Preparation of Organic-Inorganic Hybrid Vesicle "Cerasome" Derived from Artificial Lipid with Alkoxysilyl Head

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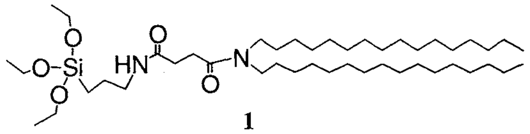
(Received April 14, 1999; CL-990295)

An artificial lipid bearing a triethoxysilyl head and a dialkyl tail has been synthesized in order to prepare a novel type of organic-inorganic hybrid, "Cerasome", which can form siloxane network on its vesicular surface. The Cerasomes were obtained upon vortex mixing of aqueous dispersion of the lipid followed by acid hydrolysis of the head moiety.

Lipid bilayer vesicles so-called liposomes have been widely employed as supramolecular assemblies to develop drug or gene delivery systems,[1,2] artificial enzymes,[3] and molecular devices.[4] Recently we have demonstrated that artificial signal transduction system as a supramolecular device can be constituted in combination with a synthetic liposome, an artificial receptor, and an enzyme.[5] A peptide lipid having a trimethylammonium head, a double-chain segment, and an amino acid residue interposed between them was employed as the lipid in the system. Since various ingenious functions observed in the living things come from the multi-cellular organisms rather than the unicellular states, it is of great interest to create superstructures of liposomes from a viewpoint of further development in supramolecular chemistry including our approach on the artificial signal transduction system. Although there are a few reports on self-organization of liposomal particles up to the present time,[6] increase in the morphological stability of liposomes seems to be more desirable for such purpose. On these grounds we are to report here on a synthetic lipid as a novel type of organic-inorganic hybrid which can form univesicular and multi-vesicular aggregates by using the sol-gel techniques.[7]

The sol-gel method is one of the most promising techniques to give organic-inorganic hybrids, because it is low-temperature solution process to prepare ceramics.[8-10] Organically modified alkoxysilane is usually employed in the sol-gel process. Accordingly, we designed a lipid with trialkoxysilyl group as a polar head. Hydrolysis of the alkoxysilyl moiety affords the corresponding amphiphilic lipid enough to form bilayer vesicles in aqueous media. In addition siloxane networks would be formed by intra-vesicular condensation among the silanol groups on the relatively hydrophobic membrane surface. The organic-inorganic hybridization probably enhances mechanical strength of the aggregates and may exhibit novel functions, which have not been observed for the conventional organic vesicles. We named this type of liposomal membrane having metal oxide surface seen in ceramics "Cerasome". In addition, under the conditions that the membrane surface is partly dehydrated by aggregation of the vesicles inter-vesicular condensation between the silanol groups would proceed to afford the multi-vesicular aggregates.

A Cerasome forming lipid, [3-(((*N*,*N*-dihexadecylamino)-succinyl)amino)propyl]triethoxysilane (**1**),[11] was synthesized by using dihexadecylamine, succinic anhydride, and 3-aminopropyltriethoxysilane in a manner similar to that reported for analogous lipid bearing different double-chain length.[12] The Cerasomes were prepared by mixing of aqueous dispersion of **1** by using a vortex mixer (VORTEX-GENE 2, Scientific Industries). In the sol-gel process, a mutual solvent such as alcohol is usually added as a homogenizing agent.[13] In the present case, however, a mutual solvent was not used to avoid possibility of vesicle destruction. When pure water at pH 7 was used as the solvent, hydrolysis of the head moiety of **1** was extremely slow at room temperature and stable dispersion sample was not obtained. It is well-known that the hydrolysis of alkoxysilyl moiety is strongly pH-dependent. Thus aqueous HCl was employed as the solvent. Upon mixing of 10 mmol of **1** with 100 mmol dm^{-3} of aqueous HCl (5.0 ml), precipitation occurred immediately. In this case, it seems that the hydrolysis and followed condensation reactions are so fast as to be unable to maintain the vesicular structure. On the other hand, the hydrolysis of the lipid head moiety proceeded gently in 1 mmol dm^{-3} of aqueous HCl and stable dispersion was obtained after the vortex mixing for 15 min at room temperature. This turbid sample was morphologically stable at least for several days. The hydrolysis process of **1** was also monitored by ^{1}H-NMR in acidic D_2O: a broad proton signal of $SiOCH_2CH_3$ (δ = 3.65 ppm) was gradually decreased to replace to a sharp quartet signal of $HOCH_2CH_3$ (δ = 3.50 ppm). Upon sonication of the dispersion with a probe-type sonicator (SONIFIER 250D, BRANSON) at a 30-W power for 10 min, the turbidity was decreased to give a transparent solution reflecting formation of smaller aggregates.



