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Neutral and acid-adapted fatty acid vesicles of conjugated linoleic acid

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ABSTRACT

FAVs (fatty acid vesicles), originated from natural biomass and created available biointerface, have advantages of biocompatibility, low-cost, and easy self-assembly in aqueous solution due to their dynamic feature. However, there is no example of applying FAVs in contact with human body since they are inherently alkaline-adapted and pH windows for the FAV formation and application are very narrow and far away from the physiological pH range. In this work an attempt to turn the alkaline-adapted FAVs into neutral and acid-adapted ones was made by fabricating the amphiphile-hybrid vesicles of CLA (conjugated linoleic acid) with a typical cosmetic emulsifier SDS (sodium dodecylsulfate) and/or a co-emulsifier DA (dodecyl alcohol). pH windows for the SDS- and/or DA-hybrid FAV formation of CLA were judged by using dynamic light scattering criterion, and confirmed by small-angle X-ray scattering and room temperature transmission electron microscopy. The experimental results show that with the aid of H-bonding and ion-dipole forces among molecules of CLA, SDS and DA within the hybrid vesicle walls that were identified by Fourier transform infrared analysis, much wider pH windows of 2.5–11.7 for the hybrid FAVs were obtained by expansion from 8.0-9.0 for FAV of CLA alone, which was composition-dependent and made the hybrid FAVs become neutral and acid-adapted.

1. Introduction

Vesicles are one of the molecular assemblies with closed thin spherical shell, including liposomes [1,2], catanionic vesicles [3], polymersomes [4], and FAVs (fatty acid vesicles) [5]. Up to date, vesicles have attracted more and more attention due to their two advantages: one is that the structure and bilayer of vesicles are similar to that of modern cells, which makes them available as model protocell membranes [6–9], and another is that interior of the usual hollow structure of vesicles is similar to environment, which provides a promising application in drug delivery systems and micro-reactors [10,11]. Among various types of vesicles, metastable liposomes and polymersomes are more concerned about because of their less dynamic features and thus relatively high stability. Liposomes have been applied as sustained release carriers in food, drug and cosmetics [12] though they have to be prepared by homogenization and with assistance of organic solvents, additionally show poor permeability in transdermal-drug delivery system [13]. Polymersomes get used to being mainly a theoretical research model of vesicles since designer polymers are of limited application value due to complex molecular structures and the resultant biological incompatibility of both monomer and polymer [14]. FAVs are self-assemblies of fatty acids, the simplest natural amphiphiles, in a suitable pH environment [5,15–18], and they are either regarded as valuable "protocell models" to investigate origin-of-life [7,19–21] for their unique dynamic features, or expected to be potential carriers for loading drugs and other sensitive items similar to other vesicles [11,22] for their green and safe characteristics. In addition, the dynamic formation is one of the most prominent features of FAVs [16,18,23], leading them to be candidates for better investigating dynamic features of vesicles. Therefore FAVs should have deserved more attention but unfortunately less attention has been paid to, which comes down to FAVs being extremely pH-sensitive [20] and stable within 1–2 pH units around its pK_a but undesired disassembly with pH fluctuation [24–27]. This nature greatly hinders them from applying in the physiological pH range, so that there is no report about the application of FAVs in food, drug, cosmetics and relevant systems since being found more than 40 years [5].

FAVs originate from natural biomass but their biointerface is hydrophobic under acidic to neutral pH conditions and thus unstable in these situations. It was reported that an acid shift of alkaline pH windows for the FAV formation could be achieved through chemical modification [28] and polymerization [29] of fatty acids, or addition of basic amino acids [30,31] at the expense of biocompatibility and other natural properties. Equimolar SDBS (sodium dodecylbenzenesulfonate) caused the FAV formation of decanoic

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acid an acid shift toward 4.3–7.5 [32] that sheds light on expanding pH windows across the physiological pH range but might have caused physiological incomparability by SDBS itself, moreover, the excessive acid shift of pH window might have made the vesicular phase disappeared easily in the desired neutral region, which comes down to the lack of a comprehensive understanding of the molecular interactions that was different from the best-known electrostatic interaction of catanionic vesicles [33,34]. It was interesting that larger organic counter-ions [17,35–38] and long-chain alcohols [39] both caused an extra alkaline shift of the original alkaline pH windows for the FAV formation, which was against crossing the physiological pH range but provided inspiration for us how to compensate the aforementioned ill effect of an excessive acid shift. In this work a typical cosmetic emulsifier SDS (sodium dodecylsulfate) and a co-emulsifier DA (dodecyl alcohol) were employed separately or together as model additives since both of them are approved cosmetic ingredients. We make an attempt to expand pH windows for the FAV formation across slightly acidic to neutral pH including the pH of human skin by taking advantage of the synergistic effect of SDS and DA on ionized/protonated fatty acids from the viewpoint of molecular interactions, and preliminarily evaluate the potential of the neutral and acid-adapted FAVs to be applied in cosmetic formulae and transdermal-drug delivery systems. Conjugated linoleic acid (CLA) is adopted in this work as a model fatty acid, or the molecular building block for FAV construction, since it has many other advantages for the FAV formation and characterization besides its natural availability and beneficial biological activities [40,41]. One of the outstanding advantages of CLA is its low T_m (melting temperature) that is benefit for the FAV formation in aqueous solution at room temperature in comparison with its counterpart saturated fatty acid. The second worthy mention is polymerization activity resulted from conjugated double bond of CLA that is contributed to chemically tethering and stabilizing FAVs in virtue of intravesicular self-crosslinking with less morphological disturbance [42–45] being convenient for the following RT-TEM characterization.

