Effect of Alkali Metal Cations on the Unilamellar Vesicle of a Squalene Bearing 18-Crown-6

Tran Ngoc Linh, Takashi Arimura,* Ken-Ichi Tominaga, Hiroko Isoda, Masato Kawasaki, and Naruhiko Adachi

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ABSTRACT: Squalene bearing 18-crown-6 was synthesized and formed unilamellar vesicles with a membrane thickness of about 6 nm and a diameter of about 0.32 μm. In the wake of the recognition of alkali metal cations, squalene unilamellar vesicles become larger as multilamellar vesicles or smaller while maintaining unilamellar vesicles depending on cations.
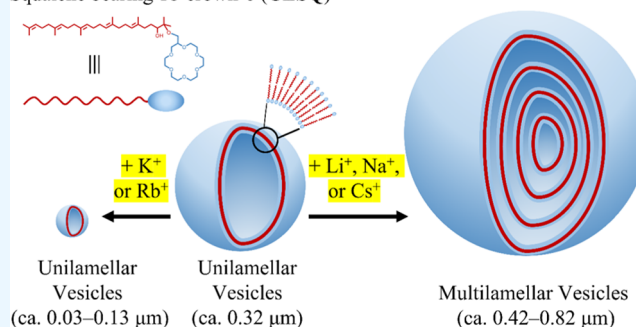
INTRODUCTION

Vesicles are essential for basic science research in life phenomena and are used as suitable models for understanding biological cellular systems and their interfacial functions.[1−6] Especially, vesicles are characterized by their internal water domain, which allows them to encapsulate bioactive substances such as drugs, but if their size can be further controlled by external stimuli or signals, the encapsulated substances can be released at will.

Micelles and vesicles utilizing crown ethers as hydrophilic groups have been extensively studied.[7−10] This is because crown ethers selectively associate with alkali metal cations, which play an important role *in vivo*. However, no examples of vesicles that systematically change in size in response to alkali metal cations have been reported. We have already found that the tetra-ethylene glycol-functionalized squalene forms vesicles with a diameter of 0.42 μm, which decreases to 0.10 μm with the addition of alkaline earth metal cations.[11]

In this study, we introduced 18-crown-6 ether as a polar moiety into hydrophobic squalene for the first time and found that it formed unilamellar vesicles of about 0.32 μm in diameter in water. Furthermore, we found that the size of vesicles systematically changed when alkali metal cations were used as external stimuli. Cryogenic transmission electron microscopy (Cryo-TEM) observations revealed the internal structure of all vesicles.

RESULTS AND DISCUSSION

2-(Oxymethyl-18-crown-6)-3-hydroxy-squalene (**CESQ**) was first synthesized from 2,3-epoxysqualene[12] in a 40% yield

(Scheme 1). The preparation method is detailed in the Supporting Information (SI).

Scheme 1. Preparation of 2-(Oxymethyl-18-crown-6)-3-hydroxy-squalene (CESQ) from 2,3-Epoxysqualene
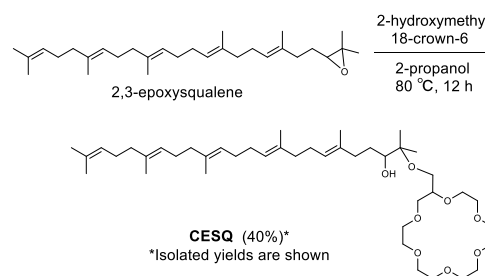
The stoichiometries and the association constants (K_a) of **CESQ** for Li$^+$, Na$^+$, K$^+$, Rb$^+$, and Cs$^+$ were estimated by ^{1}H NMR spectroscopic titrations[13] in CD$_3$CN, and the results are summarized in Table 1. The results were very similar to the previously reported values for the conventional 18-crown-6 (Figure S3, SI). Unfortunately, the association constant in water could not be determined because **CESQ** easily forms
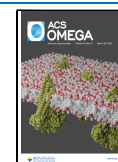
Table 1. Association Constants (K_a) for 1:1 Reactions of CESQ with Alkali Metal Cations in CD_3CN at 25 °C

cations[a]	Li+	Na+	K+	Rb+	Cs+
Log K_a[b]	3.16 ± 0.06	4.86 ± 0.08	6.10 ± 0.20	5.52 ± 0.16	4.94 ± 0.15

[a]Counter anion: SCN⁻. [b]Values are the average of three experimental determinations. The uncertainties are given as standard deviations.

aggregates. The association constants of 18-crown-6 with alkali metal cations in water have already been determined by Izatt et al.[14] and Liesegang et al.[15] (Table S1, SI). That is, 18-crown-6 shows great selectivity for K+ and Rb+, even in water, forming "nest-in-type" complexes. **CESQ**, squalene bearing 18-crown-6, is also expected to exhibit similar binding behavior toward alkali metal cations in water.

