

Supporting Information

pH-Dependent Membrane Lysis by Melittin-Inspired Designed Peptides

Ayumi Kashiwada*, Masaki Mizuno, and Jun-ichi Hashimoto

Department of Applied Molecular Chemistry, Graduate School of Industrial Technology, Nihon University, Narashino, Chiba 275-8575, Japan

E-mail: kashiwada.ayumi@nihon-u.ac.jp

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Identification of designed membrane-lytic peptides

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 min on an Inertsil ODS-3 column, 5 μ m, 250 mm \times 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 μ l/min. The spray voltage was 4000 V and flow rate of the drying N₂ gas was set to 1 psi.

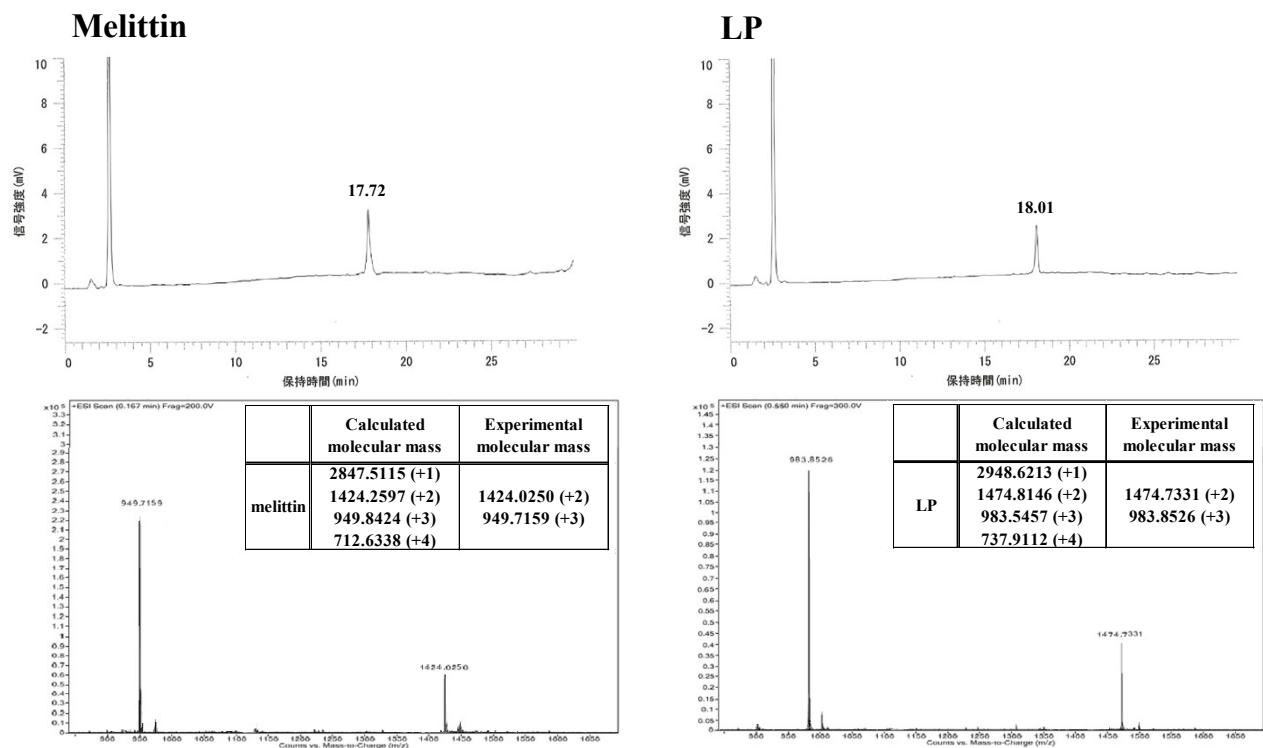


Fig. S1 Analytical HPLC chromatograms and mass spectra of melittin and a typical membrane-lytic peptide (LP).

