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Vesicle formation by non-ionic polymerizable cholesterol-based amphiphiles

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Sonication of a non-ionic polymerizable chlosterol-based amphiphile, 1-ethenyl-3-(8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctyl)-2-pyrrolidone, in water resulted in the formation of bilayer vesicles. The vesicle formation was evidenced by transmission electron microscopy and [^{14}C] sucrose entrapment. Polymeric vesicles, prepared by polymerization in the bilayer, showed increased stability.

(Keywords: vesicle formation; cholesterol-based monomer; polymerization)

Introduction

Recently, various vesicle systems have been the subject of intense scientific interest and many practical applications have been suggested[1,2]. However, these vesicles, formed by synthetic dialkyl amphiphiles, are thermodynamically unstable and undergo fusion on standing. Considerable effort has been made to construct more stabilized vesicles, for example the preparation of polymeric vesicles[3–5] or surface coating of vesicles[6]. The vesicles formed by cholesterol-based amphiphiles are also reported to be stable[7–12]. We have already reported that a cationic cholesterol-containing monomer formed highly stable unibilayer vesicles in aqueous system upon polymerization[9,10]. Even non-polymeric vesicles derived from cholesterol-based amphiphiles with appropriate polar head group exhibited high stability[8,11,12].

In the search for non-ionic, polymerizable, cholesterol-based amphiphiles to form vesicles, we have synthesized novel monomers with *N*-vinylpyrrolidone (NVP) as polymerizable moiety, 1-ethenyl-3-(8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctyl)-2-pyrrolidone (**1**) and 1-ethenyl-3-(6-(cholest-5-en-3β-yloxy)hexyl)-2-pyrrolidone (**2**). This communication describes the preliminary results on the bilayer vesicle formation by **1** and the polymerization.

Experimental

Materials. Solvents and reagents were dried or purified adequately before use by standard methods. Water (Merck, HPLC grade) was degassed with nitrogen before use. 8-(Cholest-5-en-3β-yloxy)-3,6-dioxaoctyl bromide (**3**) and 6-(cholest-5-en-3β-yloxy)hexylbromide (**4**) were synthesized according to a literature method[13], with some modifications. Chromatographic separations were carried out by use of Merck silica gel 60 of particle size 0.063–0.200 mm.

Analyses. ^{1}H n.m.r. and i.r. spectra were obtained on a Bruker AM-300 spectrometer and a Bomem FT-IR MB-100 instrument, respectively. Turbidity was recorded with a Shimadzu UV-240 spectrophotometer. Elemental analysis was performed on a Carlo Erba elemental analyser (MOD 1106). Liquid scintillation counting was performed on a Packard Tri-Carb 2000CA. Electron micrographs were obtained from a Jeol JEM-100CX II electron microscope.

Syntheses of amphiphiles 1 and 2. Amphiphile **1** was synthesized as follows. To lithium diisopropylamide (LDA; 81 mmol) in tetrahydrofuran (THF; 100 ml) and hexamethylphosphoric triamide (HMPT; 28 ml), NVP (9.0 g, 81 mmol) was added dropwise and the resulting yellow solution was maintained for 2 h at −78°C. Compound **3** (23.6 g; 40.6 mmol) in THF was added slowly to the solution at −78°C. The reaction mixture was stirred for 12 h at −78°C and overnight at room temperature. After dilution by addition of Et_2O, the reaction was quenched with aqueous 5N NH_4Cl solution. The ethereal layer was washed successively with aqueous solutions of 1N HCl, $NaHCO_3$ and saturated NaCl, and then water. The dried organic solution was evaporated to give crude dark brown syrup, which was purified by column chromatography on silica gel with petroleum ether–THF (4:1) as eluent. The desired fractions were combined, evaporated, and dried in vacuum to give waxy **1**.

Yield 10.8 g (43%). ^{1}H n.m.r. ($CDCl_3$): δ (ppm) 6.94–7.02 (m, 1H, $N\text{–}C\underline{H}{=}CH_2$), 5.22–5.23 (m, 1H, $C{=}C\underline{H}\text{–}CH_2$), 4.25–4.33 (m, 2H, $N\text{–}CH{=}C\underline{H}_2$), 3.48–3.55 (m, 8H, $O\text{–}C\underline{H}_2\text{–}C\underline{H}_2\text{–}O$), 3.25–3.44 (m, 4H, $CH\text{–}CH_2\text{–}C\underline{H}_2\text{–}O + N\text{–}C\underline{H}_2\text{–}CH_2$), 3.01–3.15 (m, 1H, $O\text{–}C\underline{H}(CH_2)_2$), 2.49–2.59 (m, 1H, $O{=}C\text{–}C\underline{H}(CH_2)_2$), 0.58–2.25 (m, 47H, cholesteryl (43H) + $N\text{–}CH_2\text{–}C\underline{H}_2\text{–}CH + CH\text{–}C\underline{H}_2\text{–}CH_2\text{–}O$). *FT*i.r. (neat): ν_{max} 2903, 1702, 1631, 1448, 1115, 980,