The critical vesicle concentration (CVC) value of **CESQ** was determined by careful examination of the vibronic band intensities of the pyrene monomer fluorescence.[16] We found that the intensity ratio of band III to band I (III/I ratio) of pyrene (5×10^{-7} M, excitation at 335 nm) in water increases with increasing **CESQ** concentration at around 1.0×10^{-6} M and reaches a saturation value (ca. 0.87) above 1.0×10^{-4} M (Figure 1). A plot of the III/I ratio for **CESQ** gave the

Figure 2. Plots of the III/I ratio in pyrene fluorescence versus the concentration of **CESQ** in water without metal cations and in the presence of alkali metal sulfates ([M+] = 0.1 M) at 25 °C; [pyrene] = 5.0×10^{-7} M, excitation at 335 nm.
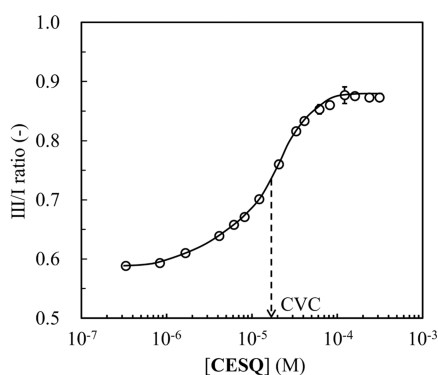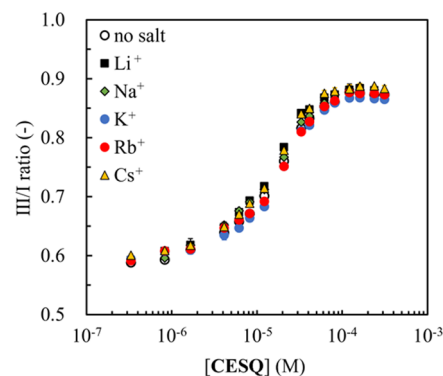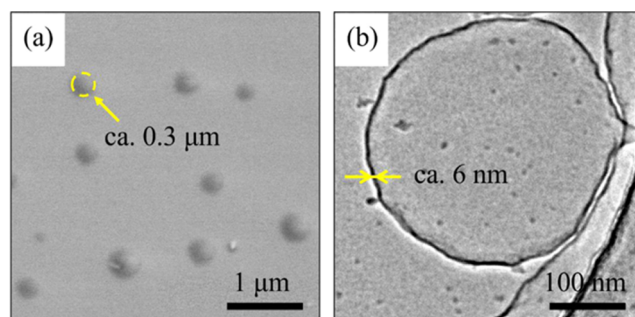

Figure 1. Plot of the III/I ratio in pyrene fluorescence versus the concentration of **CESQ** in water at 25 °C; [pyrene] = 5.0×10^{-7} M, excitation at 335 nm.

Figure 3. (a) SEM image and (b) Cryo-TEM image of **CESQ** vesicles.

continuous saturation curvature (sigmoid function), leading us to suggest that **CESQ** is organized in a hydrophobic domain in water. Thus, the CVC value was estimated as being 1.7×10^{-5} M.

We next examined the effect of metal cations on the CVC of the **CESQ** vesicle in an aqueous system. The III/I ratio plots for **CESQ** in the presence of alkali metal cations are shown in Figure 2. However, the estimated CVC values were almost unaffected by the addition of metal cations. Only with the addition of K+ and Rb+, there was a slight but increasing trend in the CVC values (Table S2, SI). This trend is compatible with the slight increase in the critical aggregation concentrations when K+ is added to 18-crown-6 bearing C_8 or C_{10} alkyl groups in water.[17,18]

The aggregation size of the **CESQ** vesicle was estimated by scanning electron microscopy (SEM) and found to be around 0.3 μm (Figure 3a). The Cryo-TEM image revealed that **CESQ** assembles as unilamellar vesicles with a membrane thickness of about 6 nm and a diameter of about 0.32 μm (Figure 3b).