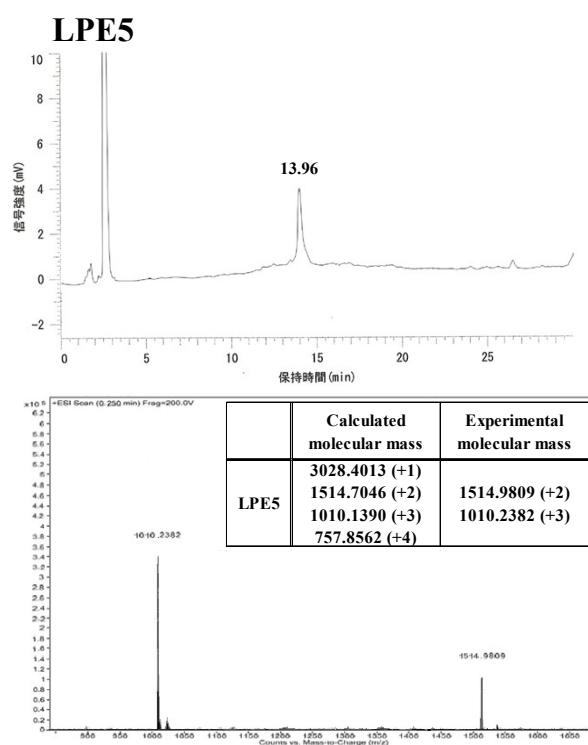
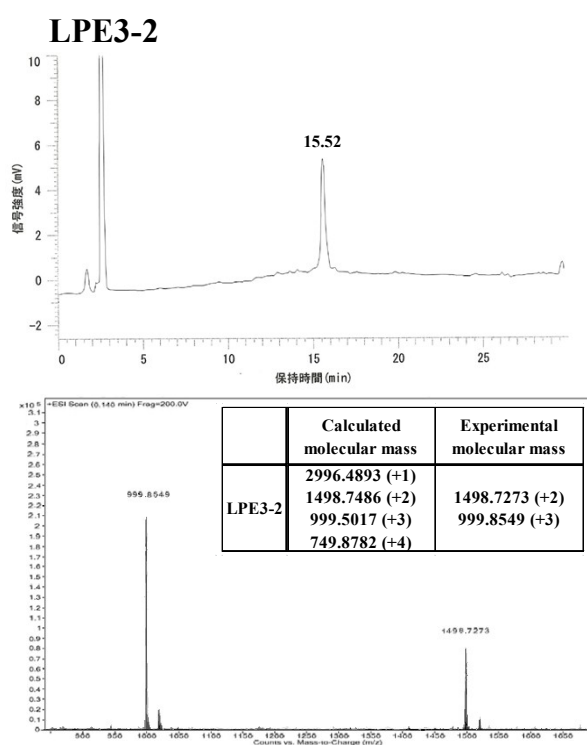
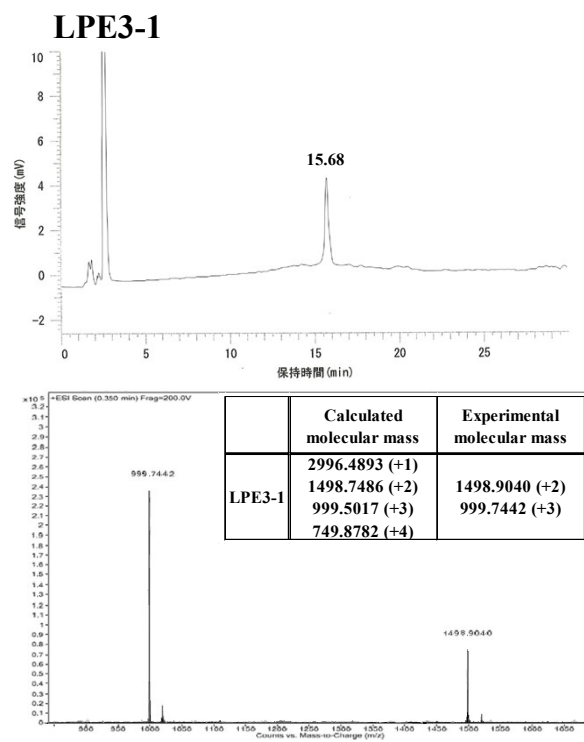
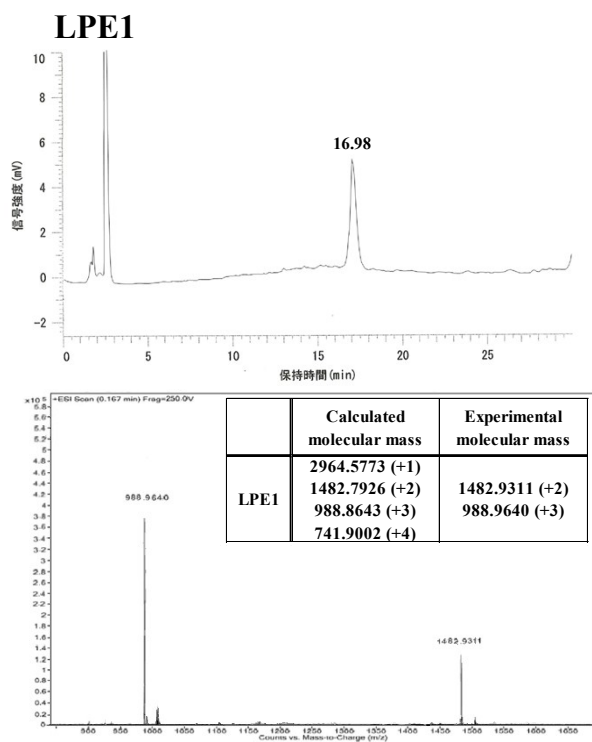


