It was ascertained that the liposome did not release 6-CF during the heating process, unless the liposome was placed in the test medium since the concentrations of 6-CF in the internal aqueous space and in the liposome suspending fluid were in equilibrium.

Three ml of the pre-dialysis liposome heated to the test temperature was placed in a dialysis tube and was dialyzed for 3 h or 16 h in 1000 ml of sodium chloride solutions of various concentrations (0.6, 0.9 and 1.35%, respectively) at constant temperatures (25, 37, 41, 47 and 57° C, respectively). The release rate was determined by measuring the remaining unreleased 6-CF during dialysis. It was ascertained that the volume of the dialyzing fluid (release test medium) was large enough and the diffusion of free 6-CF out of the dialysis tube was quick enough to assume that 6-CF release from the liposome occurred under sink condition.

Results and Discussion

Phase-transition temperature of DPPC / DSPC mixtures

Thermosensitive liposomes can be prepared from membrane-lipid compositions so as to exhibit the phase transition temperature at HT (41–45° C). It has been shown that appropriate

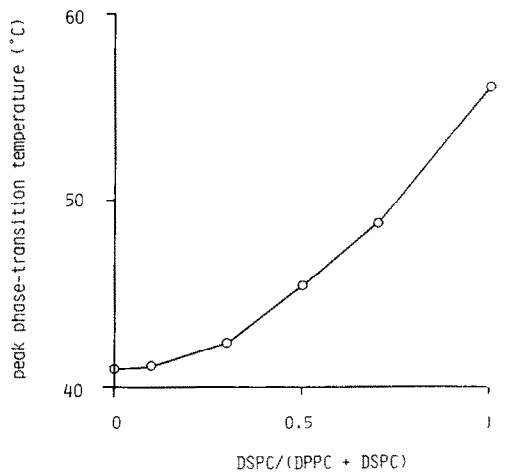


Fig. 1. Peak phase-transition temperatures of DPPC/DSPC mixtures measured by differential scanning calorimetry.

combination of DPPC with DSPC or dipalmitoylphosphatidylglycerol (DPPG) exhibited a phase transition temperature near HT temperatures. Yatvin et al. (1978) and Weinstein et al. (1979) reported the use of the combination of DPPC and DSPC in the ratio from 7/1 to 7/3 (molar ratio) for thermosensitive SUV. Magin et al. (1984) and Bassett et al. (1986) reported the use of the combination of DPPC and DPPG (4/1, w/w) for thermosensitive LUV.

In the present study, we used the combinations of DPPC and DSPC. In order to obtain an optimum combination, we examined the DSCs of mixtures of these lipids (Fig. 1). The phase diagram is similar to the previously reported one in using a mixture of DMPC and DSPC (Mabrey, 1981). As the portion of DSPC in the mixture was increased, the peak phase-transition temperature shifted to the values intermediate between those of the pure components. The peak phase-transition temperatures for DPPC/DSPC = 9/1 (w/w) and DPPC/DSPC = 7/3 (w/w) were found to be 41.5 and 42.5° C, respectively, showing close correspondence to the reported values (Mabrey, 1981). For achieving larger chemotherapeutic effect of a HT-mediated liposome delivery with minimum side effect, it is favourable that HT temperature at which the drug release occurs is as low as possible. Therefore, we chose DPPC/DSPC = 9/1 (w/w) for the lipid composition so as to get the phase

transition temperature near the lower limit of HT temperature.

Mean size and size-distribution of liposome

The negative stain electron micrograph of the SUV and the LUV are shown in Fig. 2A and B, respectively. Both of the liposomes appeared unilamellar (Franks and Lieb, 1981). The mean particle diameters of the SUV and the LUV were approximately 0.08 and 0.2 μm, respectively. The size distribution of the CDDP encapsulated LUV obtained by the filter extrusion is shown in Table 1. Almost 70% of the liposome was distributed in the size smaller than 0.45 μm but the remaining percent was distributed between 0.45 μm and 5 μm. The mean size of the LUV estimated by this method appeared larger than the size estimated by the electron micrograph. The size distribution of the same LUV determined by the light scattering is shown in Fig. 3. The profile showed a bi-modal distribution. The one peak located near 0.2 μm and the other located near 2 μm. The size of the LUV as a single particle obtained by the electron micrograph and the bi-modal size distribution obtained by the light scattering suggest that the liposome may exist in the fluid as multidispersion.

