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Modulation of the carotenoid bioaccessibility through liposomal encapsulation

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ABSTRACT

The low bioaccessibility of carotenoids is currently a challenge to their incorporation in pharmaceutics, nutraceuticals and functional foods. The aim of this study was to evaluate the modulating effects of liposome encapsulation on the bioaccessibility, and its relationship with carotenoid structure and incorporated concentration. The physical stability of liposomes, lipid digestibility, carotenoids release and bioaccessibility were investigated during incubation in a simulated gastrointestinal tract. Analysis on the liposome size and morphology showed that after digestion, the majority of particles maintained spherical shape with only an increase of size in liposomes loading β-carotene or lutein. However, a large proportion of heterogeneous particles were visible in the micelle phase of liposomes loading lycopene or canthaxanthin. It was also found that the release of lutein and β-carotene from liposomes was inhibited in a simulated gastric fluid, while was slow and sustained in a simulated intestinal fluid. By contrast, lycopene and canthaxanthin exhibited fast and considerable release in the gastrointestinal media. Both carotenoid bioaccessibility and micellization content decreased with the increase of incorporated concentration. Anyway, the bioaccessibility of carotenoids after encapsulated in liposomes was in the following order: lutein > β-carotene > lycopene > canthaxanthin. Bivariate correlation analysis revealed that carotenoid bioaccessibility depended strongly on the incorporating ability of carotenoids into a lipid bilayer, loading content, and nature of the system.

1. Introduction

Carotenoids perform several important physiological roles in human health [1,2]. However, the effective utilization of carotenoids as nutraceutical ingredients is limited by their low bioavailability associating with limited solubility and poor physicochemical stability [3]. One of the significant challenges to increase carotenoid bioaccessibility is their solubility in the mixed micelles composed of lipid digestion products and bile salts that can transport them to the epithelium cells for adsorption. It is commonly believed that carotenoids are more bioavailable when solubilized in oil carriers compared to when they are present as crystals in vegetable tissues, owing to an easier transfer to the micelle phase [4]. Recently, improvements in the oral bioavailability of carotenoids, when they are incorporated into emulsion-based structured delivery systems, are well established in the pharmaceutical and food industries [5,6].

Liposomes are colloidal structures formed by the self-association of amphiphilic lipids into enclosed aqueous compartment surrounded by a lipid bilayer membrane. Compared to structured emulsion delivery systems, liposomes exhibit outstanding advantages, including their biocompatibility, sustained release potential and relatively small size [7–9]. More recently, liposomes loading carotenoid have presented promising results optimizing disease treatments and increasing carotenoid uptake in the field of drug and nutraceuticals [10,11]. Our earlier experiments explored a new type of liposomes composed of mixed lipids including native phospholipid (egg yolk phosphatidylcholine, EYPC) and nonionic surfactant Tween 80 as carriers for four different kinds of carotenoids [12–14]. However, despite the similarity between lipid bilayers and biomembranes, there is little empirical evidence regarding the potential biological fate of carotenoid-loaded liposomes when passing through the human gastrointestinal tract (GIT).

The bioaccessibility and bioavailability of carotenoids strongly depend on the type and amount of carotenoids consumed. Due to the difference in molecular hydrophobicity, polar carotenoids (i.e. xanthophylls), which are mainly located in the lipid surface of

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the lipid droplets, are more easily transferred into micelle phase than the apolar carotenoids (i.e. carotenes), which are mainly located in the triacylglycerol core of the droplets [15]. This behavior confers a relatively high solubility to xanthophylls in the bile lipid micelles, subsequently, increasing the bioaccessibility. However, it should be noted that after liposome encapsulation, the position of carotenoids in the lipid bilayer is probably altered. For example, there is a strong evidence that carotene (e.g. β-carotene) can orient across the liposomal membrane, instead of positioning in lipid core [16]. It is also possible that xanthophyll (e.g. lutein) adopts flexible orientation in a lipid bilayer [17]. The location of lipophilic component is directly responsible for their dissolving capacities in such colloidal system. Thus, it is reasonable to hypothesize that the bioaccessibility of carotenoid encapsulated in liposome might be different from that just emulsified in oil phases or food matrix. Furthermore, an edible delivery system for nutraceutical application should be capable of encapsulating a sufficiently high amount of bioactive components. This requirement brings a question related to the efficient adsorption of a relatively large physiological dose of carotenoid in a relatively small amount of lipid. It is expected that the adsorption of isolated carotenoids present in supplements, usually less than 10 mg (carotenoid/triglyceride, w/w), whether dry tablets, oily capsules, oleoresins or beadlets, would be bioaccessible when consuming with a normal meal [18]. Therefore, in the case of a liposome system, the correlation of carotenoid loading content with their bioaccessibility was also a target of scrutiny in the current study.

On the other hand, the bioaccessibility of encapsulated nutraceuticals is closely correlated with the nature of a delivery system, including composition, particle dimension, permeability and physical integrity. These factors determine the contact area of enzyme with lipid and the accessibility of digestive enzyme to liposomal membrane [19]. In turn, incorporation of carotenoids could also modulate the physical properties of lipid membrane [20]. Therefore, there might be a mutual relationship between carotenoid modulation on liposome and their bioaccessibility, requiring more detailed knowledge.

