

TEMPERATURE-SENSITIVE RELEASES FROM LIPOSOMES CONTAINING HYDROPHOBICALLY MODIFIED POLY(*N*-ISOPROPYLACRYLAMIDE)

Jin-Chul Kim, Myung-Soo Kim* and Jong-Duk Kim[†]

Department of Chemical Engineering and Bioprocess Engineering Research Center,
Korea Advanced Institute of Science and Technology, 373-1 Kusung-Dong, Yousong-Gu, Taejon 305-701, Korea

*Korea Research Institute of Standard and Science, P.O. Box 102, Yousong, Taejon 305-600, Korea

(Received 27 February 1998 • accepted 27 August 1998)

Abstract – Novel temperature-sensitive liposomes containing hydrophobically modified poly(*N*-isopropylacrylamide) (HPNIPAM) and their release behaviors were investigated using calcein as a fluorescence probe. Above the lower critical solution temperature (LCST) of the polymer (e.g., 40°C), the degree of calcein release in 280 sec from reverse-phase evaporation vesicles (REV)s of egg phosphatidylcholine (egg PC) was 43 %, while egg PC MLVs was 16 %. Such a large difference of release may be attributed to the lamellarity of liposomes. The incorporation of dioleoylphosphatidylethanolamine (DOPE) into the PC bilayer enhanced the release by 10-13 % at 40°C, probably due to the increased instability of mixture bilayers. Meanwhile, a temperature-sensitive device of DOPE liposomes was prepared by using HPNIPAM as a stabilizer. The optimal ratio of HPNIPAM to lipid to stabilize the bilayer was 0.1. Above the LCST (e.g., 40°C), the release percentage was about 80 % of the entrapped calcein. DOPE liposomes were the most temperature-sensitive among liposomes tested. This is probably because DOPE liposomes disintegrate into a non-liposomal phase, such as hexagonal (H_n), by a thermal contraction of HPNIPAM.

Key words : Liposomes, Poly(*N*-isopropylacrylamide), Temperature-Sensitivity, Dioleoylphosphatidylethanolamine, Reverse-Phase Evaporation Vesicles

INTRODUCTION

Liposome, which is a vesicle consisting of lipid bilayers, has been proposed as a carrier of drugs [Juliano and Stamp, 1972; Bangham, 1981] including target-sensitive devices [Choe et al., 1996]. Specific functions of liposomes induced by direct interaction [Lee et al., 1992; Huang et al., 1983], pH [Choi et al., 1992; Maeda et al., 1988], and temperature [Yatvin et al., 1978; Kim et al., 1997; Kono et al., 1994] have focused on improving the delivery of drugs for cancer and gene therapy [Perez-Solar and Priebe, 1990; Gao and Huang, 1996], and infectious diseases [Cudd et al., 1990; Kim et al., 1997]. Even if it has many potential advantages as a drug carrier, a liposome itself has some drawbacks of structural instability for prolonged applications [Crommelin et al., 1994; Kim and Kim, 1988] and nonspecific interactions with target sites [Ho et al., 1986; Park et al., 1992]. Therefore, it has been proposed that the surface of liposomes be chemically modified or third molecules inserted into the lipid membrane [Chen et al., 1996; Lee and Kim, 1995]. Recently, temperature-sensitive releases of liposomes were proposed by coating the vesicles of egg phosphatidylcholine (egg PC), dipalmitoylphosphatidylcholine (DPPC), or distearoylphosphatidylcholine (DSPC) with hydrophobically modified poly(*N*-isopropylacrylamide) HPNIPAM [Yatvin et al., 1978; Kim et al., 1997]. Such hydrogels of HPNIPAM, which maintain an expanded form below its lower critical solution

temperature (LCST) of around 32°C and attain a contracted form beyond the temperature [Heskins and Guillet, 1968], were applied for controlled release devices [Bae et al., 1991] and valve membranes [Chun and Kim, 1996]. Release behaviors of materials entrapped in the inner aqueous core of liposomes can be controlled by changing the temperature, which adjusts the interaction between polymers and lipid membranes.

In this study, the release behaviors of the reverse phase evaporation vesicles of egg PC (REV)s and from the multilamellar vesicles of egg PC (MLVs) coated with HPNIPAM were observed below and above LCST of HPNIPAM. A novel device of unsaturated phosphatidylethanolamine (PE) liposomes coated with HPNIPAM was developed to obtain a high temperature sensitivity in release. Unsaturated PE, as it has been known, does not form stable bilayers under physiological conditions [Gruner et al., 1985], but it forms liposomes in the presence of complementary molecules such as palmitoyl immunoglobulin [Ho et al., 1986], palmitoyl lysozyme [Lee and Kim, 1995], and membrane glycoprotein [Tarashi et al., 1982]. Our approach to enhancing the sensitivity of dioleoylphosphatidylethanolamine (DOPE) liposomes is to use HPNIPAM as a stabilizer. Thermally induced releases of calcein and morphological changes of liposomes will be reported.