Liposome drug-encapsulating efficiency

Generally, higher drug-encapsulating efficiency of liposome is thought to be preferable for its clinical use. When an encapsulated drug is soluble in an aqueous fluid, the liposome drug-encapsulating efficiency is obviously determined by the volume of the internal aqueous fluid. Because of its larger aqueous fluid volume, LUV is thought to exhibit higher encapsulating efficiency than SUV.

In the present study, the LUV encapsulated about 25% CDDP (250 μg CDDP and 20 mg lipid/ml). On the other hand, the SUV encapsulated about 15% of the drug (150 μg CDDP and 80 mg lipid/ml). The entrapment per the lipid in the SUV was about one sixth of that in the LUV. This indicates that the injection of CDDP as LUV can save the lipid, thus offering a greater advantage for the therapeutic use of the liposome.

Long-term storage stability

Generally, liposome stability is thought to be affected by lipid composition and type of liposome. It was reported that a liposome prepared with saturated phospholipid is more stable than that prepared with unsaturated phospholipid such as Egg PC (Szoka and Papahadjopoulos, 1980). It was also reported that LUV is more stable than SUV (Szoka and Papahadjopoulos, 1980). Generally, SUV tends to coalesce. Coalescence caused by aggregation may induce drug leakage from the liposome even if it is prepared with saturated phospholipid. However, the long-term storage stability of thermosensitive liposome has not been reported.

In the present study, the stabilities of a CDDP-encapsulated thermosensitive SUV and a CDDP-encapsulated thermosensitive LUV were examined by storing the liposomes at 4°C and room temperature (RT) for 6 months (Table 2). The latencies of the LUV stored at 4°C and RT were both more than 97% even after 6 months. No coalescence was observed. On the other hand, the latencies of the SUV stored at 4°C and RT for one month were 91.8 and 9.2%, respectively. In the SUV, remarkable coalescence was observed one week after storage at RT or one month after storage at 4°C. These results indicate that the LUV prepared with DPPC/DSPC is stable even upon long-term storage at RT.

Time course of heat-specific drug release

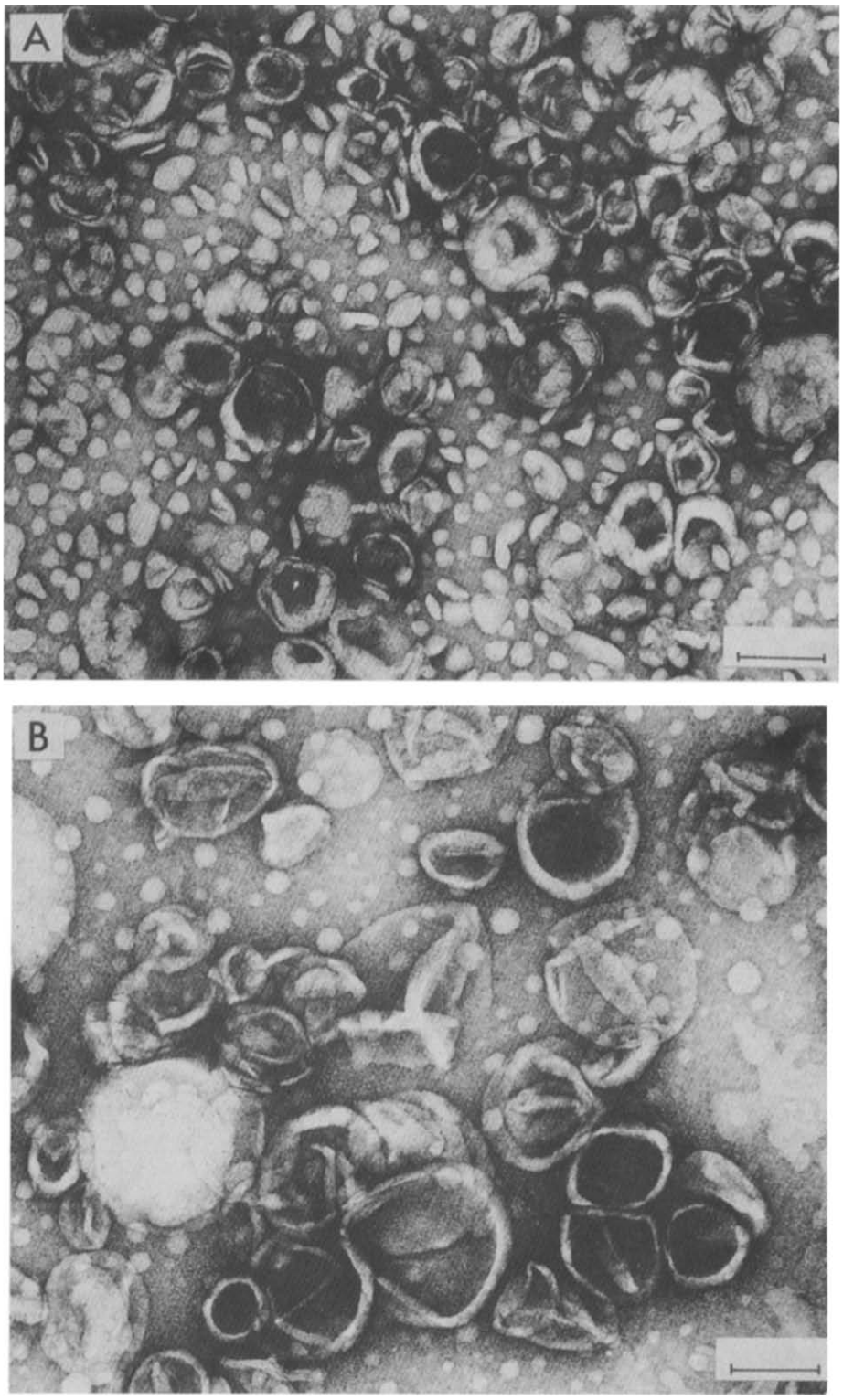
In order to achieve drug targeting by HT-mediated thermosensitive liposome, the liposome should release the highest possible amount of the drug within a short time. Magin and Niesman

