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Remarkable Long-term Stability of Cerasome as an Organic–Inorganic Hybrid Nanocontainer for Water-soluble Macromolecules

Takahiro Kawataki, Kazuma Yasuhara,* and Jun-ichi Kikuchi*
Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101

(Received February 7, 2011; CL-110104; E-mail: yasuhara@ms.naist.jp, jkikuchi@ms.naist.jp)

The encapsulation characteristics of cerasome, an organic–inorganic hybrid vesicle, were clarified using a macromolecular fluorescent marker. The cerasome successfully entrapped the marker molecule in its inner aqueous space over a prolonged period in stark contrast to unstable phospholipid liposomes. In addition, the cerasome maintained its encapsulation ability in the presence of serum containing membrane-destabilizing lipoproteins.

A variety of amphiphilic lipids form vesicles or liposomes, which are self-assembled capsules consisting of lipid bilayer in water.[1] Because of their unique hollow structure, many applications of liposomes have been explored such as drug delivery,[2] gene carriers,[3] and nanoreactors.[4] However, their practical uses are limited by their lack of long-term stability. Organic–inorganic hybrid composites appear to be a promising approach toward improving the friability of supramolecular assemblies.[5] We developed a cerasome, which is a synthetic lipid vesicle with a ceramic-like layer on the surface.[6–10] The remarkable stability of the cerasome enabled several applications, including model multicellular systems,[7] efficient gene transfection,[8] and metal-plated vesicles.[9] Encapsulation of various molecules in the cerasome is also important for realization of applications to drug delivery systems. We have previously reported that cerasomes can be used as a sophisticated, remarkably stable carrier for the hydrophobic drug paclitaxel.[10] Today, there is also a great need for encapsulation of water-soluble macromolecules such as DNA, peptides, and polysaccharides, because they are biologically important and promising drug candidates.[11] We report herein the unique characteristics of cerasomes for encapsulation of the water-soluble macromolecule FITC (fluorescein isothiocyanate)–dextran, which is a fluorescent dye-labeled polysaccharide. The encapsulation characteristics of cerasomes were compared to those of conventional phospholipid vesicles.

A cerasome-forming lipid, *N*,*N*-dihexadecyl-N^{α}-{6-[(3-triethoxysilylpropyl)dimethylammonio]hexanoyl}alaninamide bromide (**1**) (Chart 1) was synthesized in five steps according to a procedure described previously.[8] We chose a phospholipid vesicle formed with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) as a reference because of its analogous phase transition temperature, which is one of the essential characteristics of a lipid membrane.[1a,8b] Both cerasomes and DMPC vesicles were prepared by the Bangham method as follows.[12] Lipid films formed in a round-bottom flask were hydrated with HEPES buffer (pH 7.0, [NaCl] = 50 mM) containing FITC–dextran (MW: 4000 g mol^{-1}) at 40 °C to form multilamellar vesicles (MLVs). The MLVs obtained were subjected to freeze–thaw and subsequent extrusion to form large unilamellar vesicles (LUVs). To complete the formation of a siloxane layer on the vesicular surface, the suspension was incubated for 24 h at room temperature.[6] Unencapsulated FITC–dextran in the outer phase of LUV was removed by gel filtration chromatography. The hydrodynamic diameter and polydispersity index of the resultant LUVs were determined to be 160 nm and 0.085, respectively, by means of dynamic light scattering (DLS) (Figure S1[16]).

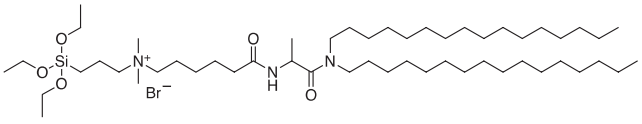

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Chart 1.

