Food Hydrocolloids 57 (2016) 20–29

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Low density lipoprotein/pectin complex nanogels as potential oral delivery vehicles for curcumin

Mingyong Zhou [a, b], Taoran Wang [a], Qiaobin Hu [a], Yangchao Luo [a, *]

[a] Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, United States
[b] College of Life Science, Sichuan University, Chengdu 610064, China

ARTICLE INFO

Article history:
Received 14 July 2015
Received in revised form
11 December 2015
Accepted 12 January 2016
Available online 18 January 2016

Keywords:
Low density lipoprotein
Egg yolk
Pectin
Nanogels
Controlled release
Curcumin

ABSTRACT

Development of delivery systems from natural protein/polysaccharide complexes is of particularly interests for applications in food, pharmaceutics and biomedicine. In this study, novel nanogels smaller than 60 nm were prepared from egg yolk low density lipoprotein (LDL) and pectin complexation via a pH- and heat-induced facile process. The nanostructure of egg yolk LDL was elucidated for the first time under different pH conditions and its complexation with pectin was comprehensively investigated. Under optimized condition, the prepared nanogels had a diameter of 58 nm and zeta potential of −41 mV, with spherical shape, smooth surface, and homogeneous size distribution, as evidenced by dynamic light scattering, scanning and transmission electron microscopes. The Fourier transform infrared spectrum revealed that hydrophobic and electrostatic interactions were the driving forces to form nanogels. The innovative Nano Spray Drying technology was studied and optimized to obtain nano-size powder of nanogels which exhibited excellent re-dispersibility in water, overcoming the drying challenge (irreversible gelation) of egg yolk LDL and greatly expanding its practical applications. Curcumin was adopted as a model compound to investigate the drug delivery potential of LDL/pectin nanogels. The mass ratio of curcumin/LDL played an essential role in determining the particle size and encapsulation efficiency. The LDL/pectin nanogels had excellent stability under simulated gastrointestinal conditions with the presence of digestive enzymes and enabled controlled release of curcumin. The novel LDL/pectin nanogels have promising features for oral delivery of nutrients and drugs.

1. Introduction

In recent decades, nanoscale oral delivery vehicles have received extensive research interests in the fields of food, pharmaceutics, and biomedicine. Among various nanoscale vehicles, biopolymer polyelectrolyte nanocomplex (PEC) is of particularly interest due to their great biocompatibility and biodegradability, as well as excellent loading capacity (Jones & McClements, 2011; Luo & Wang, 2014a, 2014b). Protein-polysaccharide nanocomplex is among the most studied PEC delivery systems for drugs and nutrients. In such PEC vehicles, proteins contribute to extraordinary binding capability to bioactive compounds through hydrogen bonding and hydrophobic interactions, whereas polysaccharides serve as coating barrier to protect proteins from enzymatic degradation in stomach and ensure the controlled release in small intestine. In our previous work, we reported a promising potential oral delivery vehicle prepared from casein/pectin complexation under facile condition (Luo, Pan, & Zhong, 2015), and many other similar protein/polysaccharide PECs have been developed recently (Luo, Zhang, Whent, Yu, & Wang, 2011; Teng, Luo, & Wang, 2013; Yu, Hu, Pan, Yao, & Jiang, 2006). The continuous efforts are still being made to search for natural nano-structured materials for fabrication of nanoscale oral delivery systems.

Egg is a key ingredient of a variety of food products and egg industry is an important segment of the US and world food industries. Egg proteins have many desirable and unique functional properties, including emulsifying, gelling, and foaming characteristics (Kiosseoglou, 2003). Egg proteins generally include egg white (mainly ovalbumin) and yolk proteins. Ovalbumin as a food protein-based biomaterial has been widely studied for its potential to encapsulate and deliver bioactives (Elzoghby, Samy, & Elgindy, 2012), while yolk protein received very limited attention in this field. However, egg yolk as a natural biomaterial may possess a

* Corresponding author. Department of Nutritional Sciences, 3624 Horsebarn Road Ext., Unit-4017 University of Connecticut, Storrs, CT 06269-4017, United States.
E-mail address: Yangchao.Luo@uconn.edu (Y. Luo).

http://dx.doi.org/10.1016/j.foodhyd.2016.01.010
0268-005X/

greater potential than egg white, given the fact that egg yolk itself is a natural carrier for many bioactives present in egg, such as unsaturated fatty acid, lipophilic vitamins, and carotenoids. Low density lipoprotein (LDL), the major constituent in egg yolk protein responsible for nutrient transport, has been shown to have a micellar structure with heterogeneous diameter of 20–60 nm, consisting of a lipid core surrounded by phospholipids and proteins (Anton et al., 2003). The extraction and purification of LDL from egg yolk has been well established, and it has been extensively studied for emulsifying properties and widely used in food products to prepare stable emulsions, such as mayonnaises, salad dressings and creams. Recent evidence indicates that daily consumption of eggs significantly increases plasma lutein and zeaxanthin in various populations (Ballesteros, Cabrera, Saucedo, & Fernandez, 2013; Blesso, Andersen, Bolling, & Fernandez, 2013). It has been postulated that the consumption of eggs may result in a better transport and delivery of these carotenoids to the blood circulation, which may be attributed to the LDL nanoparticles in egg yolk. Nevertheless, there is little research on the nanostructure of egg yolk LDL and its potential for nano-delivery applications.

