Supporting Information

Design and Construction of pH-Selective Self-Lytic Liposome System

Ayumi Kashiwada*, Kana Namiki and Haruka Mori

Department of Applied Molecular Chemistry, College of Industrial Technology, Nihon University, 1-2-1 Izumi-cho, Narashino, Chiba 275-8575, Japan
E-mail: kashiwada.ayumi@nihon-u.ac.jp

Contents:

Identification of the synthetic membrane-lytic peptides, rLP and rLPE

All products were identified by HPLC and high resolution mass analyses. Analytical HPLC chromatograms of peptides were obtained at 280 nm from a gradient of 20% to 80% (eluent B) over 30 minutes on an Inertsil ODS-3 column, 5 μm, 250 mm × 4.6 mm i.d., GL-science, Japan; eluent A: water / 0.1% TFA, eluent B: acetonitrile / 0.1% TFA; flow rate 1.0 mL/min. Mass spectra were recorded on the Agilent 6210 ESI-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The samples were dissolved in acetonitrile/water (1/1) containing 0.1% TFA and injected directly into the spray chamber by using a syringe pump with flowrates of 10 to 50 μL/minute. The spray voltage was 4000 V and flow rate of the drying N_2 gas was set to 1 psi.

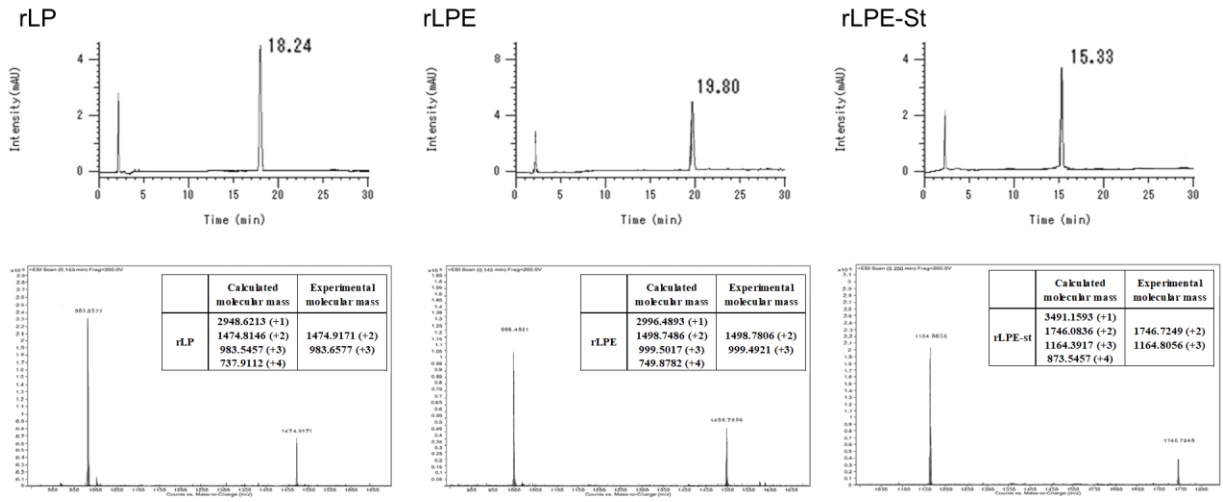

Figure 1. Analytical HPLC chromatograms and mass spectra of the synthetic membrane-lytic peptides and the designed acidic-pH-Selective membrane lytic peptide conjugate (rLPE-St).

Characterization of pH-selective lytic properties of the rLPE-St anchoring liposomes

Determination of zeta potential of the rLPE-St anchoring liposomes

The electrostatic charge of the liposome surface was characterized by the dynamic light scattering (DLS) measurements as same procedures written in "Materials and Methods".

Table 1. Zeta potentials of the rLPE-St anchoring liposomes*.

Liposomes	pH	zeta-potential (mV)
rLPE-St (1.0 mol%) / EggPC	7.4	-1.28 ± 0.22
	5.0	+1.78 ± 0.18
EggPC	7.4	-8.46 ± 0.18
	5.0	-6.49 ± 0.13

*Data are represented as mean ± SD of triplicate measurements (n = 3).

Characterization of the dependence of the rLPE-St concentrations

Membrane lytic properties of the rLPE-St anchoring liposomes were characterized by calcein-leakage assay as same procedures written in "Materials and Methods".

