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Upper critical solution temperature behavior of cinnamic acid and polyethyleneimine mixture and its effect on temperature-dependent release of liposome

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ABSTRACT

The mixture of polyethyleneimine (PEI) and cinnamic acid (CA) in HEPES buffer (pH 7.0) exhibited an upper critical solution temperature in the temperature range of 20–50 °C. CA would be electrostatically conjugated with PEI and the PEI-CA conjugate is thought to act as a thermo-sensitive polymer. On the optical microscope image of PEI/CA mixture, microparticles were found at 25 °C, disappeared when heated to 50 °C, and formed again upon cooling to 25 °C. PEI-CA conjugate was immobilized on the surface of egg phosphatidylcholine (EPC) liposome by adding PEI to the suspension of liposome incorporating CA. The size and the zeta potential of the liposome markedly increased by cooling the liposomal suspension from 50 °C to 20 °C. This could be ascribed to the cooling-induced self-assembling property of PEI-CA conjugate. The release profile of Rhodamine B base from liposome incorporating CA with PEI was investigated while the liposome suspension of 50 °C was exposed to the release medium of 20 °C, 30 °C, 40 °C and 50 °C. The release degree was higher at a lower temperature. When exposed to a lower temperature (20 °C, 30 °C, 40 °C), PEI-CA could be self-assembled and change its configuration on the surface of liposome, promoting the release from the liposome.

1. Introduction

Various kinds of temperature-responsive liposomes have been designed to render the liposomes release their contents in response to the temperature change of medium. The principle of temperature-responsive release is based on the phase transition of the liposomal bilayer membrane itself or the phase transition of the membrane-immobilized polymers (Grüll and Langereis, 2012; Al-Jamal et al., 2012; Ta and Porter, 2013; Ullrich et al., 2013; Zhou et al., 2012; Ayano et al., 2012). A phospholipid has its own phase transition temperature where the trans-to-gauche transition of the acyl chain takes place, and the phase transition temperature depends on the saturation degree and the length of the acyl chain (Chen et al., 2012; Janůšova' et al., 2011; Pippa et al., 2015). As a result of the phase transition of phospholipid, the liposomal membrane is subject to the solid gel-to-liquid crystal transition, and exhibits a high permeability to a water-soluble compound, leading to a temperature-sensitive release (Wang and Kim, 2014; Qiu et al., 2014; Wang and Kim, 2013). On the other hand, thermo-sensitive polymer was immobilized on liposomal membrane to sensitize the liposome to the temperature change of medium. Poly (*N*-isoproylacrylamide) (PNIPAM) is one of the representative thermo-sensitive polymers used for the preparation of thermo-responsive liposomes. PNIPAM exhibits a lower critical solution temperature (LCST) in an aqueous solution. When the solution is heated from below to above LCST, PNIPAM becomes insoluble in water and the polymer chains take a contracted form. Due to the thermal contraction, the liposomal membrane will be mechanically stressed and disordered, giving a rise to temperature-sensitive release (Pippa et al., 2015; Wang and Kim, 2014; Qiu et al., 2014; Wang and Kim, 2013; Zhou et al., 2012). In this study, it was demonstrated for the first time that the physical conjugate of CA and PEI, associated with each other through electrostatic interaction, exhibited an upper critical solution temperature (UCST) in an aqueous phase. And PEI-CA conjugate was immobilized on the surface of EPC liposome and the release profile of a fluorescence dye (e.g. Rhodamine B base) from the liposomes were investigated below and above the UCST.

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2. Materials and methods

2.1. Materials

L-α-Phosphatidylcholine (egg phosphatidylcholine), chloroform (Mw: 119.38), polyethylenimine (PEI; Mw: 2000), trans-cinnamic acid (CA; Mw: 148.16), rhodamine B base (Mw: 442.55), phosphotungstic acid hydrate (Mw: 2880.05) and sodium hydroxide (Mw: 40.00) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). *N*-(2-hydroxyethyl) piperazine-n′-(2-ethanesulfonic acid) (HEPES) was obtained from USB corporation (Cleveland, Ohio, USA). Sephadex G 100 was provided by GE Healthcare (Sweden). Water was doubly distilled in a Milli-Q water purification system (Millipore Corp, MA, USA) until the resistivity was 18 MΩ cm. All other reagents were in analytical grade.

2.2. Temperature-sensitivity of PEI-CA conjugate

PEI and CA were co-dissolved in 10 ml of HEPES buffer (pH 7.0) contained in a 20 ml glass vial so that the concentration of PEI/CA was 4.04 mg/ml/5.96 mg/ml, 3.03 mg/ml/6.97 mg/ml, 2.25 mg/ml/7.75 mg/ml, 1.62 mg/ml/8.40 mg/ml, and 1.11 mg/ml/8.89 mg/ml. The molar ratio of amino group to carboxyl group in the mixture solution was 7:3, 6:4, 5:5, 4:6, and 3:7, respectively. The PEI/CA mixture of which amino group to carboxylic group ratio was 7:3, 6:4, 5:5, 4:6, and 3:7 was abbreviated as PEI/CA (7/3) mixture, PEI/CA (6/4) mixture, PEI/CA (5/5) mixture, PEI/CA (4/6) mixture and PEI/CA (3/7) mixture. Table 1 shows the concentration of PEI and CA and the molar ratio of amino group to carboxylic group in PEI/CA mixture solution for the temperature-sensitivity measurement. The transmittance of the mixture solution was observed at 600 nm on a UV spectrophotometer (JENWAY 6505, UK) equipped with a temperature controller (Mettler, JENWAY Peltier Controller) in the heating and the cooling process in the temperature range of 20–80 °C.

