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Biodegradable chitosan-glycolipid hybrid nanogels: A novel approach to encapsulate fucoxanthin for improved stability and bioavailability

Hindupur Ravi, Vallikannan Baskaran*

Department of Molecular Nutrition, CSIR-Central Food Technological Research Institute, Mysore 570020, Karnataka, India

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ABSTRACT

This study aimed to improve the stability and biological availability of carotenoid fucoxanthin (FUCO) encapsulated in chitosan (CS) – sodium tripolyphosphate (TPP) – glycolipid (GL) nanogels, prepared by ionic gelation method. Scanning Electron Microscopic analysis and Dynamic Light Scattering examination revealed smooth and spherical nanogels with size range of 200–550 nm in weight ratios of CS: TPP (2.5:1), CS: GL (1:0.5). The zeta potential values (+30 to +50 mV) indicate that CS-NGs with FUCO + GL were more stable than that of CS-NGs + FUCO with no GL (+15 mV). The Fourier Transform Infrared Spectroscopy (FTIR) showed an extensive hydrogen bonding interaction between the FUCO and CS. X-ray Diffraction revealed FUCO is distributed in a disordered (amorphous) state in CS-NGs. Encapsulation efficiency, loading capacity and the yield of CS-NGs with FUCO + GL were 90%, 47% and 70%, respectively which is, significantly higher, than that of CS-NGs + FUCO without GL. Stability studies illustrated that glycolipid offers enhanced FUCO stability ($t_{1/2}$, 45 h) with CS-NGs compared to that of with no GL ($t_{1/2}$, 15 h) and standard FUCO ($t_{1/2}$, <5 h). The bioavailability of FUCO *in vitro* from CS-NGs with GL was higher (68%) compared to that of CS-NGs + FUCO without GL (51%), FUCO with GL (35.5%) and control (21.5%). In conclusion, the stability and bioavailability of FUCO was improved by nanoencapsulation of FUCO with CS + GL.

1. Introduction

Fucoxanthin (FUCO), a non-provitamin A, allenic and epoxy marine xanthophyll carotenoid, exerts beneficial effects including hypolipidemic (Woo et al., 2010), anti-obesity (Maeda, 2009), anti-diabetic (Maeda, Hosokawa, Sashima, & Miyashita, 2007) and anti-carcinogenic effects (Miyashita et al., 2011). However, FUCO has limited bioavailability due to its lipophilic nature, and its biological availability can be enhanced by incorporating lipids as carriers and polysaccharides as encapsulating/coating matrices with the fact that lipids improve carotenoid bioavailability (Gorusupudi & Vallikannan, 2012). β-Carotene bioavailability improved with the dietary fat incorporation (Ribaya-Mercado, 2002). The absorption of dietary lutein, and its accumulation in adult rats is influenced by olive oil (Lakshminarayana, Raju, Krishnakantha, & Baskaran, 2007). Lutein absorption and activity of antioxidant enzymes in rats is influenced by phospholipid, oleic acid micelles and dietary olive oil (Lakshminarayana, Raju, Prakash, & Baskaran, 2009; Nidhi, Mamatha, & Baskaran, 2013). Gorusupudi and Vallikannan (2012) studied the effect of wheat germ oil and its glycolipid fraction in mice and reported an improved lutein absorption and tissue accumulation. Similarly, entrapment of carotenoids with the polymers reported improved stability and bioavailability of lutein (Arunkumar, Harish Prashanth, & Baskaran, 2013). These studies reveal that specific lipids and encapsulation helps in improving biological potential of carotenoids.