As shown in Figures 4 and 5, when alkali metal cations (Li+, Na+, or Cs+) with K_a less than 1.0 in water were added, the average aggregation size of the **CESQ** vesicle increased to 0.82 μm for Li+, 0.5 μm for Na+, and 0.42 μm for Cs+. Interestingly,

adding alkali metal cations (K+ or Rb+) with K_a as being about 2.0 in water, the average aggregation size decreased to 0.05 μm for K+ and 0.14 μm for Rb+. The size distributions of **CESQ** vesicles in the presence of alkali metal cations were also estimated by laser diffraction particle size analysis[19,20] (Figure S9, SI) and are shown as error bars in Figure 5. In addition, the hydrodynamic diameters of **CESQ** vesicles estimated by dynamic light scattering were almost the same as those obtained from SEM and Cryo-TEM (Table S4, SI). The same trend was verified when the ζ-potential of **CESQ** vesicles was measured in the presence of alkali metal ions (Table S5, Figure S12, SI). That is, for metal ions with large association constants, the ζ-potential became positive,[21] and the vesicle particle size decreased due to repulsive electrostatic forces.

Lastly, cryo-TEM images revealed the internal structure of the **CESQ** vesicle in the presence of alkali metal cations (Li+, Na+, K+, Rb+, or Cs+). When the size of the **CESQ** vesicle increased in the presence of alkali metal cations (Li+, Na+, or Cs+), vesicle fusion resulted in the formation of large multilamellar vesicles (Figure 6b,c,f). In Figure 6d,e, they revealed that **CESQ** vesicles are unilamellar vesicles with a membrane thickness of about 6 nm, and the diameter is about 0.03 μm for K+ and about 0.13 μm for Rb+. It is reported that the vesicle size of the amphiphilic crown ether materials increases in the presence of an alkali metal cation (K+).[22−24]
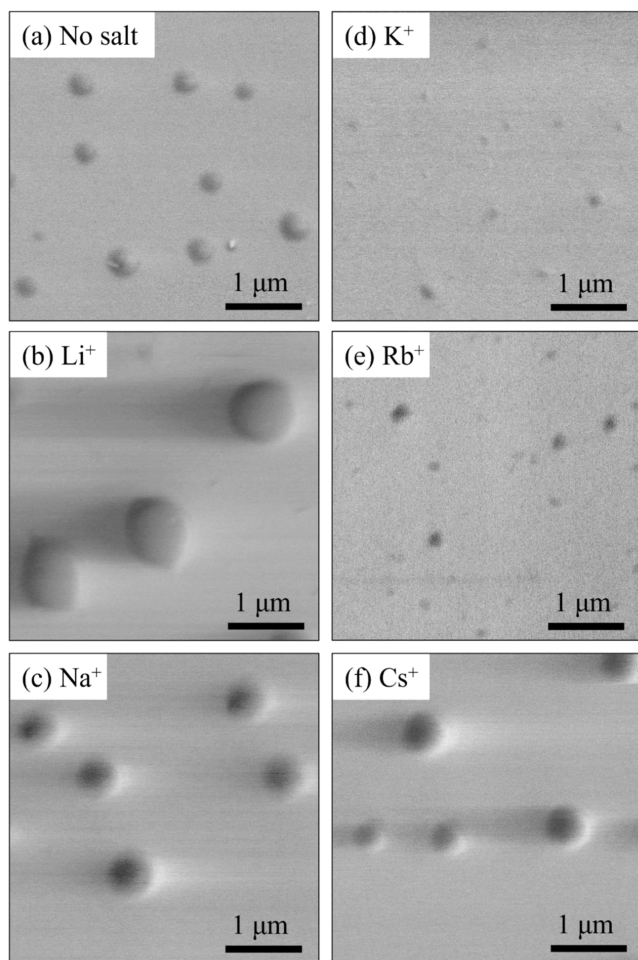
Figure 4. SEM images of **CESQ** vesicles, (a) without metal cations and in the presence of (b) Li_2SO_4, (c) Na_2SO_4, (d) K_2SO_4, (e) Rb_2SO_4, and (f) Cs_2SO_4.
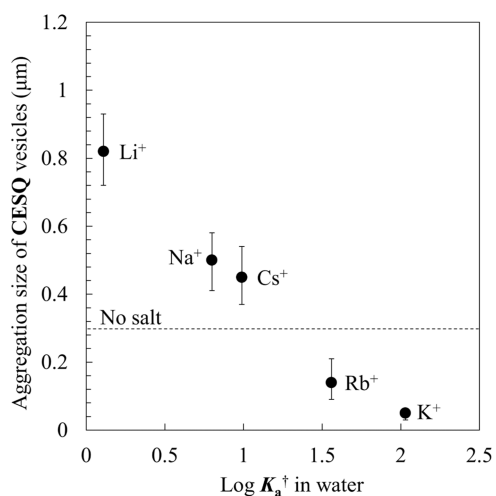