Fig. S2 Analytical HPLC chromatograms and mass spectra of the designed weakly acidic pH-selective lytic peptides mutated at the hydrophobic helical segment (LPE series).

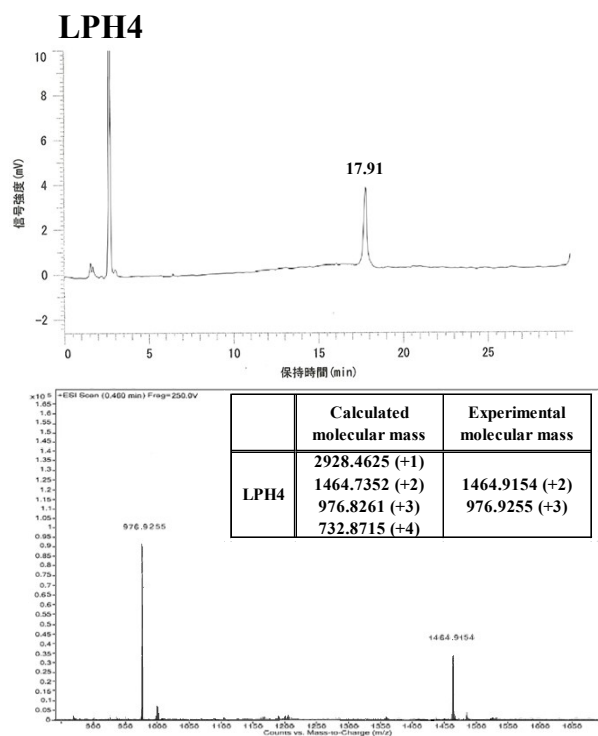


Fig. S3 Analytical HPLC chromatogram and mass spectrum of LPH4 replaced cationic arginine and lysine residues with histidine.

Characterization of membrane lytic properties of LP

Characterization of pH-dependence of membrane lytic activity of LP

Membrane lytic properties of LP were characterized by calcein-leakage assay as same procedures written in “Experimental Section”.

