Dispersion of Vesicles Composed of Industrially Produced Alkyl (Oligo) Glucoside Using Diol-Boron Complexation

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Abstract: Alkyl (oligo)glucosides (AOG) are known to be environmentally compatible amphiphiles whose commercial applicability should be broadened. The present paper describes the preparation of molecular assemblies of industrially produced AOG, which is a mixture composed of different length of alkyl chains (C9-C12) with oligoglucoside moiety with a few (1–3) of glucose units. It was also described that regulation of the dispersibility of the molecular assemblies prepared by diol-boron complexation between the sugar moiety on AOG and boric acid in a dispersion medium. The molecular assembly of AOG was successfully formed by mixing AOG and cholesterols (CH). When using a suitable amount of CH (20–40 mol% with respect to AOG), the molecular assembly formed a vesicle structure. The dispersion ability of the resulting vesicle was dependent on both the boric acid concentration and pH of the dispersion medium. The light-scattering and ζ-potential measurements revealed that high concentrations (≥10 mM) of boric acid improved dispersibility the vesicles. In contrast, the vesicle agglomerated at low concentrations of boric acid (1–7.5 mM). In the absence of boric acid in dispersion medium, the vesicles were completely agglomerated. The optimum pH range for vesicle dispersion was found to be from neutral to basic (7.4–10.1). The ¹¹B NMR study revealed that borate ester formation occurred between boric acid and the diol of the sugar moiety on AOG vesicle. The present data suggest that borate ester formation that occurred on the surface of the vesicle provided negative charge to the vesicles, contributing to their dispersion via repulsive forces.

Key words: alkyl glucoside, diol-borate interaction, dispersion stability, molecular self-assembly, vesicle

1 INTRODUCTION

Alkyl (oligo)glucosides (AOG) are amphiphilic molecules composed of hydrophilic several (or single) glucose units, which are linked each other through α(1→4)-glycosidic linkage, and hydrophobic alkyl chain, which is attached to C-1 position of the terminal glucose through ether bond. They have been often used in consumer products for daily use (e.g., dish washing detergent, cosmetics, and agrochemicals)¹, ². In such daily usage, detergents are discharged to the public sewer, and their environmental compatibility must be considered. It is known that AOG can rapidly degrade under sewage disposal conditions and, hence fulfill the criteria for “readily biodegradable” substances³. Thus, owing to their environmental compatibility, AOG are suitable as a detergent for daily as well as industrial use. The manufacturing process for AOG is also in harmony with the environment since they can be produced from renewable resources such as starch and vegetable oils⁴–⁸. Commercially available AOG are synthesized by alkylation of naturally derived sugars with fatty alcohols¹–³.

In order to broaden the applicability of AOG to other than daily household use products, we attempted to develop a molecular self-assembly process for AOG. Molecular self-assembly has remarkable applicability to materials manufacturing processes because we would then have the capability of encapsulating a wide variety of molecules and stably disperse these encapsulated molecules into various media. Due to the rapid biodegradability of AOG, molecular self-assembly of AOG is expected to result in a new class of molecular container with a low environmental load. We also focused on the dispersion stability of molecular self-assemblies in aqueous media. We utilized the diol-boron interac-
tion to generate sufficient dispersion stability. This interaction is the reversible ester formation between diol and borate with high affinity, which can occur under mild aqueous conditions (i.e., in water, neutral pH, room temperature). To date, the diol-boron complexation has been widely used in sensing or diagnosis of saccharides, nucleotides, and for separation of carbohydrates and glycoproteins. There are only a small number of examples utilizing the diol-boron complexation to stabilize a dispersion system, however. Shinkai and co-workers have demonstrated some valuable examples. They showed that organo-polymer gels consisting of vesicles are held together by polymer molecules prepared by combinations of boronic acid-appended polymers and sugar-based gelators. It has been also demonstrated that boronic acid-appended amphiphiles form aggregates. The molecular order of the aggregates was regulated by addition of saccharides, which occurred at the surface of the aggregates. Carbon nanotubes coated with polysaccharides can be dispersed and the orientation of the nanotubes controlled in the presence of boronic acid derivatives. These examples justify the use of diol-boron complexation to control the dispersion state of our materials.