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The structure of the Cerasome was observed by means of transmission electron microscopy (TEM) using a JEOL JEM-100SX electron microscope. An aqueous dispersion of the Cerasome (1 mmol dm^{-3}) was mixed with an equal volume of 1 wt% hexaammonium heptamolybdate tetrahydrate on a colldionized carbon-deposited Cu grid (200 mesh, NISSHIN EM). After the sample was dried *in vacuo* for 3 h, TEM observation was carried out. Negatively stained electron micrographs of the Cerasomes prepared by dispersion of **1** in 1 mmol dm^{-3} aqueous HCl without sonication was shown in Figure 1. Formation of multi lamellar vesicle (MLV) with the lipid bilayer thickness of ca. 4 nm and the vesicular diameter of 200 nm was clearly confirmed [Figure 1a]. On the other hand, TEM image of the porous vesicular aggregates with the vesicular diameter of ca. 200 nm was observed in the different specimen of the same dispersion sample [Figure 1b]. Although disagreement between these images presumably comes from a slight conditional difference in the drying process on the Cu grids, the porous vesicular aggregates implies the formation of

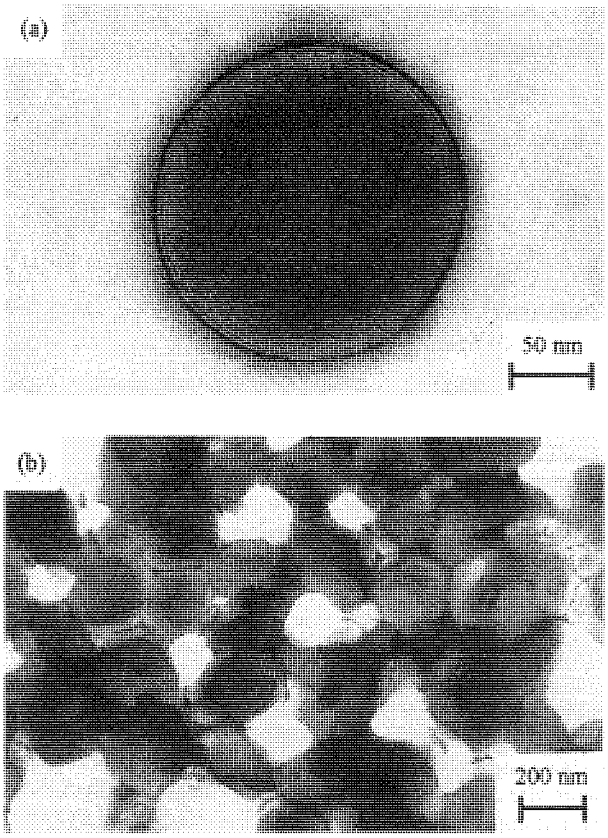

Figure 1. TEM images of the Cerasomes for **1** dispersed in 1 mmol dm^{-3} aqueous HCl. The images shown in (a) and (b) were observed in the different specimens.

the intra- and inter-membrane siloxane network. The formation of the siloxane bonds was confirmed by FT-IR measurements using a Nicolet Magna-IR E.S.P. System 560 spectrometer. The peaks assigned to Si-O-Si and Si-OH groups[14] were observed at around 1100 cm^{-1} and 950 cm^{-1}, respectively. In aqueous dispersion state of the Cerasome, the former peak was much weaker than the latter. However, these peak intensities were reversed upon drying of the aqueous sample.

Phase transition parameters (enthalpy change from gel to liquid-crystalline state, ΔH; temperature at peak maximum, T_m) for the Cerasome were measured by differential scanning calorimetry (DSC) using a Seiko-Instruments DSC-6100 calorimeter. The concentration of **1** for DSC measurements was fixed to 2 mmol dm^{-3}. The heating rate was 1.0 °C min^{-1}. ΔH and T_m for MLV of the Cerasome were 47.5 kJ mol^{-1} and 10.5 °C respectively. Upon sonication of the MLV with a probe-type sonicator for 10 min at 30 W, the ΔH value was decreased to 11.5 kJ mol^{-1}, whereas the T_m value was not shifted. We have previously clarified that transformation of MLV to the corresponding small unilamellar vesicle (SUV) is reflected in the change of DSC thermogram as decreases in both ΔH and T_m.[15] In addition, ΔH is more sensitive than T_m for such morphological changes and usual bilayer forming lipids readily transform from MLVs to SUVs under the above sonication conditions. Thus MLV of the Cerasome is hard to transform into SUV rather than analogous lipids lacking a reactive polar head. It seems that formation of the siloxane network on the vesicular surface prevents such morphological transformations.

In conclusion, we demonstrated here a novel type of bilayer vesicle, Cerasome, which can form siloxane network on its membrane surface. This organic-inorganic hybrid may perform as versatile aggregate such as multi-cellular model and metal oxide nano particles. For the present lipid, however, siloxane network can not be fully developed keeping the stable bilayer structure because Si-O-Si bond is much shorter than the diameter of the cross-section of dialkyl tail. Quantitative evaluation of the siloxane network of the Cerasome and its modification and functionalization are now in progress in our laboratory.

The authors gratefully acknowledged to Prof. Junzo Sunamoto and Mr. Shin-ichi Kawanami (Graduate School of Engineering, Kyoto University) for their help in TEM observation.

References and Notes

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11 A colorless oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.62 (t, J = 8.4 Hz, 2H, $SiCH_2$), 0.88 (t, J = 6.6 Hz, 2H, $CH_2CH_2CH_3$), 1.26 (m, 61H, CH_2, OCH_2CH_3), 1.60 (br, 6H, NCH_2CH_2), 2.51 (t, J = 6.6 Hz, 2H, $NCOCH_2$), 2.64 (t, J = 6.6 Hz, 2H, $NCOCH_2$), 3.24 (m, 6H, NCH_2), 3.80 (q, J = 7.3 Hz, 6H, $SiOCH_2$), 6.30 (br, 1H, NHCO). TLC: R_f 0.30 (Silicagel 70 FM Plate-*Wako*, Ethyl acetate-$CHCl_3$ (1:9 v/v)). Anal. Found: C, 70.31; H, 12.05; N, 3.65%. Calcd for $C_{45}H_{92}N_2O_5Si$: C, 70.26; H, 12.05; N, 3.64%.

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