Several challenges are still yet to be resolved for applications of egg yolk LDL nanoparticles. Although egg yolk LDL exist as nanoparticles in nature, many processing conditions, including heating and freezing/thawing cycle, can easily induce irreversible gelation by triggering unfolding and covalent disulfide bonds among lipoproteins (Cordobés, Partal, & Guerrero, 2004; Telis & Kieckbusch, 1997), which will significantly destroy the nanostructure of LDL. Heating may also trigger the release of lipids from the core of LDL followed by large aggregation due to disruption of micellar structures. Thus, the ideal situation for developing LDL-based nanodelivery vehicles is to induce disulfide bonds among LDL lipoprotein fractions to form a nanogel structure by partial denaturation while maintaining its intact physical structure to avoid release of core lipids and extensive gelation. To achieve this goal, a secondary coating will be needed. By coating a polysaccharide onto LDL surface during heating, the release of lipids from the core and extensive gelation can be controlled. By far, several proteins, mainly β-lactoglobulin and ovalbumin, have been extensively studied to form nanoparticles with polysaccharides, such as pectin, gum Arabic, chitosan (Jones & McClements, 2011; Schmitt & Turgeon, 2011). One recent study reported the complexation between egg yolk LDL and different polysaccharides, with a focus on separation of LDL from egg yolk using polysaccharides rather than the nutrient delivery potentials (Navidghasemizad, Temelli, & Wu, 2015).

Another big challenge for practical applications is how to transform the LDL-based nanogels dispersion into dry powder, as freeze drying and traditional spray drying will certainly destroy nanostructure and induce severe aggregation or agglomeration (Kiosseoglou, 2003). Recently, Büchi® has introduced a new generation of spray drying technology, Nano Spray Drying, enabling the production of nano-sized solid powders from liquid samples. The Nano Spray Dryer B-90 utilizes an innovative vibration mesh spray technology, creating tiny droplets (before evaporation) in a size range of a smaller order of magnitude than in traditional spray dryers. This novel technology has been recently evaluated for its capability to produce powder particles in submicron size by different scientists for drug delivery purpose (Lee, Heng, Ng, Chan, & Tan, 2011; Li, Anton, Arpagaus, Belleteix, & Vandamme, 2010). Thus, this technology might be a potential solution to dry LDL-based nanoformulations and obtain nanoscale dry powders.

The first objective of the present work was to systematically characterize the nanostructure of egg yolk LDL. The physicochemical properties of freshly extracted LDL were analyzed at different pH conditions to understand its ultrastructure. The second objective was to develop LDL-based nanogels by investigating the complexation behavior with polysaccharides using pectin as a model polysaccharide. The encapsulation and delivery potentials of nanogels were then explored, including particle size, zeta potential, encapsulation efficiency and controlled release in simulated gastrointestinal conditions, using curcumin as a model compound. Thirdly, the Nano Spray Drying technique was studied as a novel strategy to obtain nano-size solid powders of LDL-based nanogels.

2. Experimental section

2.1. Materials

Fresh hen eggs were purchased from Uconn Dairy Bar at University of Connecticut and stored at refrigerator. All eggs were used within two weeks. Citrus peel pectin (galacturonic acid content of >74%) was purchased from Sigma–Aldrich Corp. (St Louis, MO, USA). Unless noted otherwise, other chemicals were of analytical grade and purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA).

2.2. Extraction of LDL

LDL was extracted from fresh hen egg yolk according to a method previously described (Moussa, Marinet, Trimeche, Tainturier, & Anton, 2002), with minor modifications. Briefly, eggs were carefully broken and albumen was eliminated by a commercial egg separator. Yolk was slowly rolled on a filter paper until it was fully dried. The vitelline membrane was penetrated by a pipet tip and the flowing yolk was collected in a beaker cooled in ice water. An equal volume of saline solution (0.17 M NaCl) was added into yolk and stirred for 1 h in iced water. The mixture was centrifuged twice at 10,000 *g* for 45 min at 4 °C to remove granules. Then, ammonium sulfate (40%) was added to the supernatant and stirred for another 1 h at 4 °C. The resulting viscous solution was centrifuged at 10,000 *g* for 30 min at 4 °C and the yellow supernatant was collected and dialyzed using 10 kDa cut-off membrane against deionized water overnight to eliminate ammonium sulfate, changing bath hourly in the first 4 h. The desalted solution was then centrifuged at 10,000 *g* for 5 h at 4 °C to fully remove free lipids. The supernatant after centrifugation was rich in LDL and its concentration was determined by Lowry assay using bovine serum albumen as standard protein, based on the average protein content in egg yolk being 12% (Anton et al., 2003).

2.3. Physicochemical properties of LDL

The physicochemical properties of freshly prepared LDL were analyzed at different pH conditions. The pH of LDL was adjusted to 3–12 using either NaOH or HCl. Then, particle size, polydispersity index (PDI), and zeta potential of LDL at different pH conditions were measured using a Nano Zetasizer ZS model (Malvern Instruments, Ltd., Worcestershire, UK). Particle size, expressed as apparent Z-average hydrodynamic diameter, was measured at a scattering angel of 173° at 25 °C, and zeta potential was calculated from the electrophoretic mobility of the sample. The morphology of LDL was observed under transmission electron microscopy (TEM), the detailed procedures were described in the Section 2.5.

2.4. Preparation of LDL/pectin complex nanogels

LDL/pectin nanogels were prepared based on heat-induced complexation between lipoprotein and polysaccharide. The detailed procedures were modified from a previously described method for LDL/carboxymethyl cellulose nanogels (He et al., 2015). LDL and pectin were separately prepared in distilled water at 2 mg/mL as stock solutions. LDL was kept as 2 mg/mL for all samples,