TABLE 1

Size distribution of a CDDP-encapsulated LUV liposome (DPPC/DSPC = 9/1, w/w) obtained by extrusion through Acrodiscs filters of different pore sizes

Pore size of filter [a]	Percent filtered
5 μm pass	97.4 [b]
1.2 μm pass	84.9
0.45 μm pass	69.6

[a] Acrodisc. [b] Percentage of the liposome passing through the filter without disruption was evaluated by measuring the concentration of the liposomally-entrapped drug in the filtered fluid.


A
B

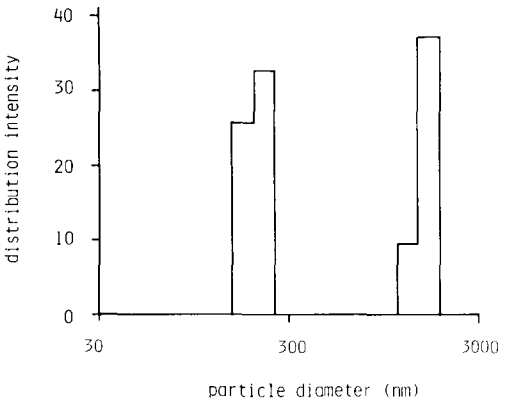


Fig. 3. The normalized particle-size distribution plotted against particle diameter for CDDP-encapsulated LUV liposome composed of DPPC/DSPC (9/1, w/w). The normalized size-distribution was obtained by size-distribution processor analysis in Coulter N4 submicron analyzer.

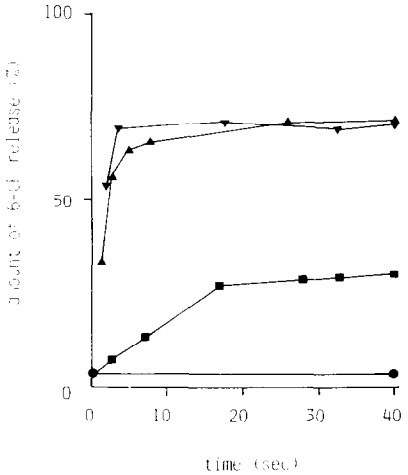


Fig. 4. Time-dependent 6-CF release from a 6-CF encapsulated thermosensitive LUV liposome (DPPC/DSPC = 9/1, w/w) when the liposome passed through a tube heated at different temperatures. The release rate was plotted against time for the liposome to pass through the heated tube. (●), 38°C; (■), 40°C; (▲), 41°C; (▼), 42°C.

TABLE 2

Stabilities of a CDDP-encapsulated SUV and a CDDP-encapsulated LUV liposome (DPPC/DSPC = 9/1, w/w) when stored at 4°C and room temperature (RT)

Liposome	Month	4°C	RT
SUV	0	97.5 [a]	–
	1	91.8 [b]	9.2 [b]
LUV	0	98.2	–
	1.5	98.2	95.1
	3	98.2	99.9
	6	97.1	96.3

[a] The latencies (%) of the liposomes were used as a measure for liposomal stability. [b] Remarkable coalescence was observed.