MATERIALS AND METHODS

1. Materials

Phospholipids of dioleoylphosphatidylethanolamine (DOPE) and egg phosphatidylcholine (egg PC), and calcein, a fluores-

[†]To whom correspondence should be addressed.
E-mail : kjd@hanbit.kaist.ac.kr

cence marker for release tests, were purchased from Sigma Chemical Co. A hydrophobic anchor for water-soluble poly(*N*-isopropylacrylamide) (PNIPAM), octadecylacrylate (ODA), was purchased from Aldrich Chemical Co. *N*-isopropylacrylamide (NIPAM) was purchased from TCI. All other reagents were of analytical grade.

2. Synthesis of Hydrophobically Modified Poly(*N*-isopropylacrylamide)

A hydrophobically modified poly (*N*-isopropylacrylamide) (poly(*N*-isopropylacrylamide-*co*-octadecylacrylate, HPNIPAM) was polymerized as previously described [Ringsdorf et al., 1991]. NIPAM (9.9 mmol), ODA (0.1 mmol), and azobisisobutyronitrile were dissolved in 20 mmol of freshly distilled dioxane. The solution was degassed by bubbling N₂ for 20 min and then heated to 65 °C for 12 h. The copolymer was precipitated when diethylether was added. For purification, the precipitated polymer was dissolved in dioxane and reprecipitated with diethylether.

3. Preparation of Liposomes Coated with HPNIPAM

3-1. Preparation of PC Liposomes

(1) Multilamellar Vesicles (MLVs)

A dry film of 20 mg of egg PC was dispersed into 2 ml of 50 mM calcein in PBS (pH 7.4) and was sonicated for 10 min with a tip-type sonicator (Sonics & Materials) at room temperature. To remove untrapped calcein, the suspension was chromatographed through Bio-gel A-0.5 m. Fractions of liposomes were mixed with HPNIPAM so that the weight ratios of polymer to phospholipid varied from 0.0125 to 0.5. The final concentrations of lipid were adjusted to 2 mg/ml. The suspensions were incubated at 4 °C for 12 hr.

(2) Reverse-Phase Evaporation Vesicles (REVs)

Dry films of 25 mg of egg PC in a 50 ml round bottom flask were dissolved into 1.5 ml of diethylether and then 50 mM calcein solution of 0.5 ml was added to the solution. The two-phase system was sonicated for 1 min in an ice bath to give a water-in-oil emulsion. The solvent was evaporated at room temperature on rotary evaporator, operated at 220 rpm, fitted with aspirator, until a collapse of gel to fluid was observed. An additional calcein solution of 1.5 ml was added and then a residual solvent was evaporated for 20 min. The liposomal suspension was mixed with HPNIPAM so that the ratio of polymer to lipid is 0.1. After incubating at 4 °C for 12 hr, the suspension was chromatographed on a Bio-gel A-0.5m to remove free calcein and polymer.

(3) Preparation of DOPE Liposomes

A dry film of 20 mg of DOPE was hydrated by addition of 2 ml of 50 mM calcein in PBS (pH 8.0), which contains varying amounts of HPNIPAM and 0.09 % deoxycholate (DOC). The mixture was sonicated in a bath sonicator (Sonics & Materials Inc.) for 30 min. The temperature of the mixture was kept below the LCST of polymer. After the suspensions were incubated at 4 °C for 12 h, the suspension was chromatographed through Bio-Gel A-0.5 m to remove DOC and free untrapped calcein. The final concentrations of lipid were adjusted to 2 mg/ml. The % quenching of liposome-entrapped calcein was determined by the formula

$$\% \text{ quenching} = (1 - F_i/F_f) \times 100$$

where F_i is the initial fluorescence, and F_f is the total fluorescence after DOC is added so that the final concentration is 0.12 %. The fluorescence intensities were measured at 17 °C.

(4) Calcein release

The liposomes containing calcein, suspended in 0.2 ml of PBS at 17 °C, were injected into a fluorescence cell containing 2.6 ml PBS preadjusted to the temperatures ranging from 24 °C to 42 °C. The change in fluorescence was monitored at 520 nm with excitation at 490 nm. The percent release of calcein was determined as follows :

$$\% \text{ release} = (F_t - F_i)/(F_f - F_i) \times 100$$

Here F_t is the intensity of fluorescence at a given temperature, and F_i is the initial intensity at 17 °C.

(5) Size Distributions

The size distributions of egg PC and DOPE liposomes with polymer to lipid ratio of 0.1 were measured at 25 °C on a dynamic light scattering (Brook Haven). After the samples were exposed to 40 °C for 80 sec and cooled to 25 °C, the size distributions of the two samples were also observed.