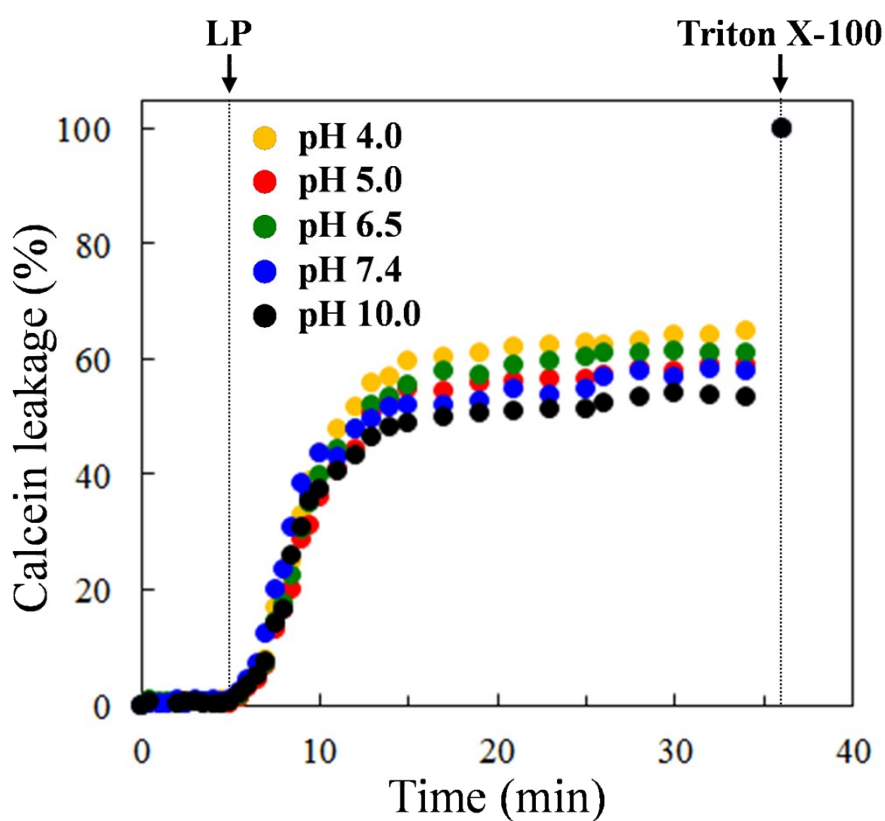
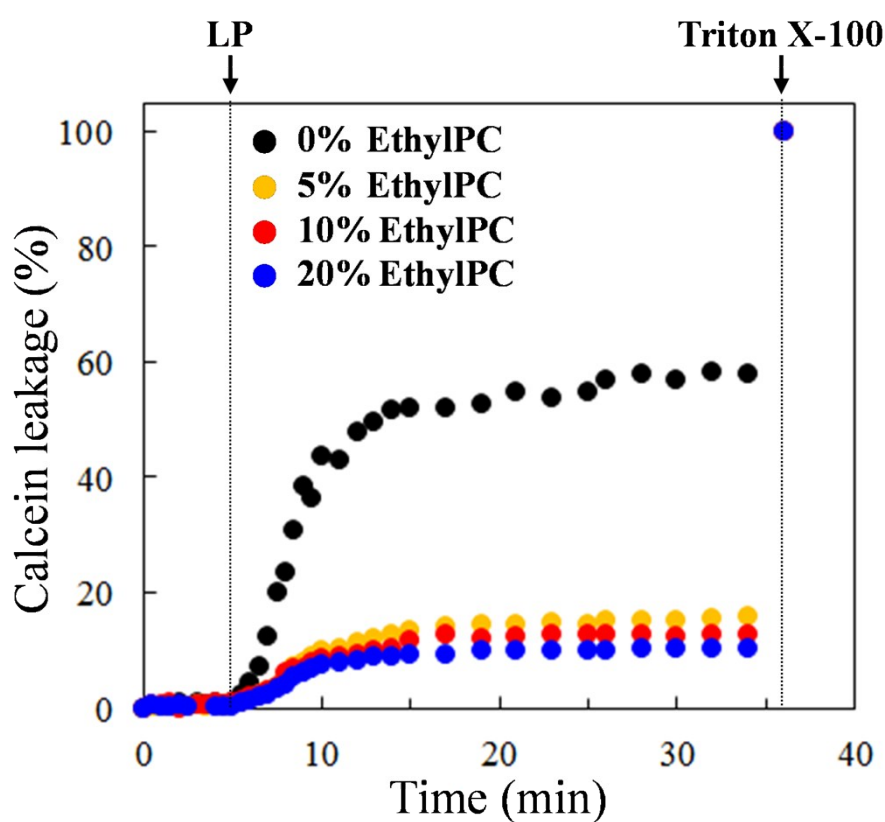


Fig. S4 Membrane lytic activities of LP in the range of pH 4.0 to pH 10.0. Calcein leakage from EggPC liposomes was measured every 30 or 60 s a following the addition of 20 μ M of LP at 25 $^{\circ}$ C.