(1984) demonstrated that drug release from a thermosensitive LUV occurred very rapidly (in a few seconds) after the liposome was heated in a small glass capillary tube. To evaluate time-dependent drug release from a thermosensitive LUV, we heated the liposome in a flow system where the liposome passed through heated thin tubes with different lengths.

Fig. 4 shows time-dependent 6-CF release from the LUV when it passed through a heated tube. At 41 and 42°C, the liposome released about 70% of entrapped 6-CF within 2 s. Thereafter, the release profile reached a plateau level. At 40°C, the liposome released about 25% of the entrapped 6-CF within 15 s, the release profile also reaching a plateau level after the initial release. However, at 38°C the liposome did not release 6-CF even after 1 min.

The data correspond to the previous report (Magin and Niesman, 1984), the drug release occurred explosively within a short time (a few seconds) in response to heating, and the rate depended on the incubation temperature rather than the incubation time. By considering the data of HT-mediated release and the increase in the tumor CDDP level (Iga et al., 1987), a few seconds heating time is probably short enough for the in vivo drug release.

Fig. 2. Negative-stain electron micrographs of a SUV liposome (A) and a LUV liposome (B) both of which were composed of DPPC/DSPC (9/1, w/w). Original magnification ×150,000; bar = 0.1 μm.

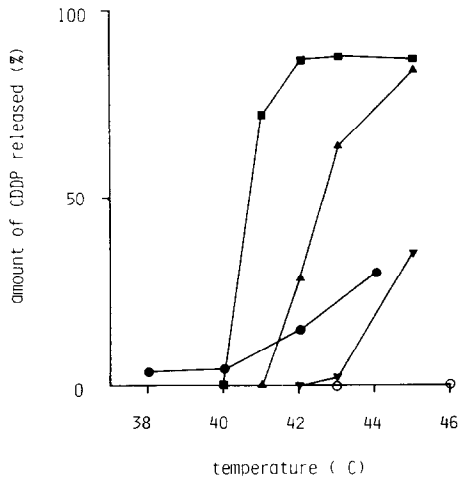


Fig. 5. Temperature-dependent release of CDDP from a CDDP encapsulated SUV liposome composed of DPPC/DSPC (9/1, w/w) and CDDP encapsulated LUV liposomes composed of DPPC/DSPC (9/1, 7/3, 5/5 and 0/10, w/w). The liposomes were diluted with saline by 10 times and incubated in a water bath maintained at constant temperatures for 15 min. The release rate was plotted against incubation temperature. (●), SUV, DPPC/DSPC = 9/1; (■), LUV, DPPC/DSPC = 9/1; (▲), LUV, DPPC/DSPC = 7/3; (▼), LUV, DPPC/DSPC = 5/5; (○), LUV, DSPC alone.

Effect of lipid composition on drug release

Heat-specific drug release is thought to be primarily determined by the lipid composition to exhibit a phase transition at HT temperatures. Temperature-dependent release of CDDP from LUVs prepared with differential ratios of DPPC and DSPC is shown in Fig. 5.

A LUV composed of DPPC/DSPC = 9/1 released the drug at and above 41°C, but did not release at 38°C. The increase in release-rate was very sharp between 40 and 41°C. The lowest temperature at which the drug release occurred, appeared slightly below the peak phase transition temperature as observed above. The amount of the drug released at 42°C was more than 80%.

As far as LUVs composed of DPPC/DSPC = 7/3 and DPPC/DSPC = 5/5, the drug-release temperature was shifted higher by approximately 1°C for DPPC/DSPC = 7/3, and by approximately 3°C for DPPC/DSPC = 5/5. The LUV composed of DPPC/DSPC = 5/5 did not show sharp drug-release increase at its phase transition temperature. The LUV composed of DSPC alone, did not show drug release at the temperature range of HT.

These results indicate that the lipid composition of DPPC/DSPC = 9/1 is optimum for HT-sensitive drug release.

Effect of liposome type on drug release

Heat-specific drug release depends on the liposomal type and the preparation method, but is primarily determined by the lipid composition. The drug release from the three classes of liposomes (SUV, LUV and MLV) were previously examined by measuring the release of Ara-C from the liposomes at temperatures near the phase transition (Maynard et al., 1985). The LUV showed sharp increase in drug-release near the phase transition temperature. On the other hand, the SUV and the MLV exhibited gradual release around the transition temperature, and the release rates were lower.