In the present study, we used an industrially produced AOG as the principal component for molecular self-assembly. AOG used in the present study contains alkyl chain different number of hydrocarbons (C9–C12) with glucose moiety with a few (1–3) glucose units, which were produced during the manufacturing process. Showing applicability of such industrially produced AOG is important in terms of future practical use. AOG is very water-soluble. Since excessive water solubility of an amphiphile limits the occurrence of molecular self-assembly, we utilized cholesterol (CH) as a second component, which supports the occurrence of molecular self-assembly of amphiphiles. Thus, the effect of CH on the molecular self-assembly of AOG was examined first. Second, we investigated the influence of boric acid concentration and pH of the dispersion medium on the dispersion stability of the molecular self-assembled AOG. Furthermore, to investigate whether the diol-borate complexation was related to the dispersion stability of the AOG molecular self-assembly, a 11B NMR spectroscopy study was conducted.

2 MATERIALS AND METHODS

2.1 Materials

AOG was kindly gifted from Kao Corporation (Tokyo, Japan). The exact composition of the AOG was determined by an electrospray ionization-mass spectrometry (ESI-MS) prior to use. It was revealed that AOG composed of mono-, di- and triglucosides with different lengths of the alkyl chain (C9–12). Distribution of m/z of AOG measured by ESI-MS was shown in Fig. S1. AOG used in this study mainly contained decyl monoglucoside, undecyl monoglucoside, nonyl monoglucoside, decyl diglucoside, and undecyl diglucoside. The detailed component of AOG was summarized in Table S1. Cholesterol (CH) and boric acid were purchased from Tokyo Chemical Industry. In confocal laser fluorescence microscopy (CLSM), a cationic porphyrin derivative or 5(6)-carboxyfluorescein (CF, Sigma) was used as a hydrophilic dye to stain an aqueous region. CF is an anionic fluorescence dye, which is a conventionally used to assess dye-retention ability of vesicles. On the other hand, the porphyrin was 5,10,15,20-tetra(N-methylpyridinum)porphyrins, which was previously synthesized in our laboratory because of its applicability in a medical field (i.e., photodynamic diagnosis). 1,6-Diphenylhexatriene (DPH) was also used as a hydrophobic dye to stain lipid region in CLSM.

2.2 Preparation of molecular assemblies of AOG

A conventional sonication method was used to form molecular assemblies of single unilamellar vesicles (SUV). AOG and CH were added to a round-bottom 10 mL glass vial and dissolved in chloroform. Then, the chloroform solution was slowly evaporated on a rotary evaporator under reduced pressure to form a uniformly thin lipid film on the bottom of the vial. The dried lipid film was completely hydrated with 5 mL of aqueous boric acid solution (0–100 mM) for 0.5 h. In the present study, the lipid concentration remained constant at 20 mM. If the pH of the boric acid solution needed changing, sodium hydroxide solution was added and then used as hydration of the lipid film. The hydrated lipid film was vigorously stirred with a probe-type sonicator (30 W) for 0.5 h in a water bath. Various examinations of the molecular assembly were carried out after one hour-incubation at room temperature.

2.3 Size distribution measurement

To determine the size distribution of the molecular assembly, dynamic light scattering (DLS) measurements were performed using a Nicomp 380 (Particle Sizing Systems, FL, USA) equipped with a helium–neon (He–Ne) laser (5 mW) operating at a wavelength of 632.8 nm. The scattered light was collected at a 90° angle at room temperature. The measurement was conducted without stirring the sample dispersion.

2.4 ζ-potential measurement

The ζ-potential of the molecular assembly was determined using a Nicomp ZLS380 (Particle Sizing Systems, FL, USA).

2.5 Confocal laser scanning microscopy

To confirm lipid layers, a typical characteristics of vesicles, confocal laser scanning microscopy (CLSM) was
carried out using a FLUOVIEW Fv10i (Olympus, Tokyo, Japan). In this microscopy, hydrophobic and hydrophilic fluorescent dyes, described in Section 2.1, were used to stain lipid layers of molecular assembly and aqueous interspace between the lipid layers, respectively. Such multi-staining technique using CLSM is useful to observe lipid layers and the interspace simultaneously in an aqueous medium, which is an inherent condition of an aqueous dispersion containing vesicles. The preparation procedure of an experimental sample for the CLSM was the same as that described in Section 2.2 except for method of stirring hydrated lipid film. That is, hydrated lipid film was stirred with a vortex mixer instead of a probe-type sonicator to form the multi-lamellar vesicles (MLV).