(6) Transmission Electron Microphotograph

Before and after being exposed to 40 °C for 80 sec, a structural change of egg PC and DOPE liposome with polymer to lipid ratio of 0.1 was observed. Microphotographs of liposomes negatively stained with freshly prepared 2 wt% phosphotungstic acid solution (pH 6.8) were taken on an electron microscopy (Fillips) with magnification of 115,000.

RESULTS AND DISCUSSION

1. Release from Egg PC REVs and Egg PC MLVs

Fig. 1 shows the degrees of release from MLVs and REVs at HPNIPAM to lipid ratio of 0.1. In 280 sec, MLVs released 16 % of encapsulated calcein at 40 °C and 4 % at 24 °C. REVs released 43 % of encapsulated calcein at 40 °C and 7 % at 24 °C, respectively. In both cases, the % releases at high temperature were higher than those at 24 °C. Such higher releases may be attributed to the strong interaction between HPNIPAM and membrane. Since the LCST of the polymer is around 31 °C, the transition from an expanded form to a contracted form as temperature increases to 40 °C may cause membrane defects [Kim et al., 1997; Kono et al., 1994].

The degree of release of REVs at 40 °C proved to be much greater than that of MLVs at the same temperature. Depending on the relative amount of lipid, oil and water, the REV method results in uni- or multilamellar vesicles, and a large internal aqueous phase [Szoka and Papahadjopoulos, 1978]. As in Fig. 2, the REVs were larger vesicles than MLVs, but the number of lamellae was reduced from that of MLVs. Thus, the defect in the bilayer of REVs would be induced by thermal contraction of the polymer more easily than in case of MLVs. This could explain why REVs release more significantly than MLVs do.

2. Release from DOPE/Egg PC MLVs

Fig. 3 shows the effect of DOPE contents in egg PC liposomes on the % releases in 80 sec at 24 °C and 40 °C. As seen in Fig. 1, the releases from egg PC liposomes almost reached saturation by 80 sec, and therefore the data points in

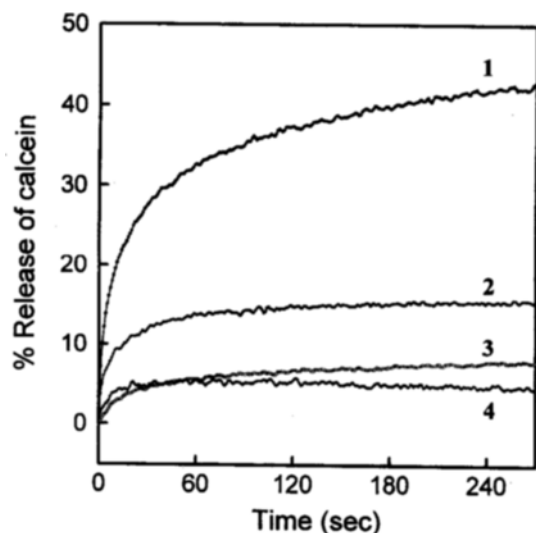


Fig. 1. Calcein release, at 24°C (plots of no. 3 and 4) and 40°C (plots of no. 1 and 2), from egg PC MLVs (plots of no. 2 and 4) and egg PC REVs (plots of no. 1 and 3) method. The weight ratio of HPNIPAM to lipid was 0.1.