We first examined the long-term stability of the cerasome encapsulating FITC–dextran in the inner aqueous space in comparison with DMPC vesicles. Since FITC–dextran displayed self-quenching behavior at high concentration (Figure S2[16]), leakage of the marker from the inner phase of the vesicles was monitored as the recovery of the fluorescence due to dilution in the bulk phase (the procedure for the determination of entrapped fraction is described in detail in the Supporting Information[16]). Thus, concentrated FITC–dextran (3 mM) was entrapped in both the cerasomes and the DMPC vesicles for the stability test. After the complete disruption of vesicles by the addition of Triton X-100, the cerasomes exhibited ca. 1.5-fold higher fluorescence intensity than DMPC vesicles. This indicates that the cerasome has slightly higher encapsulation efficiency than DMPC liposomes under the experimental conditions. Figure 1 shows the time-dependent retention of entrapped FITC–dextran in the cerasome and the DMPC vesicle. It is noteworthy that cerasome successfully retained 90% of entrapped FITC–dextran over 100 days after preparation. In contrast, the entrapped marker spontaneously leaked from DMPC vesicles and eventually reached less than 10% retention in the first month. DLS measurement revealed that the cerasome maintained almost the initial size distribution over 140 days, whereas DMPC vesicles easily formed large aggregates (Figure S3[16]). At the time of vesicle preparation, the difference in osmotic pressure across the membrane was adjusted in advance to diminish the rupture of vesicles by osmolality imbalance. Thus, the observed leakage of FITC–dextran from DMPC vesicles seems to be originated in the decrease of membrane integrity due to the aggregation and following slow membrane fusion.[13] The lack of 24 h incubation of cerasomes resulted in two-fold increase in background fluorescence, reflecting the spontaneous leakage of entrapped FITC–dextran during the gel filtration (Figure S4[16]). We have previously reported that the progress of siloxane bond formation depends on the incubation time.[6a] The short-time incubation

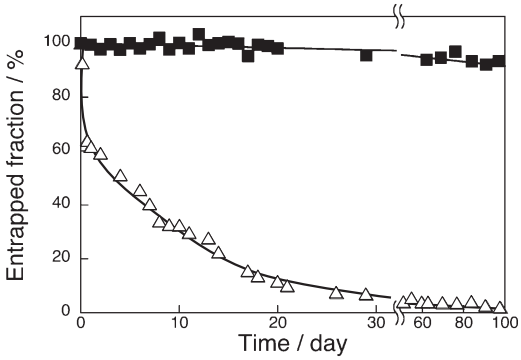

Figure 1. Time dependence of the entrapped fraction of FITC–dextran (MW: 4000 g mol^{-1}) in cerasome (filled squares) and DMPC vesicles (triangles). [lipid] = 33 μM in 10 mM HEPES buffer (pH 7.0, [NaCl] = 50 mM), at 25 °C. The estimation of the entrapped fraction is described in the Supporting Information.[16]

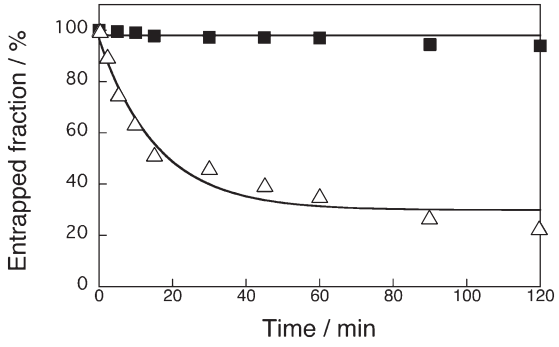

Figure 2. Serum-induced release of FITC–dextran (MW: 4000 g mol^{-1}) entrapped in cerasome (filled squares) and DMPC vesicles (triangles). An aliquot (10 μL) of vesicle suspension was added to FBS (990 μL) at 0 min. [lipid] = 33 μM, at 25 °C.