2.3. Temperature-dependent self-assembling of PEI-CA conjugate

The temperature-dependent self-assembling phenomenon of PEI-CA conjugation was observed on a photomicroscope using a hot stage (FP-82HT, Mettler Toledo). A rubber O-ring (0.6 cm in diameter, 0.2 cm in thickness) was immobilized to the surface of a cover class using glue, the space in the O-ring was filled with PEI/CA (7/3) mixture solution (1.11 mg PEI/ml and 8.89 mg CA/ml in HEPES buffer (pH 7.0)), and it was covered with another clover class. The replica was mounted on the heating equipment and the self-assembling phenomenon of PEI-CA conjugation was investigated by heating and cooling the suspension in the temperature range of 20–50 °C.

2.4. Preparation of liposome

Liposome was prepared by film swelling and sonication method (Seo et al., 2013). 20 mg of EPC with and without 5 mg of CA was dissolved in 2 ml of chloroform and the solution was put in a 50 ml-round bottom flask. The organic solvent was removed on a rotary evaporator operated at 50 °C and 110 rpm. 2 ml of Rhodamine B base solution (0.1 mg/ml, in HEPES buffer (pH 7.0)) was added to the dry lipid film, and the flask was swirled at room temperature until the lipid film was completely detached from the glass wall. Then, the suspension was sonicated at room temperature using a bath type sonicator (Sonics & Materials, USA) for 10 min with 10 s of pulse-on and 10 s of pulse-off. Free dye was removed by a column chromatography using Sepadex G 100 filled-glass column (1.6 cm × 38 cm). After the liposome suspension was gel-filtered, the concentration of EPC was determined by Bartlett assay. In order to determine the concentration of CA, the liposomal suspension was mixed with ethanol in the volumetric ratio of 1:9, then the absorbance of the solution was determined at 271 nm on a UV spectrophotometer (JENWAY 6505, UK).

2.5. Determination of dye loading efficiency

The encapsulation efficiency of Rhodamine B base in liposome was obtained by calculating the percent of the amount of dye entrapped in liposome with respect to the total amount of dye used for the preparation of liposome. The amount of dye entrapped in liposome was determined by measuring the dye fluorescence intensity at 573 nm using the excitation wavelength of at 555 nm on a fluorescence spectrophotometer (Hitachi F2500, Japan) after Triton X-100 was added to the liposome suspension so that the concentration was 0.5% (w/v). The specific loading of dye in liposome, defined as the mass of dye entrapped in liposome per the unit mass of phospholipid, was also reported.

2.6. TEM of liposome

1 ml of the suspension of liposome incorporating CA (2 mg EPC/ml, 0.5 mg CA/ml, in HEPES buffer (pH 7.0)) or 1 ml of the suspension of liposome free of CA (2 mg EPC/ml, in HEPES buffer (pH 7.0)) was mixed with 1 ml of PEI solution (0.0624 mg/ml, in HEPES buffer (pH 7.0)) or 1 ml of blank HEPES buffer (pH 7.0). In the mixture suspension of liposome incorporating CA and PEI, the molar ratio of amino group to carboxylic group was 3:7. For the negative staining of the liposomes, the liposome suspensions were mixed with phosphotungstic acid solution (2%, pH 6.8) in equi-volumetric ratio and the mixture was allowed to stand for 3 h at room temperature. The suspensions were deposited on the formvar/copper-coated grid and dried at room temperature. The TEM photos were taken on a transmission electron microscope (LEO 912AB OMEGA, Germany) installed at Korea Basic Science Institute (KBSI, located in Chuncheon, Republic of Korea).

2.7. Measurement of size and zeta potential of liposome

1 ml of the suspension of liposome incorporating CA (2 mg EPC/ml, 0.5 mg CA/ml, in HEPES buffer (pH 7.0)) or 1 ml of the suspension of liposome free of CA (2 mg EPC/ml, in HEPES buffer (pH 7.0)) was preheated to 50 °C. The preheated liposome suspension was mixed with 1 ml of PEI solution (0.0624 mg/ml,

Table 1
Concentration of PEI and CA and molar ratio of amino group to carboxylic group in PEI/CA mixture solution for temperature-sensitivity measurement.

PEI/CA mixture	Concentration of PEI (mg/ml)	Concentration of CA (mg/ml)	Amino group to carboxylic group molar ratio in PEI/CA mixture
PEI/CA (7/3)	4.04	5.96	7:3
PEI/CA (6/4)	3.034	6.966	6:4
PEI/CA (5/5)	2.25	7.75	5:5
PEI/CA (4/6)	1.622	8.378	4:6
PEI/CA (3/7)	1.11	8.89	3:7