Specific Fixed Aqueous Layer of Three-component Hybrid Liposomes Related to Inhibition of Hepatoma Cells Growth

Yoko Matsumoto,* Yoshihiro Tanaka, Koichi Goto, and Ryuichi Ueoka
Graduate Course of Applied Life Science, Sojo University, 4-22-1 Ikeda, Kumamoto 860-0082

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It is noteworthy that the thickness of fixed aqueous layer (TFAL) of three-component hybrid liposomes (THL) composed of L-α-dimyristoylphosphatidylcholine (DMPC), polyoxyethylene (20) sorbitan monolaurate (Tween 20), and decyl-β-lactopranoside (LactC_{10}) (DMPC:Tween 20:LactC_{10} = 65:7:28) was about twice that of two-component hybrid liposomes (HL) composed of DMPC and Tween 20. It is also attractive that THL could remarkably inhibit the growth of hepatoma cells.

Biological membranes provide compartments of defined sizes, shapes, and microenvironments. They organize living matter in the cell, create a fluid two-dimensional matrix. There are wide variations in the types of lipids including phospholipids and proteins as well as in their ratios. Such variations in lipids should take an important role for the activation of bioactive substances. In the course of our study of esterase models, remarkably stereospecific catalysis was observed in the hydrolysis of amino acid and/or dipeptide esters carried out by functional molecular assemblies composed of surfactants and catalytic species.[1–3]

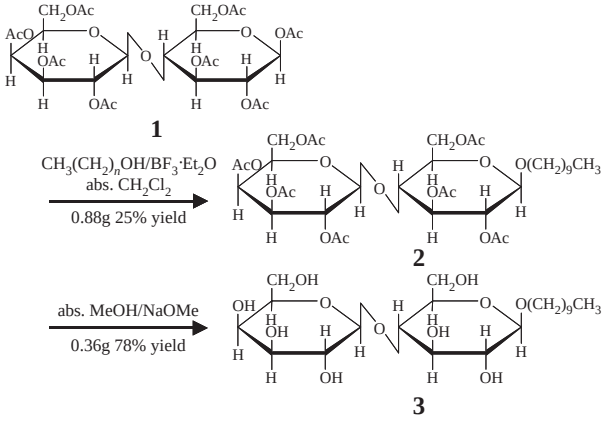
It is well known that saccharides play important roles in adhering to cells, transmitting information, recognizing molecules on the cell membranes thought receptors including lectin.[4] For example, lactose was found in molecular recognition in vivo.[5] Hydration of saccharides with hydrogen bonds provides stability to the structure of water. The hydration of sugar derivatives was discussed in relation to the hydration of the parent sugars.[6] Recently, the preparation and characterization of glyco-liposomes have been reported.[7]

We have developed HL composed of vesicular and micellar molecules have been produced.[1,8] HL without drugs inhibited the proliferations of various tumor cells along with apoptosis in vitro and in vivo.[9–12]

In this study, we report that the lactose surfactant was synthesized by glycosylation and deacetylation, which were allowed to experiment as the relevance to hydration and antitumor effects of THL composed of L-α-DMPC, polyoxyethylene (20) sorbitane monolaurate (Tween 20), and decyl-β-lactopranoside (LactC_{10}) on the growth of hepatoma cells in vitro.

Firstly, we prepared LactC_{10} (decyl-β-lactopranoside) from Ac-LactC_{10} (decyl-β-lactose heptaacetate), which was obtained by glycosylation and deacetylation of Ac-Lact (β-lactose octaacetate)[13,14] (Scheme 1). **3** was prepared from **1** by glycosylation at C-1 site of D-glucose unit with 1-decanol and boron trifluoride diethyl etherate in dry dichloromethane, followed by deacetylation of acetyl groups by treatment with sodium methoxide in absolute methanol.

Satisfactory elemental, IR, and ^{1}H NMR analyses were obtained for **3** and we successfully produced the lactose surfactant. Anal. Calcd for $C_{22}H_{42}O_{11}$: C, 55.88; H, 9.13%. Found: C, 55.45; H, 9.13%. 0.36 g, 78% yield. mp: 185.6–186.5 °C. IR (KBr): ν (cm^{-1}) 3424, 2921, 2853, 1064. ^{1}H NMR (270 MHz in DMSO-d_6): δ 5.10 (1H, s, OH), 5.08 (1H, s, OH), 4.80 (1H, br s, OH), 4.66 (2H, s, OH × 2), 4.54 (2H, m, OH × 2), 4.18 (1H, d, J = 7.91 Hz, CH), 4.15 (1H, d, J = 7.92 Hz, CH), 3.76–3.27 (13H, m, CH × 9, CH_2 × 2), 2.98 (1H, m, CH), 1.51 (2H, m, CH_2), 1.25 (14H, br s, CH_2 × 7), 0.85 (3H, t, J = 6.92 Hz, CH_3).



Scheme 1. Synthesis of **3** (decyl-β-lactopyranoside: LactC_{10}).