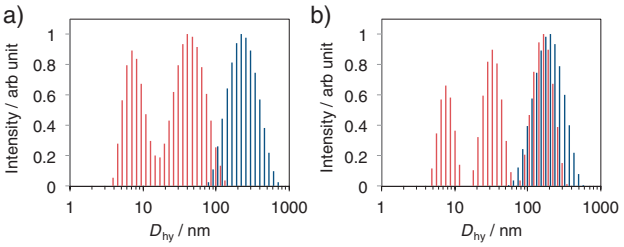

Figure 3. Size distribution of (a) DMPC vesicle and (b) cerasome in HEPES buffer (blue) and FBS (red) determined by DLS. [lipid] = 33 μM, at 25 °C. The serum shows two peaks at 9.5 and 53 nm in the absence of vesicles (see Supporting Information Figure S6[16]).

of the prepared cerasome for 15 min resulted in the formation of dimer and trimer species, whereas high-molecular weight species (tetramers and pentamers) were formed during prolonged incubation for 10 h. Thus, it was clarified that the degree of polymerization markedly affects the encapsulation ability of cerasomes. Our previous study showed that the cerasome displayed similar long-term stability for the encapsulation of a hydrophobic drug, in contrast to the unstable phospholipid vesicles.[10] Therefore, it was demonstrated that the introduction of an organic–inorganic framework to a vesicle is an effective approach to achieving long-term stability for entrapment of not only hydrophobic drugs but also hydrophilic macromolecules.

Generally, the stability of drug carriers in a physiological environment is a key property toward their in vivo use. However, it is well known that conventional phospholipid liposomes lose their membrane integrity in the presence of serum, resulting in leakage of entrapped molecules.[14] Thus, we next evaluated the stability of FITC–dextran-entrapping cerasome in fetal bovine serum (FBS). Figure 2 shows the serum-induced leakage of entrapped FITC–dextran from the DMPC vesicle and the cerasome. The addition of FBS immediately induced leakage of FITC–dextran from DMPC vesicle (Figure S5[16]), and the fraction leaked reached 77% in 2 h, reflecting destabilization of the lipid membrane by serum components. On the other hand, serum-induced leakage was significantly diminished for the cerasome. Specifically, the fraction leaked reached only 6% after 2 h incubation in the presence of FBS. To elucidate the mechanism of serum-induced leakage of FITC–dextran, we evaluated the effect of serum on the size distribution of vesicles using DLS as shown in Figure 3. In the case of DMPC vesicles, the initial peak at about 200 nm disappeared upon addition of FBS. This is consistent with a previous report that the vesicular lipids are removed to form a complex with lipoproteins in serum.[14] In contrast, the cerasome maintained the initial size distribution despite the addition of FBS. Hence, it was confirmed that a crosslinked siloxane network on the surface of the cerasome contributes to achieving resistance against membrane solubilization by lipoproteins, resulting in their significant stability in serum. Although the cerasomes displayed excellent stability during long-term storage as well as toward solubilization by the membrane-disrupting component in serum, the complete leakage of the entrapped FITC–dextran was observed by irradiation of weak ultrasound, which is widely used in medical applications.[15] Thus, it was confirmed that the cerasomes achieved robustness originating in an organic–inorganic hybrid composite and stimuli-responsive release of entrapped molecule simultaneously.

In conclusion, we demonstrated that the cerasome, a synthetic lipid vesicle with organic–inorganic hybrid structure, maintained its encapsulation ability for a significantly long time in contrast to a fragile conventional phospholipid vesicle. The cerasome also exhibited remarkable stability in serum solution, which contains various membrane-destabilizing components. These unique properties are expected to enable applications of cerasome as an excellent vehicle for in vivo delivery of various water-soluble bioactive macromolecules.

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References and Notes

1 a) *Liposomes: A Practical Approach*, ed. by V. P. Torchilin, V. Weissig, Oxford University Press, New York, **2003**. b) T. Kunitake, in *Comprehensive Supramolecular Chemistry*, ed. by J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, J.-M. Lehn, J.-P. Sauvage, M. W. Hosseini, Pergamon, Oxford, **1996**, Vol. 9, p. 351.