2.6 FF-TEM observation

Aqueous dispersions of the molecular assembly were frozen rapidly in liquid nitrogen and fractured using a cold knife (FR-7000A, Hitachi High-Technologies, Tokyo, Japan). The molecular assembly replica was prepared by first exposing the cross-section of the frozen dispersion to platinum vapor, then the cross section was treated with carbon vapor to build the replica. Molecular assemblies on the replica were washed out with methanol and distilled water, after which the replica was transferred onto a copper grid. The molecular assembly replica was visualized using a transmission electron microscope (H-7650, Hitachi High-Technologies) with 120 kV of acceleration voltage at −160°C.

2.7 ¹¹B NMR

Sample for ¹¹B NMR measurement was MLV, which was prepared by the same method with sample for the CLSM. It should be noted that there was no signal in ¹¹B NMR when preparing samples by the sonication method. The reason for this defect is probably contamination of trace amount of paramagnetic titanium derived from a sonicator tip. If the pH of the sample needed changing, an aqueous solution containing sodium hydroxide or hydrogen chloride was added to boric acid solution prior to dissolving AOG. The samples were added to a PTFE sample tube and measurements were performed at various pH values at 25.0°C. The spectra were obtained using a JNM-ECP500 (JEOL, Tokyo, Japan). Chemical shifts in ppm were referenced with respect to an external BF₃·(Et₂O) standard at 0.0 ppm. To acquire proper spectrum in the present NMR, a number of times accumulation needed (at least ≥ 10,000).

3 RESULTS AND DISCUSSION

3.1 Effect of CH content on vesicle formation composed of AOG

In order to form vesicles from AOG, which is applicable for use as a molecular container, we adopted a published technique[21-23]. This method is quite simple and effective for the formation of vesicles using water-soluble amphiphiles, i.e., for mixing CH with the amphiphiles. First, we investigated the effect of CH content on the formation of vesicle-structured molecular assemblies in terms of the size of the molecular assembly (Fig. 1a). The size of the assembly changed depending on the CH content. Without CH, the mean diameter of the assembly was relatively small (12 nm), and the appearance of the solution was clear and colorless (Fig. 1b). This result indicated that AOG existed in the form of micelles. When the CH content ranged from 10 to 40 mol% with respect to AOG, the mean diameter of the assembly ranged from 80 nm to 200 nm (Fig. 1a), and the appearance of the dispersion was translucently opalescent (Fig. 1b). This is a typical phenomenon for the formation of vesicles. In addition, CLSM images showed that when the CH content was 30 mol%, the molecular self-assembly of AOG formed vesicle structures (Fig. 2). The CLSM image showed that AOG vesicles could encapsulate hydrophilic dyes in the inner water phase without leakage of the dyes (Fig. 2a). Since the present vesicles in the CLSM were in the form of multi-lamellar vesicle, hydrophilic dyes existed in aqueous interspaces between hydrophobic lipid layers (Fig. 2a). In addition, hydrophilic dyes were observed at the outer surface of the vesicle, indicating that adsorption of the dyes on the lipid layer (Fig. 2c). Due to the cationic nature, the hydrophilic dyes should be electrostatically adsorbed on anionic surface of the lipid layer (see also result of ζ-potential measurement, Fig. 4c). When using a typical hydrophilic dye, 5(6)-carboxyfluorescein.
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(Fig. S2), the dyes did not adsorbed on outer surface of the vesicle(Fig. S2). This should be due to anionic nature of CF. These results indicate that electrostatic interaction between dyes and the vesicle surface plays a key role in adsorption of the dyes on the vesicle surface. The CLSM image also visualized thin-lipid layers stained with hydrophobic dye (Fig. S2). Hydrophilic and hydrophobic regions were alternately visualized in the CLSM images (Fig. S2), suggesting that the resulting molecular assemblies had typical characteristics of multi-lamellar vesicles. Although there may be the adsorbed hydrophilic dyes on the outer surface, the present molecular assemblies could encapsulate hydrophilic dyes in the water phase, which is one of important abilities of vesicles as molecular container.

Manosroi et al. reported that addition of a suitable amount of CH allows water-soluble nonionic amphiphiles (e.g., Tween-61, diglyceryl monolaurate, etc.) to form vesicles. In Manosroi’s report, CH stabilized the molecular geometry of the amphiphiles, and their hydrophobicity was suitable for vesicle formation. In the present study, we assumed that the addition of CH had the same effect on vesicle formation. At a low CH content (10 mol%), however, a bimodal size distribution was observed, indicating that micelles coexisted with vesicles. At a high CH content (50 mol%), size distribution was multimodal, indicating that the amount of CH exceeded the capacity of the vesicles to encapsulate CH. Given the above results, an optimized CH content was determined to be 30 mol% for our study.