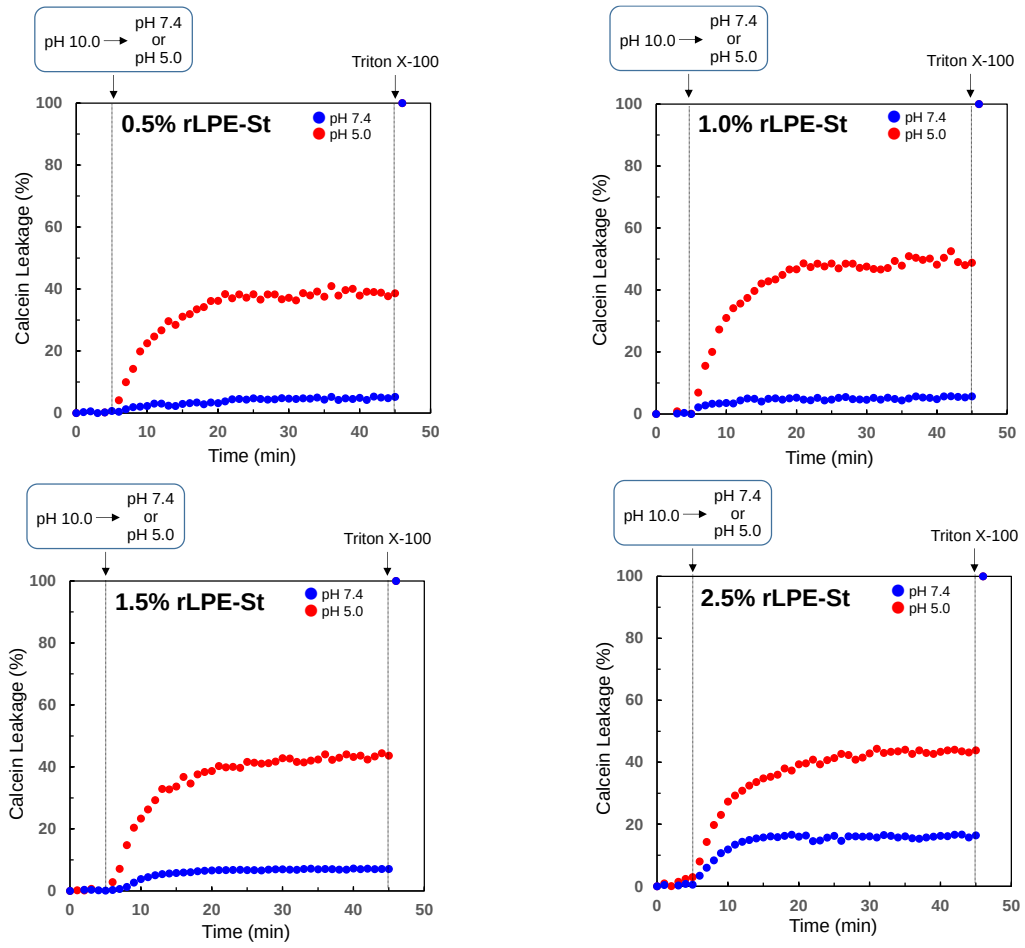

Figure 2. pH-dependent calcein release behavior from liposomes anchoring the various amounts (0.5 mol% to 2.5 mol%) of rLPE-St. The calcein-entrapped liposomes were prepared at pH 10.0. After 5 minutes incubation, the pH was lowered to a value of 7.4 or 5.0.

Characterization of the pH-dependence of membrane lytic activity of the rLPE-St anchoring liposomes

The pH-dependence of the membrane lytic properties of the rLPE-St anchoring liposomes were characterized by calcein-leakage assay as same procedures written in "Materials and Methods".

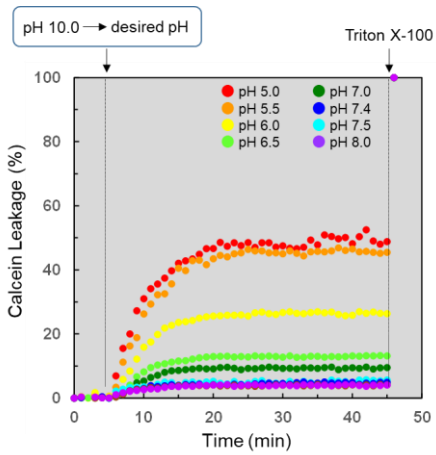

Figure 3. pH-dependent calcein release behavior from liposomes anchoring the 1.0 mol% of rLPE-St. The calcein-entrapped liposomes were prepared at pH 10.0. After 5 minutes incubation, the pH was lowered to a target pH.