2 a) Y. Barenholz, *Curr. Opin. Colloid Interface Sci.* **2001**, *6*, 66. b) D. Stepensky, *Pharm. Res.* **2010**, *27*, 2776. c) T. Lian, R. J. Y. Ho, *J. Pharm. Sci.* **2001**, *90*, 667.

3 a) *Self-assembling Complexes for Gene Delivery: From Laboratory to Clinical Trial*, ed. by A. V. Kabanov, P. L. Felgner, L. W. Seymour, Wiley, Chichester, **1998**. b) P. L. Felgner, T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold, M. Danielsen, *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7413. c) T. Nagasaki, K. Wada, S. Tamagaki, *Chem. Lett.* **2003**, *32*, 88.

4 a) P.-A. Monnard, *J. Membr. Biol.* **2003**, *191*, 87. b) P. Walde, S. Ichikawa, *Biomol. Eng.* **2001**, *18*, 143.

5 a) A. Shimojima, K. Kuroda, *Chem. Rec.* **2006**, *6*, 53. b) S. Inagaki, S. Guan, Y. Fukushima, T. Ohsuna, O. Terasaki, *J. Am. Chem. Soc.* **1999**, *121*, 9611. c) J. H. Jung, S. Shinkai, T. Shimizu, *Chem. Rec.* **2003**, *3*, 212. d) T. Ogoshi, Y. Chujo, *Compos. Interfaces* **2005**, *11*, 539.

6 a) K. Katagiri, M. Hashizume, K. Ariga, T. Terashima, J. Kikuchi, *Chem.—Eur. J.* **2007**, *13*, 5272. b) K. Katagiri, K. Ariga, J. Kikuchi, *Chem. Lett.* **1999**, 661.

7 a) K. Katagiri, R. Hamasaki, K. Ariga, J. Kikuchi, *J. Am. Chem. Soc.* **2002**, *124*, 7892. b) K. Katagiri, R. Hamasaki, K. Ariga, J. Kikuchi, *Langmuir* **2002**, *18*, 6709.

8 a) K. Matsui, S. Sando, T. Sera, Y. Aoyama, Y. Sasaki, T. Komatsu, T. Terashima, J. Kikuchi, *J. Am. Chem. Soc.* **2006**, *128*, 3114. b) Y. Sasaki, K. Matsui, Y. Aoyama, J. Kikuchi, *Nat. Protoc.* **2006**, *1*, 1227.

9 D. Minamida, S. Okada, M. Hashizume, Y. Sasaki, J. Kikuchi, N. Hosoito, T. Imori, *J. Sol-Gel Sci. Technol.* **2008**, *48*, 95.

10 Z. Cao, Y. Ma, X. Yue, S. Li, Z. Dai, J. Kikuchi, *Chem. Commun.* **2010**, *46*, 5265.

11 a) S. D. Patil, D. G. Rhodes, D. J. Burgess, *AAPS J.* **2005**, *7*, E61. b) V. P. Torchilin, A. N. Lukyanov, *Drug Discovery Today* **2003**, *8*, 259. c) T. Ghosh, K. Chattopadhyay, M. Marschall, P. Karmakar, P. Mandal, B. Ray, *Glycobiology* **2009**, *19*, 2.

12 A. D. Bangham, R. W. Horne, *J. Mol. Biol.* **1964**, *8*, 660.

13 a) G. Cevc, H. Richardsen, *Adv. Drug Delivery Rev.* **1999**, *38*, 207. b) J. H. Prestegard, B. Fellmeth, *Biochemistry* **1974**, *13*, 1122.

14 a) T. Ishida, H. Harashima, H. Kiwada, *Biosci. Rep.* **2002**, *22*, 197. b) C. Kirby, J. Clarke, G. Gregoriadis, *FEBS Lett.* **1980**, *111*, 324.

15 A. Schroeder, J. Kost, Y. Barenholz, *Chem. Phys. Lipids* **2009**, *162*, 1.

16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