### 3.2 Effect of boric acid on the dispersion state of AOG vesicles

The effect of boric acid concentration on the dispersion state of AOG vesicles was investigated at constant pH. The observation of vesicles by means of FF-TEM clearly revealed the effect of boric acid on the dispersion state of the vesicles. When using aqueous boric acid solution as the dispersion medium, the primary particles of the AOG vesicles maintained a dispersed state without agglomeration (Fig. 3a). On the other hand, in the absence of boric acid, the primary particles of the AOG vesicles agglomerated (Fig. 3b). These results proved that boric acid was useful for improving dispersibility AOG vesicles in aqueous media.

Size distributions and photographs of the AOG vesicles in aqueous solution with various boric acid concentrations are shown in Fig. 4. In the absence of boric acid in the dispersion medium, the primary particles of the vesicles completely agglomerated and the agglomerates were recognized at a visible level (Fig. 4a, 0 mM). Whereas, at high boric acid concentrations (10–100 mM), the size distribution of the vesicles was monomodal, showing a mean diameter of around 150 nm (Fig. 4a, 10–100 mM), which is identical to the size of the primary particle observed by FF-TEM (Fig. 3a). The appearance of the dispersion was translucently opalescent (Fig. 4b). At moderate boric acid concentrations (1–7.5 mM), although the size distribution was broader compared to those at the higher boric acid concentrations (10–100 mM) (Fig. 4a), it was difficult to recognize the agglomerated particles at a visible level and...
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The AOG vesicles, we compared the amount of agglomerates, which were obtained by centrifugation of dispersion (Fig. S3). The amount of agglomerates tended to decrease with an increase in boric acid concentration. However, there was no significant difference among the amount of agglomerates at moderate boric acid concentrations (1–7.5 mM). In addition, the amount of agglomerates from samples prepared without boric acid was similar to those prepared at moderate boric acid concentrations. On the other hand, there was a small amount of agglomerates at 100 mM of boric acid. These results indicate that dispersibility of the AOG vesicles at moderate boric acid concentrations should be remarkably reduced compared to those at the high boric acid concentrations (10–100 mM).

This study also showed that the presence of boric acid influenced the \( \zeta \)-potential of the vesicles. Values for the \( \zeta \)-potential tended to increase in the negative direction with an increase in the concentration of boric acid (Fig. 4c). The vesicle dispersion state being related to \( \zeta \)-potential and boric acid concentration was depicted in Fig. 4c. When the boric acid concentration ranged from 1 mM to 7.5 mM, values of the \( \zeta \)-potential ranged from \(-5 \) mV to \(-28 \) mV. These relatively small absolute values of \( \zeta \)-potential indicate low dispersibility of the vesicle. The dispersion should be an intermediate state coexisting the dispersed primary particles and the agglomerates. In fact, when inducing precipitation of the agglomerates by centrifugation, a larger amount of agglomerates was observed in this concentration range as described above. In the case of high concentrations of boric acid (10–100 mM), the value of the \( \zeta \)-potential was ranged from \(-29 \) mV to \(-48 \) mV and the vesicles completely dispersed. Hence, the larger absolute value of the \( \zeta \)-potential (\( \geq 29 \) mV) may be necessary to disperse the primary particles of the AOG vesicles. It was found to be more than 10 mM of boric acid to achieve this stably dispersed condition. Moreover, given the dependence of boric acid concentration on \( \zeta \)-potential, it can be speculated that boric acid molecules bound to the surface of the vesicles in the form of the borate anion. Binding of the borate anions should contribute to dispersing the vesicles by providing a repulsive force among the vesicles. In fact, the \( \zeta \)-potential of AOG vesicles in different buffers was smaller than that in boric acid solution (\( \zeta \)-potential: \(-5.9 \pm 2.3 \) mV in phosphate buffer (100 mM, pH 7.4)), and aggregated particles were observed. This fact supports the formation of borate esters between the diol and boric acid on the surface of AOG vesicles, which provides a negative charge on the surface.