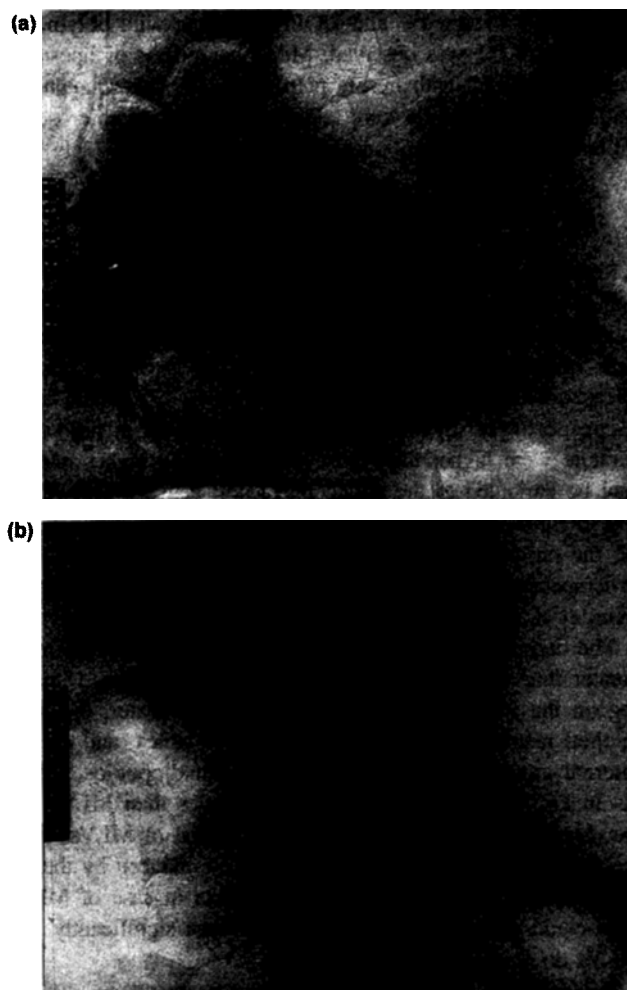


Fig. 2. Transmission electron microphotograph of negatively stained egg PC REVs (a) and egg PC MLVs (b). The weight ratio of HPNIPAM to lipid was 0.1. Magnification was 115,000 times.

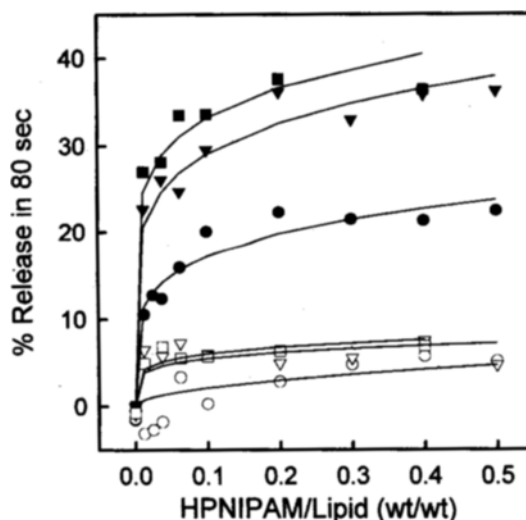


Fig. 3. The degree of release, at 24°C (blank symbols) and 40°C (filled symbols), from MLVs composed of egg PC and DOPE (7:3 ▼, ▽; 5:5 ■, □) and pure egg PC (●, ○) MLVs. The weight ratio of HPNIPAM to lipid was 0.1.

Fig. 3 designate the maximal releases. In the absence of the polymer, the liposomes at 40°C exhibited almost no release, while the degree of release increased in a saturation pattern with the increase of polymer to lipid ratio. Obviously, HPNIPAM enhances the release of calcein, because the thermal contraction of the polymer may lead to a packing defect of liposomal membrane [Kono et al., 1994]. The maxima in releases were obtained near an HPNIPAM/lipid ratio of 0.1 where the degree of releases increased following the order of egg PC liposome, egg PC/DOPE (70/30, wt/wt) liposomes and egg PC/DOPE (50/50, wt/wt) liposomes. For the liposomes of 50 wt% DOPE, the degree of release at 40°C in 80 sec was 34% at the polymer to lipid ratio of 0.1. This value is about 13% greater than in the case of pure egg PC liposomes. The *cis* double bonds in unsaturated PE prevent close approach of adjacent molecules in the bilayers; thus, the presence of DOPE increases inherent instabilities of liposomes [New, 1990]. Therefore, induction of the defect and/or breakdown of the liposomal membrane could be easier. In all of three samples, the % releases at 24°C were less than 7%.

3. Preparations of DOPE Liposomes with HPNIPAM

For a high sensitivity of release, a novel 100% unsaturated phosphatidylethanolamine (DOPE) liposome coated with HPNIPAM was prepared. Fig. 4 shows quenching of liposome-entrapped calcein fluorescence with the varying ratio of HPNIPAM to lipid. When polymer was not included, the quenching was almost 0%, indicating no formation of liposomes. As the ratio increased to 0.1, the quenching increased up to 74% and thereafter reduced. The degree of quenching in fluorescence is an indication of liposomal formation [Ho et al., 1986]. Therefore, the optimal condition for liposomal formation was achieved at a ratio of 0.1.

4. Releases from DOPE Liposomes with HPNIPAM

Fig. 5 shows release behaviors of DOPE liposomes with polymer to lipid ratio of 0.1. Below the LCST (e.g., 29°C),

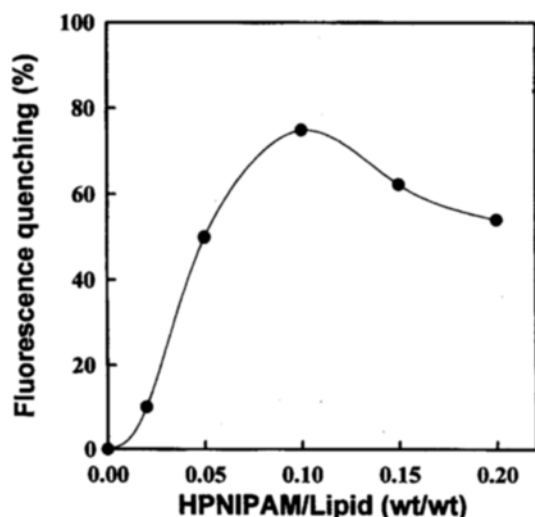


Fig. 4. Quenching of liposome-entrapped calcein with ratio of HPNIPAM to lipid.