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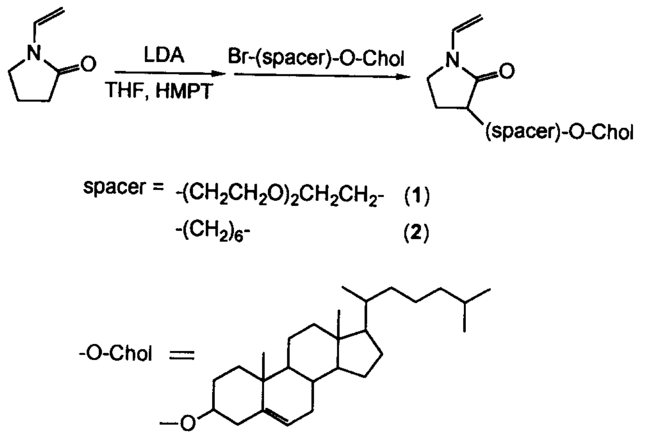

Scheme 1

843 cm^{-1}. Anal. calcd for $C_{39}H_{65}NO_4$: C, 76.55; H, 10.71; N, 2.29. Found: C, 76.64; H, 11.04; N, 2.68.

Amphiphile **2** was prepared by a similar method to that employed for **1**, starting with **4** (20.8 g, 37.8 mmol).

Yield 11.2 g (51%). ^{1}H n.m.r. ($CDCl_3$): δ (ppm) 7.01–7.09 (m, 1H, $N{-}C\underline{H}{=}CH_2$), 5.30 (m, 1H, $C{=}C\underline{H}{-}CH_2$), 4.31–4.39 (m, 2H, $N{-}CH{=}C\underline{H}_2$), 3.31–3.45 (m, 4H, $CH_2{-}C\underline{H}_2{-}O + N{-}C\underline{H}_2{-}CH_2$), 3.06–3.09 (m, 1H, $O{-}C\underline{H}(CH_2)_2$), 2.44–2.46 (m, 1H, $O{=}C{-}C\underline{H}(CH_2)_2$), 0.63–2.36 (m, 55H, cholesteryl (43H) + $N{-}CH_2{-}C\underline{H}_2{-}CH + CH{-}(C\underline{H}_2)_5{-}CH_2{-}O$). *FT*i.r. (KBr): ν_{max} 2906, 1696, 1632, 1465, 1104, 986, 865 cm^{-1}. Anal. calcd for $C_{39}H_{65}NO_2$: C, 80.77; H, 11.30; N, 2.42. Found: C, 80.77; H, 11.48; N, 2.88.

Vesicle formation and polymerization. Monomer **1** (15.3 mg, 0.025 mmol) was dissolved in chloroform and the solvent was removed under vacuum. Water (5.0 ml) was added and the mixture was sonicated with a Branson 2200 bath-type sonicator (117 W) at 60°C for 10 min. The vesicles formed in 5.0 ml of water containing 0.5 mg of potassium persulfate were polymerized at 70°C for 12 h with stirring.

[^{14}C] sucrose entrapment and electron microscopy. Vesicle solution prepared in water containing [^{14}C] sucrose was chromatographed on a Sephadex G-50-80 column. The eluent fraction was radio-assayed. Aqueous sample solution applied to a carbon-coated copper grid was negatively stained with 0.2% phosphotungstenic acid solution for 1 min. The grid was blotted and dried before measurement.

Vesicle stability to ethanol addition. The stability of non-polymeric and polymeric vesicles was estimated according to the method of Regen *et al.*[3]. The aqueous vesicle solutions were diluted with ethanol, mixed thoroughly with a vortex mixer, and then allowed to stand for 24 h. The absorbance at 400 nm of each sample solution was corrected for the volume change.