Secondly, we examined the thickness of fixed aqueous layer (TFAL) of THL from zeta potential (ζ).[15] THL was prepared by sonication of a mixture of DMPC, Tween 20, and **3** (DMPC:Tween 20:**3** = 65:7:28) with a sonicator (VS-N300, VELVO-CLEAR) at 45 °C under a nitrogen atmosphere with 300 W in phosphate-buffered saline (PBS(−)) solution containing various concentrations of NaCl (10, 50, 200, and 400 mM), followed by filtration with a 0.45-μm filter. ζ of the sample solutions were measured by laser doppler photometry method using an electrophoretic light scattering spectrophotometer (ELS-8000, Otsuka Electronics) with a He–Ne laser as light source (633 nm, 10 mW) at the scattering angle of 20°. ζ was calculated from electrophoretic mobility (U: m^2 Volt^{-1} s^{-1}) applying the eq 1 (smoluchowski equation).

$$\zeta = 4\pi\eta U/\varepsilon \quad (1)$$

where η (Pa.s) and ε (N/Volt^2) are the viscosity and permittivity of solvent, respectively. ζ was measured at 37 °C. ζ is defined as the electrostatic potentials at the position of the slipping plane Δ (nm), which occurs just outside the fixed aqueous layer of THL. Then, ζ is expressed as eq 2.

$$\ln(\zeta) = \ln A - \Delta\kappa \quad (2)$$

where κ is Debye–Hückel parameter (=3.3$\sqrt{c}$, c: M for NaCl). If the ζ is measured in various concentrations of NaCl and plotted against κ, the slope gives the position of the slipping plane or the

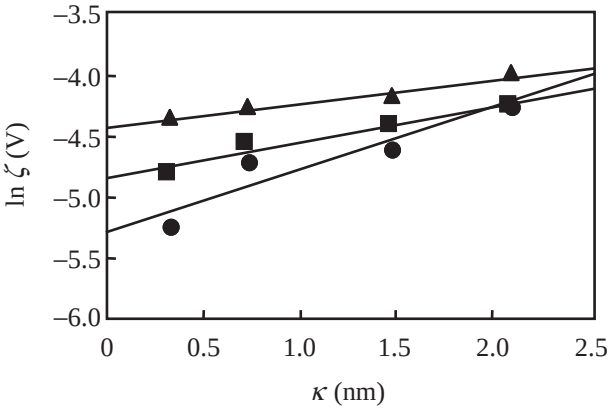


Figure 1. Plot of ln zeta potential (ζ) versus Debye–Hückel parameter (κ) for THL. ▲: DMPC liposomes; $y = -4.43 + 0.21x$ ($R^2 = 0.96$), ■: HL; $y = -4.83 + 0.31x$ ($R^2 = 0.94$), ●: THL; $y = -5.31 + 0.58x$ ($R^2 = 0.88$). [DMPC] = 3000 μM, [Tween 20] = 333 μM, [$LactC_{10}$] = 1296 μM (DMPC:Tween 20:$LactC_{10}$ = 65:7:28). Data presented are mean values.

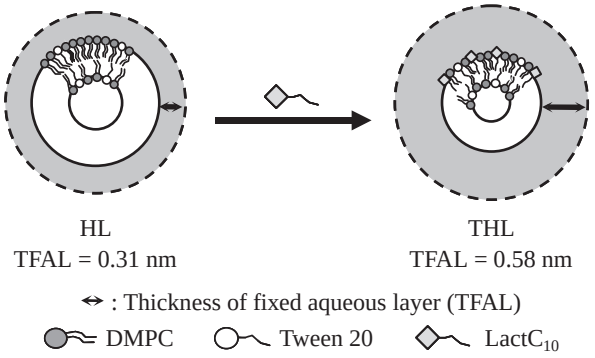


Figure 2. Schematic representation of the TFAL of THL.

TFAL in nm units. On the basis of this theory, the TFAL of THL was estimated. Figure 1 shows the plots of ln ζ versus κ with THL containing various concentrations of NaCl. Schematic representation of the TFAL of THL are shown in Figure 2. It is noteworthy that the TFAL of THL (0.58 nm) was about twice that of DMPC liposomes (0.21 nm) and HL (0.31 nm). This result suggests that THL having larger TFAL could be hydrated with water molecules on the membrane surface.

In addition, we examined the fifty percent inhibitory concentration (IC_{50})[16] of THL on the growth of human hepatoma (Hep-G2 and Huh-7) cells on the basis of the WST–1 assay.[17] It is noteworthy that greater inhibitory effects of THL on the growth of Hep-G2 (IC_{50} = 250 μM) and Huh-7 (IC_{50} = 180 μM) cells compared with DMPC liposomes (IC_{50} = 360 μM for Hep-G2, IC_{50} = 400 μM for Huh-7) and HL (IC_{50} = 340 μM for Hep-G2, IC_{50} = 250 μM for Huh-7 were obtained on the basis of IC_{50}. These results indicate that THL should be effective for inhibiting the growth of Hep-G2 and Huh-7 cells. The degree of construction of water molecules in tumor tissue was shown to be less than that in normal tissue using the spin–lattice relaxation time (T_1) for proton in water molecules.[18] It was suggested that the motions of water of the tumor cells surface might be moved more active and disordered state than normal cells. So, the inhibitory effects of THL could be related to hydration in tumor cells.

In conclusion, we successfully obtained for **3** (decyl-β-lactopranoside: $LactC_{10}$) from satisfactory elemental, IR, and ^{1}H NMR analyses. It is noteworthy that the TFAL of THL composed of DMPC, Tween 20, and **3** (DMPC:Tween 20:**3** = 65:7:28) was about twice that of two-component HL. Inhibitory effects of THL on the growth of hepatoma (Hep-G2 and Huh-7) cells were obtained. Thus, this study demonstrated the importance of relation between physicochemical characteristics (TFAL of THL including sugar surfactants) and biological specifics (inhibitory effects of THL on the growth of hepatoma cells) for the first time.

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