Characterization of contribution of electrostatic interactions at the surface of the liposomes to membrane lytic activity of LP



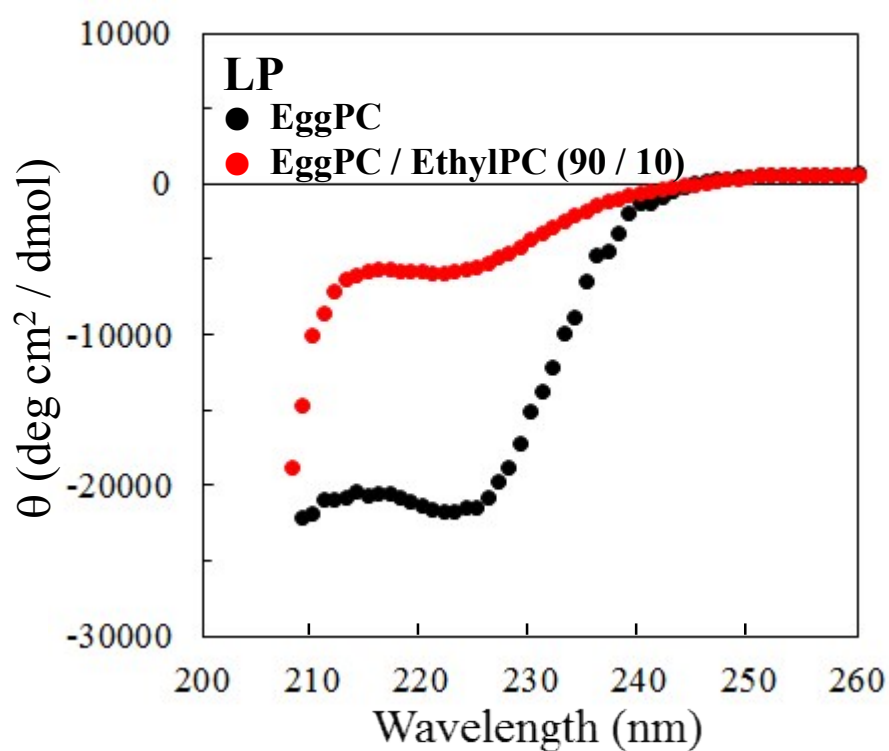


Fig. S6 Circular dichroism spectra of LP in the presence of EggPC and cationic charged (EggPC/EthylPC = 90/10) liposomes at pH 7.4. These spectra were taken for solutions of 20 μ M LP in the presence of 2.0 mM lipids at 25 $^{\circ}$ C.

Characterization of pH-selective (or -responsive) membrane lytic properties of designed peptide, LPE3-1

Membrane-penetrating property of lytic peptides characterized by fluorescence spectroscopy

Membrane-penetrating properties of lytic peptides (LP and LPE3-1) were characterized by the fluorescence of the Trp residue as same procedures written in “Experimental Section”.