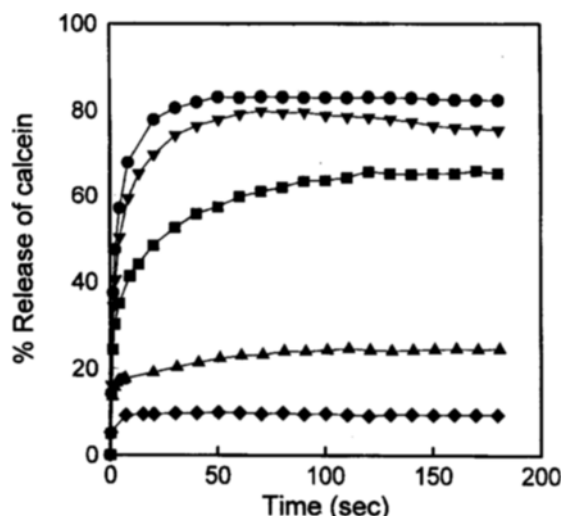


Fig. 5. Calcein release from DOPE liposomes with HPNIPAM to lipid ratio of 0.1 at 28.9 (◆), 32 (▲), 35.8 (■), 39 (▼) and 41 (●) °C.

the release was observed less than 10 % in 180 sec. Above the LCST, the liposome released an increased amount with temperature and reached approximately 80 % of a total entrapment at 39°C. Such an extensive release above the LCST may come from a thermal contraction of HPNIPAM and, in turn, the interaction of liposomes and HPNIPAM.

5. Comparison of Liposomes

Fig. 6 shows the degree of release in 80 sec from DOPE and egg PC liposomes with polymer to lipid ratio of 0.1. The turbidity change of HPNIPAM is also represented with dotted line. The turbidity of polymer solution started to increase at 29°C, and 50 % of the light was blocked around 32°C, a typical LCST behavior of PNIPAM [Lee and Kim, 1995]. Percent releases of egg PC and DOPE liposomes are plotted in squares and triangles, respectively. For egg PC liposomes, the degree of release above the LCST (e.g., around 39°C) was about 20 %, but below the LCST, it was about 5 %. In the whole tempera-

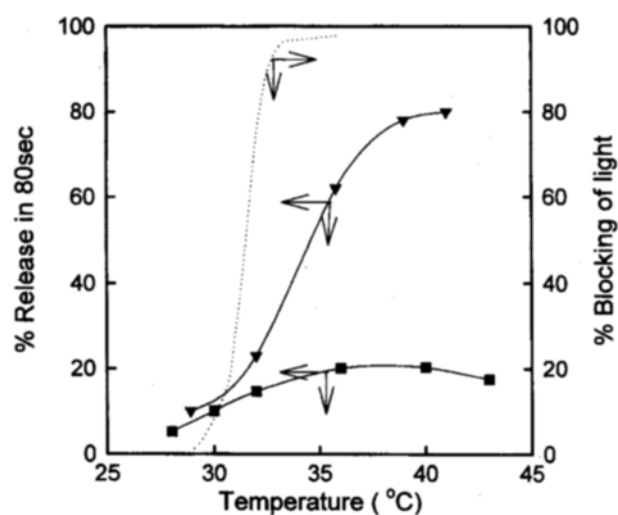


Fig. 6. The degree of release from egg PC (■) and DOPE (▼) liposomes. The weight ratio of HPNIPAM to lipid was 0.1. Dotted line represents change in turbidity of HPNIPAM in PBS (pH 8.0).

ture range tested, the release of egg PC was not so extensive as was that of DOPE. However, the DOPE liposome shows a significant release of calcein at the elevated temperature even if delayed up to several degrees.