Results and discussion

Cholesterol-based monomers **1** and **2** were synthesized by α-alkylation of NVP with the corresponding bromides (*Scheme 1*). In spite of possible polymerization of NVP[14], the expected monomers were obtained in good yield. The chemical structures were characterized by ^{1}H n.m.r., i.r. and elemental analysis.

Thin films of **1** were sonicated in degassed water, as described earlier, to yield a turbid solution. The chemical structure of **1** is comparable with 8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctanol, which was reported to form vesicles by sonication[8], in that the hydroxy group of the latter is replaced by an NVP moiety. However, **1** could not be dispersed only by stirring at 60°C. Transmission electron micrographs of the resulting solution showed the formation of spherical bilayer vesicles. Unilamellar and multilamellar vesicles with diameters in the range 200–1000 Å were observed. The layer width was about 50 Å. Closed vesicle formation was further evidenced by [^{14}C] sucrose entrapment. With **2**, all the efforts to prepare vesicles failed. Monomer **2** was not dispersed by sonication. Although it was possible to obtain an opaque dispersion of the equimolar mixture of **2** and cholesterol by the injection of an ethanol solution of the mixture into water at 60°C[7], no layered structure was observed in the electron micrographs.

Initially, vesicles formed by **1** were polymerized with potassium persulfate at 70°C for 12 h with stirring. Some aggregates of **1** appeared during polymerization and this made the solution less turbid. *Figure 1* shows the vesicles of which the structure and size were retained after polymerization. The polymerization through NVP moieties of the vesicles was confirmed by ^{1}H n.m.r. and i.r. In the ^{1}H n.m.r. spectrum, the vinyl protons at 6.94–7.02 and 4.25–4.33 ppm disappeared completely. The disappearance of the i.r. absorption band at 1631 cm^{-1}, which is due to the vinyl double bonds, also indicated polymerization.

The non-polymeric vesicles were stable, but began to precipitate after a week. However, the polymeric vesicles showed more enhanced long-term stability. There was no observed change in the polymeric vesicle solution for several weeks. The stability difference between the non-polymeric and polymeric vesicles could also be estimated by comparison of their response to ethanol (*Figure 2*)[3]. The turbidity of the polymeric vesicle solution decreased slightly upon addition of 40% v/v ethanol, while non-polymeric vesicles responded more sensitively to ethanol addition. Addition of more ethanol decreased

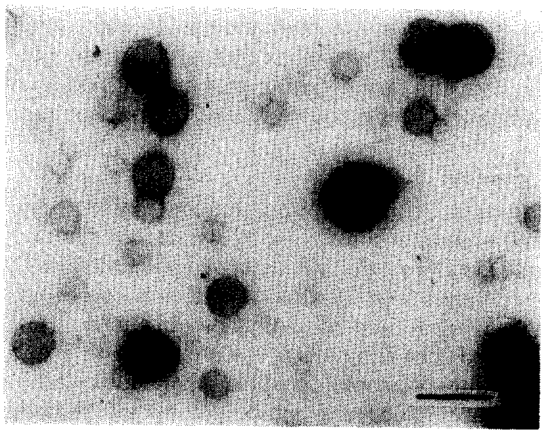

Figure 1 Transmission electron micrograph of polymeric vesicles formed by **1**, stained with 0.2% phosphotungstenic acid solution. Scale bar represents 1000 Å

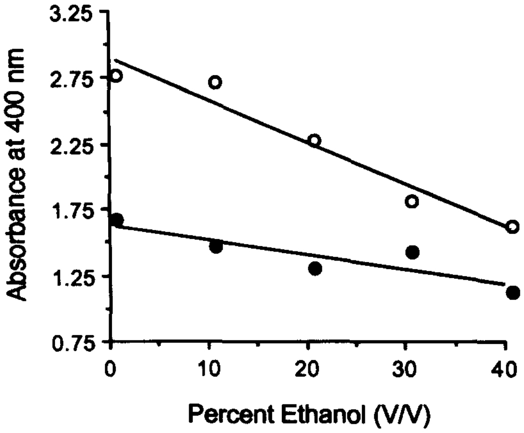

Figure 2 Plot of absorbance at 400 nm as a function of volume percentage ethanol for non-polymeric (○) and polymeric (●) vesicles formed by **1**

abruptly the turbidity of both the non-polymeric and polymeric vesicles.

The polymeric vesicles prepared using potassium persulfate may not be absolutely non-ionic. Polymerization of the non-ionic vesicles by photoinitiation or by using non-ionic radical initiators, as well as detailed characterization, is being planned.

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