Monitoring the liposome integrity during the membrane lytic process

Size distribution of liposomes during membrane lytic process was characterized by the dynamic light scattering (DLS) measurements as same procedures written in "Materials and Methods".

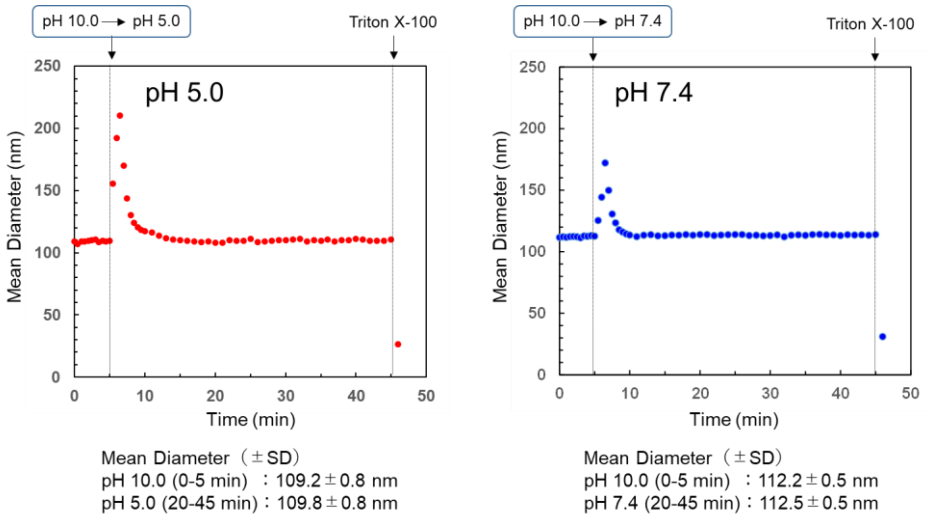

Figure 4. Mean diameter of the liposomes anchoring the 1.0 mol% of rLPE-St from DLS measurements during pH-activated membrane lytic process.

Secondary Structure of the rLPE Segment Anchored into the Liposomal Membranes

The pH-selective conformational changes of the rLPE-St segment were applied at both physiological and endosomal pHs was characterized by circular dichroism (CD) measurements as same procedures written in "Materials and Methods".

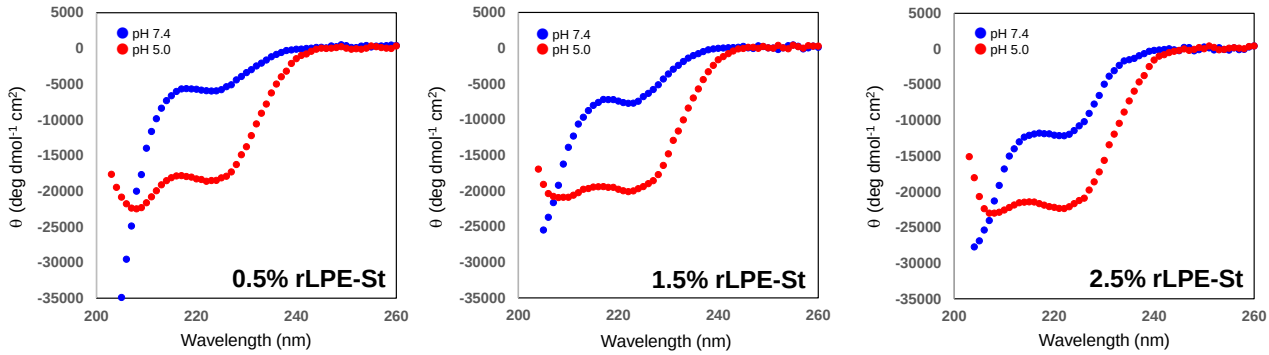

Figure 5. Circular dichroism (CD) spectra of the rLPE-St (0.5, 1.5, and 2.5 mol%) anchored into the liposomal membranes at pH 7.4 and pH 5.0. Measurements were carried out at 25 °C.