6. Thermally Induced Change in Size

Fig. 7 shows the size distribution of egg PC and DOPE liposomes before and after the suspensions were exposed to 40 °C for 80 sec. The distributions of all samples were observed at 25 °C. For egg PC liposomes, no significant change in distribution and mean diameter was observed. The mean diameter was 291 nm and 301 nm, before and after. For DOPE liposomes, however, the mean size increased from 161 nm to 481

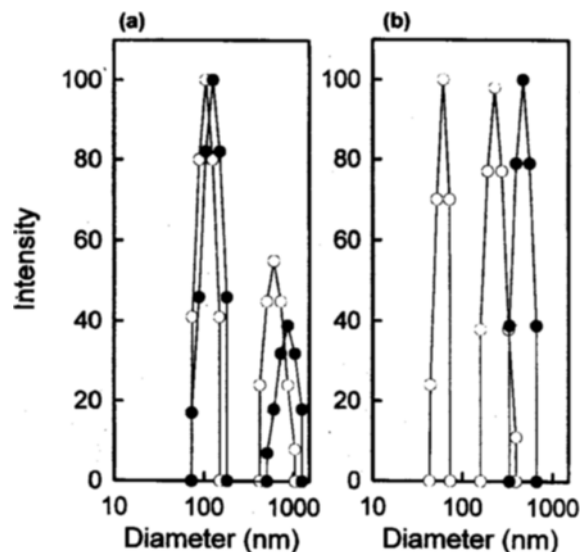


Fig. 7. Size distribution of egg PC (panel a) and DOPE (panel b) liposomes. The weight ratio of HPNIPAM to lipid was 0.1. Unfilled and filled circles in each panel represent the distributions before and after exposing the samples to 40 °C in 80 sec. All distributions were observed at 25 °C.

nm. In addition, as all DOPE liposomes underwent phase transition into the hexagonal phase upon disintegration, the size distribution of DOPE liposomes no longer showed unimodality. Following an extensive release in Fig. 6 and a marked size change in Fig. 7, we believe that DOPE liposomes stabilized with HPNIPAM may be disintegrated.

7. Thermally Induced Change in Structures

Fig. 8 shows the structures of egg PC and DOPE liposomes before and after the suspensions were exposed to 40°C for 80 sec. The distributions of all samples were observed at 25°C. For egg PC liposomes, no significant structural change was observed even after exposure to 40°C. For DOPE liposomes, however, a non-liposomal phase appeared after the heat treatment.

Much attention has been paid to target-sensitive DOPE immunoliposome constructed of membrane-bound ligands [Ho et al., 1986; Lee and Kim, 1995; Tarashi et al., 1982]. The ligands are capable of stabilizing a membrane of otherwise inherently unstable DOPE. When the ligand-bearing vesicle meets its target these ligands are aggregated by binding to a target, leaving a large free domain of the membrane exposed to de-

stabilization. Thus, by the receptor-mediated destabilization the liposomes disintegrate into hexagonal phase (H_H) with release of contents [New et al., 1990]. Temperature-sensitive DOPE liposomes stabilized with HPNIPAM could follow the same mechanism of release as the target-sensitive liposomes. HPNIPAM stabilize DOPE membrane below its LCST. When HPNIPAM undergoes a phase transition at its LCST, the polymers are aggregated by a thermal contraction, leaving a large polymer-free portion of membrane. In fact, HPNIPAM is contracted and aggregated freely in a fluid liposomal membrane as in the aqueous bulk phase [Ringsdorf et al., 1991]. Therefore, by thermally induced aggregation of the polymer liposomes destabilize into hexagonal phase (H_H). This mechanism could explain an extensive release from DOPE liposomes in Fig. 6, but is not clear yet. It is also assumed that a bundle of hexagonals is responsible for the increased size of DOPE liposomes in Fig. 7. On the other hand, since egg PC by itself does form a stable bilayer, egg PC liposomes maintain bilayer structure, even when PNIPAM is contracted [Ringsdorf et al., 1991]. Thus, unlike DOPE liposomes, no significant changes in size and structure were observed with egg PC liposomes (See Fig. 7 and Fig. 8) and the release was not as extensive as that of DOPE liposomes (See Fig. 6).

In summary, we attempted to increase temperature-sensitivity of liposomal bilayers containing HPNIPAM using egg PC REV's and DOPE liposomes. Egg PC REV's exhibited more extensive release than egg PC REV's, probably due to the lamellarity of the each liposome. The incorporation of dioleoylphosphatidylethanolamine (DOPE) of 30 wt% or 50 wt% into egg PC MLVs enhanced the release at 40°C by 10-13%. This may be attributed to the instability of the mixture bilayer. This result led to preparing 100% DOPE liposomes for a more temperature-sensitivity in release. The optimal ratio of HPNIPAM to lipid for stabilizing the DOPE into a stable bilayer was around 0.1. DOPE liposomes are more temperature-sensitive than are egg PC liposomes. This is probably because DOPE liposomes are disintegrated into non-liposomal phase, probably hexagonal phase (H_H), by a thermal contraction of PNIPAM. From the above results, it is concluded that DOPE liposomes of HPNIPAM are the most temperature-sensitive among the liposomes tested and that a microstructural arrangement is induced by thermal contraction of polymer.