In the present study (Fig. 5), a similar difference of the release characteristics between the SUV and the LUV was also found between a CDDP encapsulated SUV and a CDDP encapsulated LUV. Unlike the LUV, the SUV showed only a slight increase in drug release as the temperature was increased through the phase-transition temperature. The release rate at 42°C was about one sixth of that found in the LUV. Smaller rate of the drug release from the SUV as compared with the LUV is probably due to the larger lipid-membrane curvature of the SUV.

These results indicate that the LUV is more favourable for thermosensitive drug release than the SUV.

Effect of differential osmotic pressure between internal and external fluids on drug release

The difference of the osmotic pressures between the internal aqueous fluid in the liposome and the release test medium (external fluid of the liposome) is also a factor influencing thermosensitive drug release. There have been few reports on the effect of the differential osmotic pressure, and the osmotic permeability of a liposome have been reported (Blok et al., 1976; Yoshikawa et al., 1985; Neitchev et al., 1986).

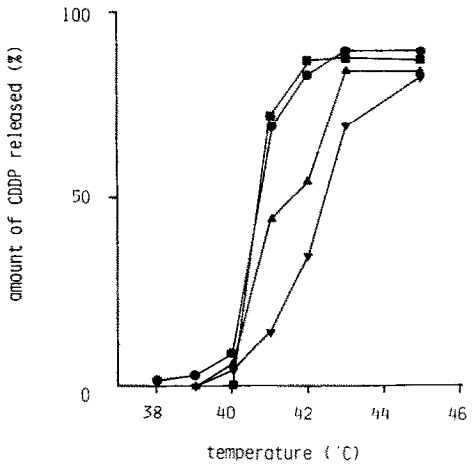


Fig. 6. Temperature-dependent release of CDDP from a CDDP encapsulated thermosensitive LUV liposome (DPPC/DSPC = 9/1, w/w) in media with different concentrations of sodium chloride. The liposome was diluted with the media by 10 times and incubated in a water bath maintained at constant temperatures for 15 min. The release rate was plotted against incubation temperature. (●), 0.72% NaCl; (■), 0.9% NaCl (saline); (▲), 1.08% NaCl; (▼), 1.35% NaCl. The data of saline were the same as the data of the LUV composed of DPPC/DSPC = 9/1 (w/w) in Fig. 5.

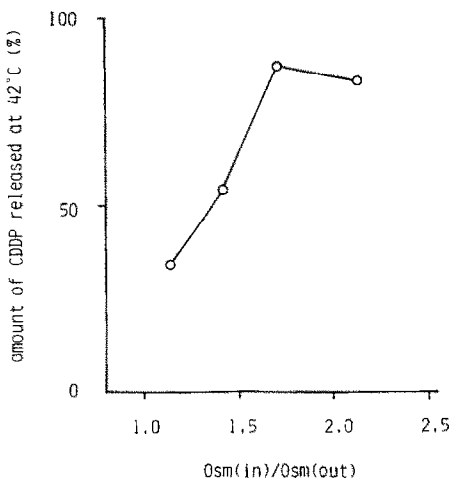


Fig. 7. The plots of the rates of CDDP release from a CDDP-encapsulated thermosensitive LUV liposome (DPPC/DSPC = 9/1, w/w) at 42°C versus the ratios of the internal aqueous space osmotic pressure to the external fluid osmotic pressure. The release rate data were the same as presented in Fig. 6, and the internal fluid osmotic pressure was assumed to be 1.7 times the saline osmotic pressure.