Encapsulation in the form of liposomes, micelles or nanogels, offers targeted delivery of nutrients/drugs to the body. Nanogels (NGs) are known to transport and deliver drugs, which are unstable in the biological systems and cannot readily diffuse across the intestinal mucosal barrier (Prego, Torres, & Alonso, 2005). Oral NGs are promising nutrient/drug delivery systems due to improved bioavailability, targetability, bioadhesion which execute the controlled release of them in the gastrointestinal tract (Chaudhry et al., 2008; Mozafari et al., 2008). They can be directly adhered to the mucosa, which is a pre-requisite step before the translocation process of nanogels to occur. Further, encapsulation of carotenoids enhances the stability of bioactives by protecting them from light, heat, low pH (gastric) and ionic variation (during food processing) when interact with other molecules (Yurdugul & Mozafari, 2004). In addition, the

* Corresponding author. Tel.: +91 8212514876; fax: +91 8212517233.
E-mail addresses: basrev@yahoo.co.in, baskaranv@cftri.res.in (V. Baskaran).

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advantages of encapsulation are to (1) reduce the reactivity of the core material with the environment (light, heat and water), (2) reduce the loss or degradation of the core compound, (3) promote easier handling/processing, (4) control the release of active compound and (5) mask the core taste (Wilson & Shah, 2007).

Astaxanthin was microencapsulated with the chitosan matrix (Higuera-Ciapara, Felix-Valenzuela, Goycoolea, & Argüelles-Monal, 2004) for stability enhancement (Kittikaiwan, Powthongsook, Pavasant, & Shotipruk, 2007). Chitosan-dextran sulphate nanoparticles have been used for controlled delivery of bioactive molecules and cells in bone regeneration (Valente, Gaspar, Antunes, Countinho, & Correia, 2013). Low molecular weight chitosan has been used to improve the bioavailability of lutein (Arunkumar et al., 2013). The available literature vividly demonstrates the potential application of the polymer chitosan for the protection of carotenoids.

Chitosan (CS), a cationic polysaccharide, is derived from deacetylation of naturally occurring biopolymer chitin, which is composed of glucosamine namely, 2-amino-2-deoxy-(1,4)-β-D-glucopyran. It is the most widely distributed biopolymer, and it is non-toxic, exhibit biocompatibility, biodegradability, non-immunogenic, non-carcinogenic and antibacterial properties (Sun & Wan, 2007). The phenomenon that CS dissolves in low acidic solution at pH less than 6.5 may perhaps be exploited in formulating CS-NGs by cross-linking with anionic compounds such as tripolyphosphate (TPP), polyaspartic acid sodium salt (Hu et al., 2008). In brown seaweed (*Phaeophyceae*), FUCO, is in association with the complex sulphated polysaccharide fucoidan, which protects FUCO from various environmental factors. So, similar approach is applied in this study using a biodegradable polymeric CS that complexes with FUCO thereby rendering its protection from light, heat, etc. Hence, CS would be the ideal polysaccharide for encapsulation of lipophilic carotenoid FUCO. It is expected that the CS with FUCO encapsules are nano in size and may favour the mucosal and epithelial cells uptake and intracellular trafficking of FUCO.

The present study aims at the preparation of nanoencapsulated FUCO with CS and glycolipid as a surfactant and carrier. Earlier, we have reported that wheat germ oil and its constituent glycolipid fraction enhance the bioavailability of lutein in mice model (Gorusupudi & Baskaran, 2013). Hence, in this study, wheat germ oil, being the rich source of glycolipids, has been exploited for dispersion of FUCO before being encapsulated to augment its stability and bioavailability (simulated digestion model). Further, this is the first study developed on hybrid nanogels for encapsulation of FUCO with chitosan-glycolipid as carriers for improved stability and bioavailability. The findings will have an impact in nutritional and pharmacological applications, where improved FUCO absorption is essential.