ACKNOWLEDGMENTS

We thank Jeong-Hwan Park for the measurements of dynamic light scattering and Korea Science and Engineering Foundation for financial support.

NOMENCLATURE

Abbreviations

HPNIPAM : hydrophobically modified poly(*N*-isopropylacrylamide)
 PC : phosphatidylcholine
 DOPE : dioleoylphosphatidylethanolamine
 MLVs : multilamellar vesicles
 REV : reverse-phase evaporation vesicles

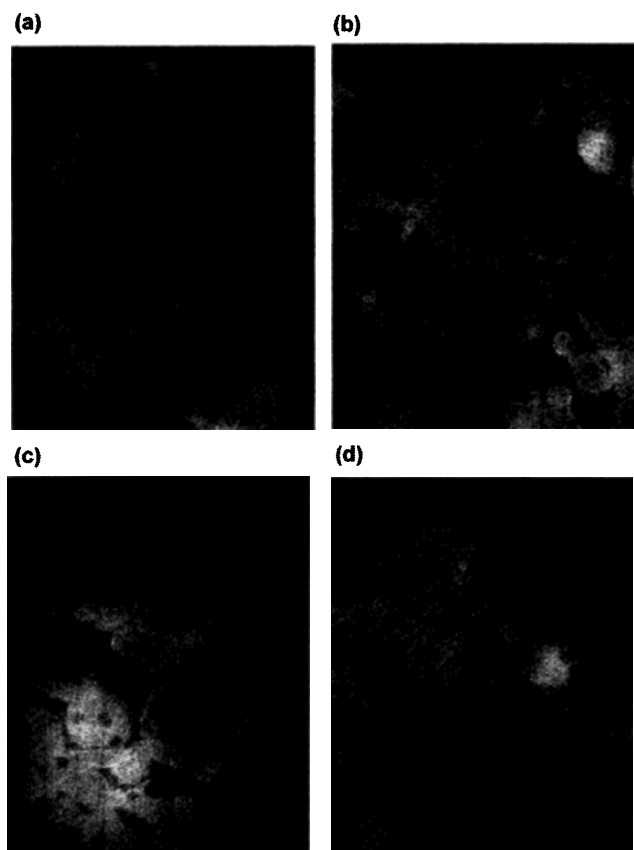


Fig. 8. Transmission electron microphotographs of negatively stained egg PC and DOPE liposomes. The weight ratio of HPNIPAM to lipid was 0.1. Photographs of A and B are egg PC liposomes before and after exposed to 40°C for 80 sec. Photographs of C and D are DOPE liposomes before and after being exposed to 40°C for 80 sec. The photographs were taken at 25°C, and magnification was 115,000 times.