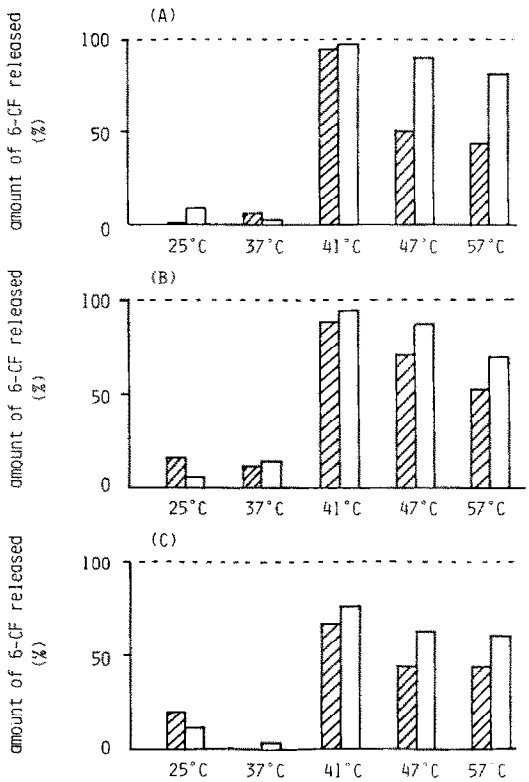


Fig. 8. The liposomal-membrane permeabilities of a 6-CF encapsulated thermosensitive LUV liposome at the gel phase, the transition phase and the liquid-crystalline phase as evaluated by 6-CF-release from the pre-dialysis LUV liposome when it was dialyzed at different temperatures using aqueous sodium chloride solutions with different concentrations. Panels A, B and C show the percentage of released 6-CF when dialyzed with media of 0.6%, 0.9% and 1.35% sodium chloride, respectively. Hatched columns and open columns show the results in 3 and 16 h dialysis, respectively.

Fig. 6 shows the temperature-dependent release of CDDP from the thermosensitive LUV in media with different osmotic pressures (sodium chloride solution). The heat-specific drug release from the LUV depended on the osmotic pressure of the medium. Higher osmotic pressure of the medium as compared with physiological osmotic pressure remarkably decreased the heat-specific drug release.

The internal-fluid osmotic pressure for the CDDP-encapsulated LUV estimated by the present method was about 1.7 times the physiological osmotic pressure. Fig. 7 showed the dependency

of the release rate on the ratio of the internal fluid osmotic pressure to the external fluid osmotic pressure. There seemed to be a critical point at a ratio near 1.5.

On the other hand, DSC profiles of the liposome suspensions showed that the phase transition was influenced by the ratio of the osmotic pressure of the internal aqueous fluid to the osmotic pressure of the external fluids in the liposome (liposomal suspension fluid). The higher osmotic pressure of the internal fluid shifted the transition temperature to a lower value as compared to the transition temperature of the hydrated lipid mixture of the same composition (data not shown). One reason may be that the membrane of the liposome with higher inside osmotic pressure is flexing outward by the internal fluid and the outer lipid layer is tensioned by this force. This may contribute to the mechanism for heat-specific drug release (discussed below).

Liposomal membrane permeability

Fig. 8 shows the membrane permeability of the 6-CF encapsulated thermosensitive LUV at the gel phase, the transition phase and the liquid-crystalline phase. This was evaluated by measuring 6-CF release from the predialysis liposome and after dialysis at various temperatures using media with different osmotic pressures.

At room temperature and 37°C (gel phase), the amounts of 6-CF released were very low even after 16 h-dialysis in any medum. However, some erroneous data existed in estimating the released amount from the unreleased amount. This indicates that, at the gel phase, the liposome membrane was not permeable and it was not changed by the osmotic pressure of the medium.

At 41°C (transition phase), however, the released amount was large, particularly in lower osmotic pressure media. Upon dialysis with 0.6% sodium chloride and saline the release amounts were more than 90%. In contrast, the released amount when dialyzed with 1.35% sodium chloride was smaller. This indicated that the liposomal membrane permeability was high at the transition phase but depended on the osmotic pressure of the test medium.

At 47 and 57°C (liquid-crystalline phase), the released amounts were smaller than those at the phase transition temperature. The results indicate that at this phase, 6-CF release depended on the time of incubation rather than on the temperature or the osmotic pressure of the medium.

These results indicate that (a), the lipid membrane at the gel phase is impermeable and osmotically insensitive rendering the stability to the liposome; (b), the lipid membrane at the transition phase is permeable and osmotically sensitive so that the liposome releases 6-CF very rapidly under lower osmotic pressure of the suspension medium; (c), the lipid membrane at the liquid crystalline phase is somewhat permeable but not much osmotically sensitive, and the liposome is not much stable.

Postulated mechanism of thermosensitive drug release

The findings in the above experiments suggest that higher inside osmotic pressure contributes to the mechanism of thermosensitive drug release and is a major driving force of the release (Prescott and Nimmo, 1981). It is possible that at the phase-transition temperature, the high inside osmotic pressure enlarges 'aqueous pores' existing at the lipid bilayer, and thereby causes the internal aqueous fluid to diffuse out through the pores explosively in a short period. The drug release occurs concurrently with this fluid flow. After diffusion of the inside fluid, the pores may shrink or close and the explosive drug release will not occur any more. This mechanism can explain the plateau level of the release rate as observed in the temperature release-rate profile (Figs. 4–6).

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