Therefore, the objective of the current study was to explore the potential of a liposomal delivery system to modulate the bioaccessibility of carotenoid. Four dietary carotenoids, that is, lycopene, β-carotene, lutein, and canthaxanthin are selected in a wide concentration range to understand the influence of molecular structure and loading content. The changes in physical properties and microstructure of the liposomes were characterized during the digestion in a simulated gastrointestinal tract. At the same time, the release behavior of carotenoids from liposomes was monitored when they passed through the various digestion stages. Ultimately, a bivariate correlation analysis was introduced to identify the main factors governing the bioaccessibility of carotenoid by liposome encapsulation.

2. Materials and methods

2.1. Materials

Egg yolk phospholipid (EYPC) was purchased from Chemical Reagent Plant of East China Normal University (Shanghai, China). Carotenoids including lycopene, β-carotene, lutein and canthaxanthin (all 98% purity) were a gift from Zhejiang Medicine Co., Ltd (Zhejiang, China). Polyoxyethylene sorbitan monooleate (Tween 80) was obtained from China Medicine (Group) Shanghai Chemical Reagent Co. (Shanghai, China). All other reagents were of analytical grade.

2.2. Preparation of carotenoid-loaded liposomes

Multilamellar liposomes loading carotenoids were prepared by the thin-film evaporation method [13]. Briefly, carotenoid was dissolved in chloroform together with the lipids composed of EYPC and Tween 80 at the fixed mass ratio of 1:0.72. The initial concentrations (IC = $m_{carotenoid}/m_{lipids}$, % w/w) of carotenoids were selected at 0.5%, 1.0%, 1.5%, and 2.0%, respectively. After dissolution, the liposomal system was transferred to a round-bottom flask attaching to a rotary evaporator at 55 °C for removing the organic solvent until a thin film was obtained. The film was vacuum-dried in oven to ensure removal of the solvent completely, and hydrated with 40 mL hydration media (0.01 M phosphate buffer solution, 150 mM NaCl, PBS, pH 7.4) by vortexing for 60 min at 55 °C. In order to prepare nanoliposomes, the liposomal suspension was then subjected to sonication process. The final samples were filled into vials and kept in the refrigerator (4 °C in the dark).

2.3. Size measurement

Size measurements were performed at (25 ± 0.1) °C using a zetasizer (Nano-ZS90, Malvern Instruments Ltd., United Kingdom) with a He/Ne laser (λ = 633 nm) and scattering angle 90°. All samples were diluted with 10 mL with deionized water to avoid multiple scattering phenomena. Immediately, the diluted sample was transferred into the polystyrene cuvette for size determination, and the particle size distribution was recorded by dynamic light scattering (DLS). The measurements were repeated three times.

2.4. Atomic force microscopy

Atomic force microscopy (AFM) was carried out with a commercial AFM (Dimension icon, Bruker Co., USA). The ScanAsyst mode was applied using silicon tip (TESP, Bruker, nom. frep. 320 kHz, nom. Spring constant of 42 N/m) with scan resolution of 512 samples per line. Just before the analysis, the liposomal samples were diluted in water to obtain a less sticky fluid. An aliquot of 1 μL of diluted sample was deposited on the clean mica surface and incubated for 30 min at room temperature. Then, samples were carefully washed with ultrapure water to eliminate salts and non-adsorbed vesicles, and dried for 24 h at room temperature. Height mode images are acquired simultaneously at a fixed scan rate (0.997 Hz) with a resolution of 512 × 512 pixels in the dimension of 5 μm × 5 μm. Processing of images and analysis was carried out using NanoScope™ software (Digital Instruments, version V614r1). All height images are presented after the second-order two-dimensional flattening.

2.5. In vitro digestion

The digestion of the liposomal samples, under simulated gastrointestinal tract conditions, was conducted with slight modifications of a previously described method [21]. An aliquot of 1.5 mL of the carotenoid-loaded liposomes was mixed with 13.5 mL of basal saline (140 mM NaCl, 5 mM KCl, and 150 μM BHT) for 10 min. To initiate the gastric digestion, the mixture was treated with 4.5 mL simulated gastric fluids (SGF) (containing 3.2 g/L pepsin in 1 M HCl), followed by adjusting pH to 2.0 using 1.0 M NaOH. After 1 h incubation at 37 °C, the pH of the sample was adjusted to 7.5 with 1.0 M NaOH. Then, 4.5 mL simulated intestinal fluid (SIF) (containing 4.76 mg/mL pancreatin and 5.16 mg/mL porcine bile extract in PBS, pH 7.5) was added. During 2 h of the intestinal digestion process, to neutralize the free fatty acids (FFAs) released from lipid digestion, the pH of the solution was maintained at 7.5 by adding 0.1 M NaOH manually. The volume of NaOH added over time was recorded throughout the digestion. It was assumed that the