2. Materials and methods

2.1. Materials

CLA (conjugated linoleic acid) was prepared by alkali isomerization of linoleic acid come from saponification of safflower oil (Cofco Co. Ltd.) and enriched by urea inclusion [42]. SDS with a purity of 99% was purchased from Acros Organics Co. Ltd. DA (dodecyl alcohol), ITX (2-isopropylthioxanthone) and other reagents are all analytical reagent grade except for 5-FU (5-fluorouracil) biochemical reagent grade, purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received without further purification. Ultrapure Millipore water(18.2 MΩ cm) was used throughout.

2.2. Dynamic light scattering

The binary or ternary aqueous solutions were prepared by dissolving different ratios of CLA, SDS and DA in 0.1 mol L^{-1} of NaOH solution on the base that the concentration of CLA was remained constant at 3 mmol L^{-1}, in which the mole fractions of CLA, SDS, and DA was referred to x_C, x_S and x_D, respectively. Each 5 mL of the aqueous solution was pipetted into a separate test tube, and then adjusted with a trace amount of 5 mol·L^{-1} of HCl solution to obtain a series of sample solutions at different pH. After equilibration for 3 days at room temperature, the accurate pH values of the samples were measured and recorded. The appearances of the samples (colorless, bluish fluorescence, cloudy or phase separation) were also recorded in digital photos. After the samples were filtered by Millipore filter(0.8 μm), the *SI* (scattering intensity) and the corresponding D_h (hydrodynamic diameter) of the samples were determined at 25 °C using DLS (dynamic light scattering) (ALV/DLS/SLS-5022F spectrometer, ALV-Laser Vertriebsgesellschaft mbH, Langen, Germany) with a 5 mW laser light source at 632.8 nm and the scattering angle being 90°.

2.3. Small-angle X-ray scattering

FAVs of CLA(40 mmol L^{-1}) at pH 8.6 were characterized by SAXS (small-angle X-ray scattering) technique and performed at 25 °C using a SAXS instrument (SAXSess mc2 spectrometer, Anton Paar GmbH, Austria), which mounted at a PW3830 X-ray generator with a long fine focus sealed glass X-ray tube operated at 40 kV and 50 mA. The ranges of q (scattering vectors) were 0.07–5 nm^{-1}. The SAXS data were analyzed by the GIFT (generalized indirect Fourier transformation) technique [46,47] and shown in Fig. S1.

2.4. Transmission electron microscopy

After adding 20 μL of aqueous ITX solution(0.22 mol L^{-1}) and purged by N_2 for 30 min, let each of the above DLS samples corresponding to different pH levels intravesicular self-crosslinking induced by UV irradiation [44] (PowerArc UV 100 mercury arc lamp, 365 nm, Blue Sky Special Lamps Development Co. Ltd., China) for 1 h at ambient temperature under N_2 atmosphere to obtain the relevant self-crosslinking FAVs solution that was convenient for RT-TEM (room temperature transmission electron microscopy) characterization. The morphology of each sample was imaged with TEM (JEM-2100, 200 kV, JEOL Ltd., Japan), in which a drop of the self-crosslinked FAVs solution(5 μL) was sprayed onto a carbon-coated copper grid, and the excess solution was blotted away using a strip of filter paper immediately and repeated three times, finally the copper grid was left to dry at room temperature.

2.5. Fourier transform infrared spectroscopy

The samples for FT-IR (Fourier transform infrared) measurement were prepared according to literature [48]. The typical SDS-hybrid FAVs formed in CLA(3 mmol L^{-1})/SDS(x_S = 0.5) system at pH 6.5 and the DA-hybrid FAVs formed in CLA(3 mmol L^{-1})/DA(x_D = 0.05) system at pH 9.2 were frozen in liquid nitrogen promptly, and the water was removed by freeze-drying in a freeze-dryer (LYOQUEST PLUS-85 freeze-dryer, Telstar, Spain) at −80 °C as samples, or directly dried at 80 °C as control samples. Then FT-IR spectra were performed on a FT-IR spectrometer (Nicolet iS50 FT-IR spectrometer, Thermo Fisher Scientific Co. Ltd., USA).

2.6. Hydrolysis percentage of SDS at acidic condition

An aqueous SDS solution(3 mmol L^{-1}) was prepared by dissolving SDS in CPBS (citrate-phosphate buffered saline, pH 2.5), and then the concentration of SDS was determined by two-phase mixed indicator titration method [49] both for before and after equilibration for 3 days at ambient temperature. The hydrolysis percentage of SDS to DA was calculated by the difference between the above two concentrations, which was used as reference for DA dosage in hybrid FAVs fabrication.

3. Results and discussion

3.1. A DLS criterion judging pH windows for the SDS-hybrid FAVs of CLA supported by TEM images

pH window for the FAV formation of CLA could be normally estimated by a usual combination of turbidity observation with acid-base titration [29,50,51] and the resulted pH range of 8.0-9.0