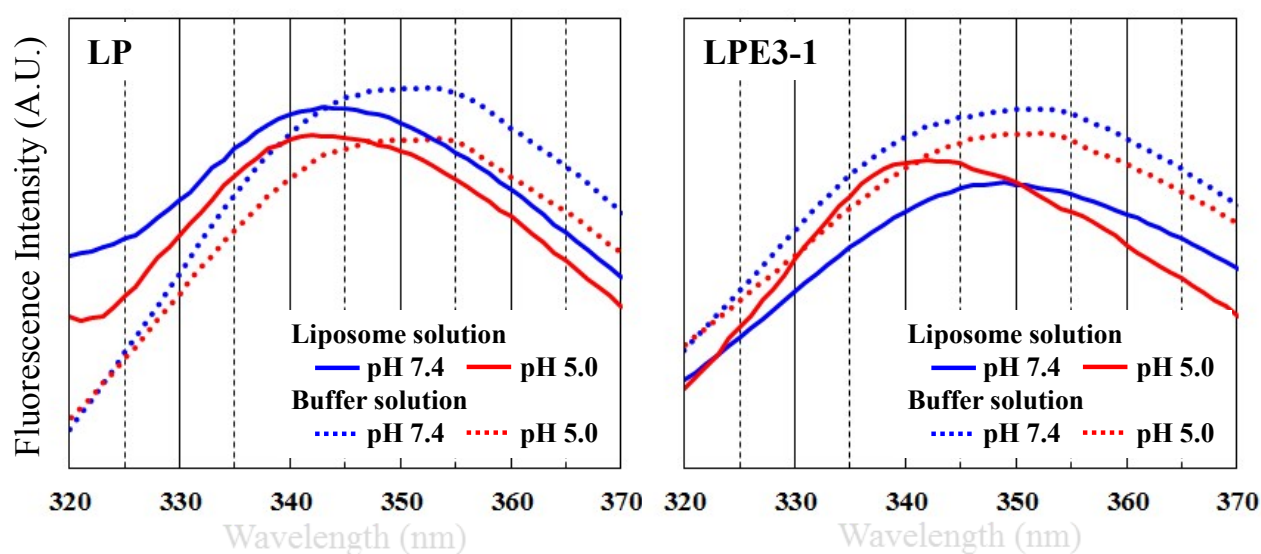


Fig. S7 Fluorescence spectra of LP and LPE3-1 in the presence or absence of liposomes at pH 7.4 and pH 5.0. These spectra were taken for 20 μ M peptide solutions at 25 $^{\circ}$ C.

Characterization of pH-responsive membrane lytic properties of LPE3-1 by membrane accessibility assay

Membrane lytic properties of LPE3-1 were characterized by membrane accessibility assay as same procedures written in “Experimental Section”.