REFERENCES

- Bae, Y.-H., Okano, T. and Kim, S.-W., "On-Off Thermocontrol of Solute Transport. II Solute Release from Thermosensitive Hydrogels," *Pharm. Res.*, **8**, 624 (1991).
- Bangham, A. D., "Liposomes: from Physical Structure to Therapeutic Applications," ed., Knight, C. G., Elsevier/North-Holland, 1 (1981).
- Chen, H., Torchilin, V. and Langer, R., "Lectin-bearing Polymerized Liposomes as Potential Oral Vaccine Carriers," *Pharmaceutical Research*, **13**, 1378 (1996).
- Choe, T. B., Ku, J. K., Jung, S. H., Yang, J. W. and Kim, J.-D., "The Selective Delivery of Anticancer Agents Using Target-Sensitive Liposomes," *Korean J. Chem. Eng.*, **13**, 393 (1996).
- Choi, M.-J., Han, H.-S. and Kim, H., "pH-Sensitive Liposomes Containing Polymerized Phosphatidylethanolamine and Fatty Acid," *J. Biochem.*, **112**, 694 (1992).
- Chun, S.-W. and Kim, J.-D., "A Novel Hydrogel-dispersed Composite Membrane of Poly(*N*-isopropylacrylamide) in a Gelatin Matrix and Its Thermally Actuated Permeation of 4-Acetamidophen," *J. Controlled Release*, **38**, 39 (1996).
- Crommelin, D. J. A., Grit, M., Talsma, H. and Zuidam, N. J., "Liposomes as Carriers for Drugs and Antigens: Approaches to Preserve Their Long Term Stability," *Drug Development and Industrial Pharmacy*, **20**, 547 (1994).
- Cudd, A., Noonan, C. A., Tossi, P. F., Melnick, J. L. and Nicolau, L., "Specific Interaction of CD4-Bearing Liposomes with HIV-infected Cells," *J. AIDS*, **3**, 109 (1990).
- Gao, X. and Huang, L., "Potentiation of Cationic Liposome-mediated Gene Delivery by Polycations," *Biochemistry*, **35**, 1027 (1996).
- Gruner, S. M., Cullis, P. R., Hope, M. J. and Tilcock, C. P. S., "Lipid Polymorphism: the Molecular Basis of Nonbilayer Phases," *Annu. Rev. Biophys. Chem.*, **14**, 211 (1985).
- Heskins, M. and Guillet, J. E., "Solution Properties of Poly(*N*-isopropyl-acrylamide)," *J. Macromol. Sci. Chem.*, **A2**, 1441 (1968).
- Ho, R. J. Y., Rouse, B. T. and Huang, L., "Target-Sensitive Immunoliposome: Preparation and Characterization," *Biochemistry*, **25**, 5500 (1986).
- Huang, A., Tsao, Y. S., Kennel, S. J. and Huang, L., "Characterization of Antibody Covalently Coupled to Liposomes," *Biochim. Biophys. Acta*, **716**, 140 (1983).
- Juliano and Stamp, D., "Lectin-Mediated Attachment of Glycoprotein-Bearing Liposomes to Cells," *Nature*, **261**, 235 (1972).
- Kim, J.-C., Bae, S. K. and Kim, J.-D., "Temperature-sensitivity of Liposomal Lipid Bilayers Mixed with Poly(*N*-isopropylacrylamide-co-acrylic Acid)," *J. Biochemistry*, **121**, 15 (1997).
- Kim, J.-C., Lee, E.-O., Kim, J.-Y., Bae, S.-K., Choi, T.-B. and Kim, J.-D., "Hemolytic and Antifungal Activity of Liposome-entrapped Amphotericin B Prepared by the Precipitation Method," *Pharm. Develop. Technol.*, **2**, 275 (1997).
- Kim, J.-G. and Kim, J.-D., "Turbidity and Stability of Sonicated Lecithin Liposomes," *HWAHAK KONGHAK*, **26**, 171 (1988).
- Kono, K., Hayashi, H. and Takagishi, T., "Temperature-Sensitive Liposomes: Liposomes Bearing Poly(*N*-isopropylacrylamide)," *J. Controlled Release*, **30**, 69 (1994).
- Lee, E. O. and Kim, J.-D., "Palmitoyl Lysozyme-induced Stabilization of PE(phosphatidylethanolamine) Liposomes and Their Interaction with *Candida albicans*," *J. Biochem.*, **117**, 54 (1995).
- Lee, E. O., Kim, J. G. and Kim, J.-D., "Induction of Vesicle-to-micelle Transition by Bile Salts for DOPE Vesicles Incorporating Immunoglobulin G," *J. Biochem.*, **112**, 671 (1992).
- Maeda, M., Kumano, A. and Tirrell, D. A., "H⁺ Induced Release of Contents of Phosphatidylcholine Vesicles Bearing Surface-Bound Polyelectrolyte Chains," *J. Am. Chem. Soc.*, **110**, 7455 (1988).
- New, R. R. C., Black, C. D. V., Parker, R. J., Puri, A. and Scherphof, G. L., "Liposomes: a Practical Approach," New, R. R. C. ed., Oxford University Press, New York, 221 (1990).
- New, R. R. C., "Liposomes: a Practical Approach," New, R. R. C. ed., 1-32 Oxford University Press, New York (1990).
- Park, J. H., Lee, E.-O. and Kim, J.-D., "Preparation and Characteristics of Phosphatidylcholine Liposomes Covalently Coupled with Immunoglobulin Fragments," *J. Kor. Pharm. Sci.*, **22**, 115 (1992).
- Perez-Solar, R. and Priebe, W., "Anthracycline Antibiotics with High Liposome Entrapment: Structural Features and Biological Activity," *Cancer Research*, **50**, 4260 (1990).
- Ringsdorf, H., Venzmer, J. and Winnik, F. M., "Fluorescence Studies of Hydrophobically Modified Poly(*N*-isopropylacrylamide)," *Macromolecules*, **24**, 1678 (1991).
- Szoka, F. and Papahadjopoulos, D., "Procedure for Preparation of Liposomes with Large Internal Aqueous Space and High Capture by Reverse-phase Evaporation," *Proc. Natl. Acad. Sci.*, **75**, 4194 (1978).
- Tarashi, T. F., Van Der Steen, T. M., DeKruiff, B., Tellier, C. and Verkleij, A. J., "Lecithin-receptor Interactions in Liposomes: Evidence that Binding of Wheat Germ Agglutinin to Glycoprotein Phosphatidylethanolamine Induced Nonbilayer Structure," *Biochemistry*, **21**, 5756 (1982).
- Yatvin, M. B., Weinstein, J. N., Dennis, W. H. and Blumenthal, R., "Design of Liposomes for Enhanced Local Release of Drugs by Hyperthermia," *Science*, **202**, 1290 (1978).