3.3 Effect of pH on the dispersion state of the vesicles

It is known that diol-borate complexation depends on the solution pH since there are equilibria (e.g., protonation-deprotonation of diols, boric acid-borate anion), which may change the extent of complexation\(^{-3}\). Thus, the effect of pH on the dispersion state of the vesicles was investigated at a constant boric acid concentration (100 mM). When the pH in the boric acid solution was higher than 7.4, the dispersions were translucently opalescent, and the size distribution of the vesicle was monomodal (Fig. 5ab), indicating that the vesicles dispersed in the form of primary particles. Under these conditions, the \( \zeta \)-potential was negatively large (\(-30\)–\(-45 \) mV) (Fig. 5c), which adequately maintained the dispersion state. On the other hand, at pH 6.2, the appearance of the dispersion was turbid with coexistence of larger particles (diameter>1 \( \mu \)m), indicating that the vesicles agglomerated in these acidic conditions. At
this time, the absolute value of the mean $\zeta$-potential was relatively small (\(-22\) mV) (Fig. 5c). The present pH range in which the AOG vesicle is sufficiently dispersed was consistent with that occurring for the complexation between diol and boric acid. These results strongly indicate that diol-borate complexation between the sugar moiety on AOG and borate anions can provide negative charges on the vesicles, contributing to the dispersion of the vesicles. Moreover, the $\zeta$-potential in basic dispersion media (pH 10.1) without boric acid was \(-10.6\) mV, which is smaller than that in basic medium (pH 10.1) containing boric acid. This indicates that the cause of the decrease in the $\zeta$-potential was not deprotonation of the sugar moieties in AOG in basic media, but diol-borate complexation between the sugar moiety and boric acid. Furthermore, temporal size stability of the AOG vesicle was investigated. The AOG vesicles could almost maintain their original size for at least 18 days in the optimal boric acid concentration (100 mM, pH 8.2) (Fig. S4). The slight changes in vesicle size observed in Fig. S4 might be attributed to slow equilibrium shift due to the non-equilibrium system.

3.4 Confirmation of complexation between the sugar moiety and boric acid using $^{11}$B NMR

Considering the above results and discussion, it can be assumed that boric acid binds to the diol on AOG, and eventually the vesicles will disperse in aqueous media. Thus, we attempted to confirm complexation between boric acid and the diol on AOG by means of $^{11}$B NMR spectroscopy. The possible structures for the complexes are shown in Fig. 6. All species are in the form of a borate ester with a negative charge. The $^{11}$B NMR spectra of these complexes were shown in Fig. S5. In the NMR measurement of the AOG vesicle dispersed in boric acid solution, small peak derived from $B_5$ was observed at 4.1 ppm (Fig. 7). This result suggests that complexation between boric acid and the diol on AOG occurred on surface of the vesicles. Moreover, the $^{11}$B NMR measurement determined the complexation mode between AOG and borate. On the AOG vesicles, formation of $[B_{5}]$ predominantly occurred compared with the $[B_{6}]$ and $[B_{52}]$. In the case of the vesicles system, formation of $[B_{52}]$ may be limited by steric hindrance due to immobilization AOG in the lipid layer by intermolecular association. The reason for the weak intensity for $[B_{6}]$ can be explained in terms of thermodynamics. The formation of a six-membered borate ester is more unfavorable in terms of entropy change compared with the five-membered one. Therefore, in the case of vesicle formation involving boric acid and AOG, complexation should occur in the form of $[B_{5}]$, and this complexation should be optimum.
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Results of the NMR study and acid and the sugar moiety on alkyl self-assemblies of alkyl glucoside provide a technical application

4 CONCLUSIONS

We demonstrated vesicle formation for industrially produced alkyl(oligo)glucoside, which is an amphiphile with good environmental compatibility. Vesicle formation readily occurred by addition of cholesterol to alkyl(oligo)glucoside. It was found that the dispersion ability of the vesicles could be regulated by addition of boric acid in the dispersion medium. $^{11}$B NMR revealed complexation of the boric acid and the sugar moiety on alkyl(oligo)glucoside. The results of the NMR study and $\zeta$-potential measurements suggest that binding boric acid to the sugar moiety of an alkyl(oligo)glucoside provides negative charge to the vesicles, contributing to stable dispersions of the vesicles via repulsive forces. This study shows the practical application of industrially manufactured alkyl(oligo)glucoside. Moreover, the formulations presented here for the molecular self-assemblies of alkyl(oligo)glucoside provide a technique to produce a molecular container with low environmental toxicity.

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Supporting Information

This material is available free of charge via the Internet at http://dx.doi.org/jos.65.10.5650/jos.ess.15215

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