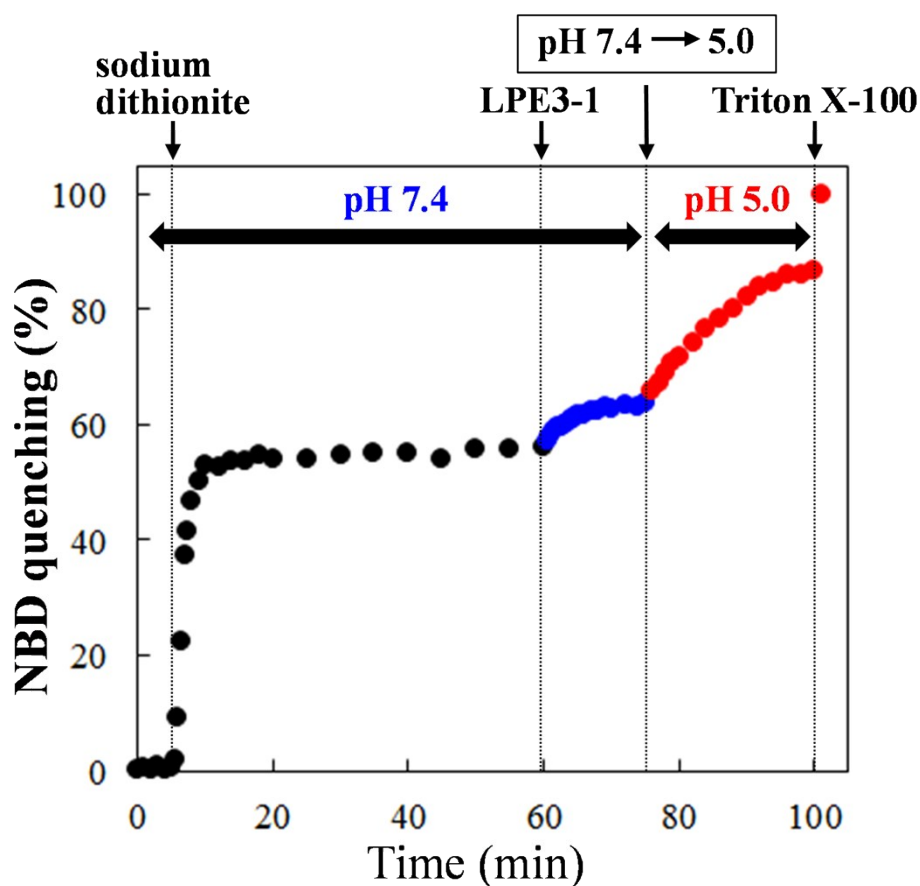


Fig. S8 pH-activated membrane lysis of LPE3-1 characterized by the membrane accessibility assay. LPE3-1 was added to liposome (inner-leaflet of liposome was labeled by NBD) solution at pH 7.4. After 15 min incubation, the pH was lowered to a value of 5.0 by adding hydrochloric acid.

Monitoring the liposome integrity during the membrane lytic process by LPE3-1

Size distribution of liposomes during membrane lytic process was characterized by the dynamic light scattering (DLS) measurements as same procedures written in “Experimental Section”.

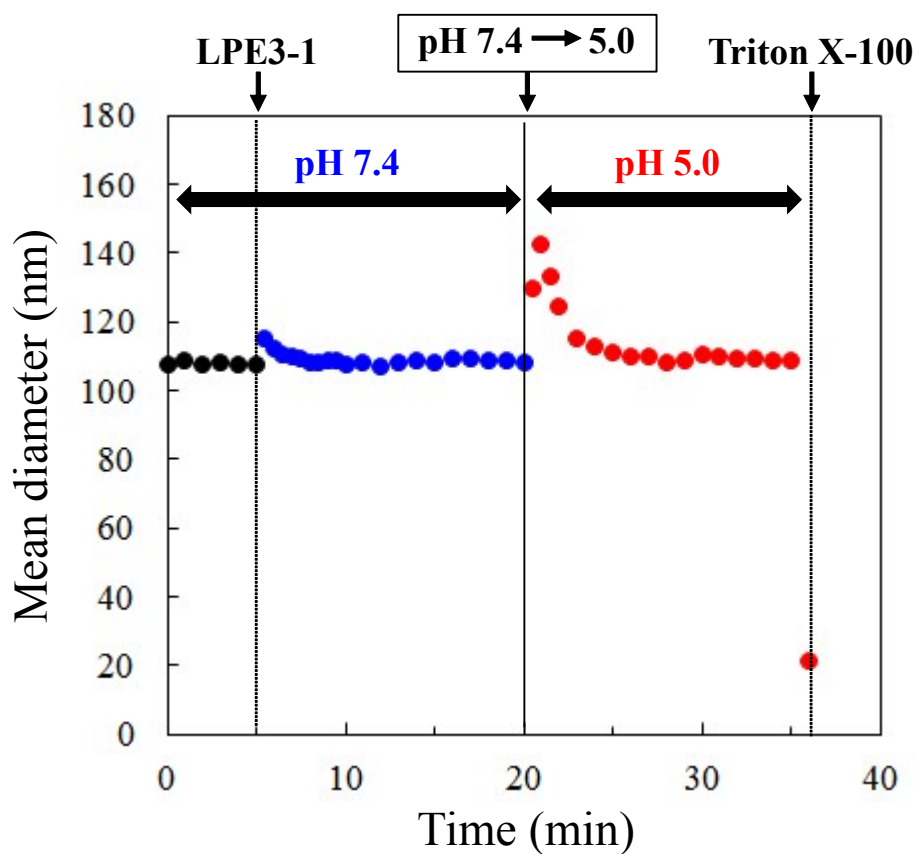


Fig. S9 Mean diameter from DLS measurements during pH-activated membrane lytic process by LPE3-1. LPE3-1 was added to liposome solution at pH 7.4. After 15 min incubation, the pH was lowered to a value of 5.0 by adding hydrochloric acid.