2. Materials and methods

2.1. Materials

Chitosan (CS) (deacetylation degree $\geq$ 75%) derived from crab shell was purchased from Himedia laboratories, India. Methanol, acetonitrile (HPLC grade), sodium sulphate, ammonium acetate, acetone, methanol, diethyl ether, ethyl acetate, acetic acid, chloroform and silica (60–120 mesh) were purchased from Sisco Research Laboratory, Mumbai, India. Hexane was purchased from Rankem Laboratories, Mumbai, India. Penta-sodium triphosphate (TPP), pepsin (porcine), bile extract (porcine) and pancreatin (porcine) were purchased from Sigma–Aldrich, St. Louis, USA. Groundnut oil (GNO), food grade, was obtained from the super market (Mysore, India). Standard FUCO from brown seaweed wakame was donated by Dr. T. Sugawara, Kyoto University, Japan. Indian brown seaweed for the FUCO extraction was collected from Mandapam coastal region, Tamilnadu (9.28° N, 79.12° E), India. Millipore water was used for HPLC analysis. Other chemicals were of analytical grade unless otherwise mentioned.

2.2. FUCO extraction from Indian brown seaweed Padina tetrastomatica

Seaweed was collected off the coastal region of Mandapam (9.28° N, 79.12° E, Tamilnadu, India), washed in de-ionised water, dried on blotting paper followed by air drying and then ground to a fine powder. FUCO was extracted from the dried seaweed powder (100 g) with the method developed in this study, with ice cold acetone: methanol (7:3) by swirling at 100 rpm in shaking water bath (Scigenics Orbitek, India) three times at 4 °C for 2 h (modified procedure of Haugan, Aakermann, & Liaaen-Jensen, 1992). The pooled extract was evaporated to dryness at 30 °C in a flash evaporator (Hahn-Shin, HS-2005V-N, Korea) and redissolved in methanol (200 mL) followed by the addition of water (20 mL) and hexane (200 mL) in a separatory funnel and swirled 4 times. To the lower methanol-water phase, water (300 mL) and diethyl ether (400 mL) were added, swirled and the upper ether phase with FUCO and other carotenoids was collected, flash evaporated at 30 °C, and the crude extract obtained was dissolved in 5 mL of hexane (a few drops of acetone were added if a residue is formed and isopropanol was added to remove water moiety, if any).

2.3. FUCO purification by open column chromatography

FUCO was purified from the crude extract by Open Column Chromatography (OCC) by the use of specific solvent systems with silica gel (particle size 60–120 mesh) (Sangeetha, Bhaskar, & Baskaran, 2009). In brief, an aliquot of crude extract (5 mL) was applied to open column chromatography (20 cm × 1.5 cm), the chlorophylls were eluted first with the hexane (200 mL) (modified procedure of Maeda, Hosokawa, Sashima, Funayama, & Miyashita, 2005) followed by hexane: acetone (9:1) and hexane: acetone (8:2), respectively to elute β-carotene. Finally, hexane: acetone (7:3) was used to elute FUCO as the brownish yellow band. The FUCO elute was concentrated by flash evaporator and made to 5 mL with the same solvent system and analysed by HPLC (procedure given). The respective spectra and absorption maxima (λ_{max}) of FUCO were recorded and used for the confirmation of purity by photodiode array (PDA) detector, and the same has been compared with the standard FUCO based on the peak area.

2.4. HPLC analysis of FUCO

An aliquot of crude extract, and the purified FUCO by OCC and TLC was evaporated under a stream of nitrogen and redissolved (100 μL) in acetonitrile: methanol: water 60:35:5 (v/v/v) containing 0.1% ammonium acetate (mobile phase) and the extract (20 μL) was injected to HPLC system (LC-10Avp; Shimadzu, Kyoto, Japan) equipped with photodiode array (PDA) detector (SPD-M20A, Shimadzu). FUCO was separated on a Princeton C_{30} (ODS) column (250 mm × 4.6 mm; 5 μm) isocratically eluting with 1 mL/min of mobile phase. FUCO was monitored at 446 nm using Shimadzu Class-VP version 6.14SP1 software. The peak identity of FUCO was confirmed by their UV–Vis spectra recorded with the PDA detector.