Figure 5. Plot of aggregation sizes of **CESQ** vesicles in the presence of alkali metal cations determined by SEM images (Figure 4) versus the association constants (log K_a) of 18-crown-6 for alkali metal cations in water at 25 °C; †cited from refs 14, 15.

Lehn and co-workers have reported that the formation of multilamellar vesicles is driven by metal cation-selective binding with a surface energy gain γ.[25] However, so far, it is not exactly clear why the unilamellar vesicles change to
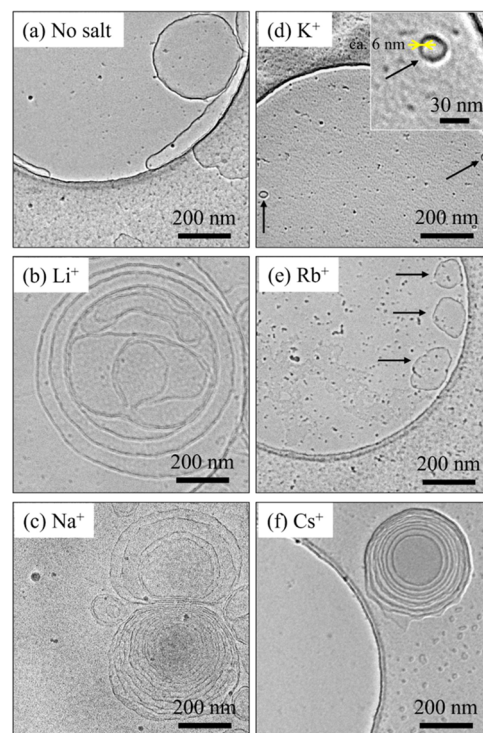

Figure 6. Cryo-TEM images of **CESQ** vesicles in water, (a) without metal cations and in the presence of (b) Li_2SO_4, (c) Na_2SO_4, (d) K_2SO_4, (e) Rb_2SO_4, and (f) Cs_2SO_4.

multilamellar vesicles in the presence of metal cations. This is because the size of the **CESQ** vesicle decreased as small unilamellar vesicles with the addition of alkali metal cations (K^+ or Rb^+) which strongly bind to 18-crown-6 by hole-size selectivity in water (vide ante, Figure 5 and Table S1, SI). Van Tamelen has reported that squalene, a kind of triterpenoid, exists in a highly coiled, unusually compact conformation instead of a fully extended state in hydrophilic solvent,[12] leading us to suggest that the aggregation ability of squalene as a hydrophobic moiety to form vesicles may be relatively different from that of an ordinary triterpenoid or a long aliphatic group in water. Consequently, we infer that the decrease in the size of the **CESQ** vesicle reflects improvements in solubility in water and the electrostatic repulsions between individual **CESQ**-K^+ or **CESQ**-Rb^+ subunits within the ensembles.

■ CONCLUSIONS

We found for the first time that the recognition of alkali metal cations (Li^+, Na^+, K^+, Rb^+, or Cs^+) causes **CESQ** vesicles to become larger as multilamellar vesicles or smaller while maintaining unilamellar vesicles. Further applications of the amphiphilic squalene system are currently being pursued in our laboratories.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00744.

> Materials and instruments, synthesis procedure and characterization, determination of association constants by NMR titration, determination of critical vesicle concentration, preparation of vesicles, laser diffraction

particle size analysis, dynamic light scattering, ζ-potential, SEM and Cryo-TEM observation (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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