Food Hydrocolloids 70 (2017) 363–370

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Biopolymer nanoparticles for vehiculization and photochemical stability preservation of retinol

Flavia F. Visentini [a, b], Osvaldo E. Sponton [a, b], Adrián A. Perez [a, b], Liliana G. Santiago [b, *]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, CONICET, Argentina
[b] Área de Biocoloides y Nanotecnología, Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, 1 de Mayo 3250, Santa Fe 3000, Argentina

ARTICLE INFO

Article history:
Received 30 December 2016
Received in revised form
15 April 2017
Accepted 15 April 2017
Available online 18 April 2017

Keywords:
Retinol
Ovalbumin
Heat-induced nanoparticle
High methoxyl pectin
Biopolymer nanoparticle
Photochemical stability

ABSTRACT

This paper gives experimental information about the production of biopolymer nanoparticles (BNPs) for vehiculization and photochemical preservation of retinol (RET). BNPs production involved the combination of two biopolymer functional properties: (i) protein ability for binding RET, in order to form protein-RET nanocomplexes, and (ii) polysaccharide deposition onto the protein-RET nanocomplexes surface so as to obtain BNPs. A particular set of biopolymer materials was employed: native ovalbumin (OVA), OVA nanosized aggregate (OVAn) and high methoxyl pectin (HMP). Absorbance, particle size distribution and ζ potential measurements, suggested OVA aggregation mainly influenced the production of colloidal stable BNPs. At protein:HMP ratio ($R_{Prot:HMP}$) 2:1, the most appropriated pH values for obtaining BNPs were: 4.0 for OVA-HMP system, and 6.0 for OVAn-HMP system. RET photochemical decomposition in BNPs was examined over 30 h. It was observed that HMP deposition on protein-RET nanocomplex surface improved the RET photochemical stability. OVAn-RET-HMP nanoparticle formed at pH 6.0 promoted a lower RET photochemical decomposition (14.1%) in comparison with the one registered for OVA-RET-HMP nanoparticle formed at pH 4.0 (25.0%). Because of denatured/aggregated state, the OVAn particular features to bind RET and to interact with HMP could explain the observed BNPs performance. Information derived from this work could be extrapolated for vehiculization and protection of other photosensitive lipophilic compounds.

1. Introduction

Protein-polysaccharide interactions find several applications in a lot of research and development sectors, e.g. food, pharmaceutics, cosmetics, etc. In food industry, these interactions had been widely exploited for enhancement of biopolymer functionality in complex food, and for the obtention of supramolecular structures so as to control texture and stability of colloidal dispersed systems (Fioramonti, Martínez, Pilosof, Rubiolo, & Santiago, 2015; Perez, Carrera Sánchez, Rodríguez Patino, Rubiolo, & Santiago, 2012). More recently, studies about biopolymer interactions have been focused on the creation of biopolymer nanoparticles (BNPs) which can be used as delivery systems for vehiculization and protection of bioactive compounds (Fioramonti, Perez, Aringoli, Rubiolo, & Santiago, 2014; Noshad, Mohebbi, Shahidi, & Koocheki, 2015; Perez, Sponton, Andermatten, Rubiolo, & Santiago, 2015; Zhang et al., 2015). This challenge involves to know aqueous medium conditions (e.g. pH, ionic strength, biopolymer relative concentration, cosolutes presence) which promote the appropriate biopolymer self-assembly (Chanasattru, Jones, Decker, & McClements, 2009; Li & McClements, 2013; Qiu et al., 2017; Zeeb, Stenger, Hinrichs, & Weiss, 2016). This phenomenon is governed by different intermolecular forces, e.g. electrostatic, hydrophobic, van der Waals, hydrogen point, etc., which occur under certain environmental conditions in close relationship with physicochemical features of involved macromolecules. In nanotechnology field, the strategy employing biopolymer self-assembly principles is named *bottom-up* technology, being this one a powerful tool to obtain tailor-made particles with defined sizes, surface and delivery properties (Chen, Remondetto, & Subirade, 2006; Jones & McClements, 2010; Joye & McClements, 2014; Lesmes & McClements, 2009). BNPs for lipophilic bioactive compound delivery can be designed by applying the following functional properties:

* Corresponding author.
E-mail address: lsanti@fiq.unl.edu.ar (L.G. Santiago).

http://dx.doi.org/10.1016/j.foodhyd.2017.04.020
0268-005X/

(i) Ligand binding to protein for obtaining inclusion complexes (Joye & McClements, 2014; Perez, Andermatten, Rubiolo, & Santiago, 2014; Visentini, Sponton, Perez, & Santiago, 2016). Hydrophobic forces at specific protein domains mainly conduct molecular binding, which usually promote the ligand solubilization in aqueous medium. Besides, lipophilic ligand binding can be increased if protein conformation is altered by denaturation/aggregation process, e.g. controlled heat treatment (Sponton, Perez, Carrara, & Santiago, 2015a). In this case, a greater protein surface hydrophobicity could favor an increased ligand loading capacity per inclusion complex unit (Sponton, Perez, Carrara, & Santiago, 2016).

(ii) Polysaccharide deposition onto the inclusion complex surface (Perez et al., 2015). Electrostatic forces mainly govern this phenomenon, depending on polysaccharide chemical nature and protein conformational state (Fioramonti et al., 2014). In some cases, this leads to a cover formation which promote ligand protection against several injurious environmental factors (Perez et al., 2015; Zimet & Livney, 2009).

In this framework, the present paper is aimed to get experimental information about appropriate protein-polysaccharide interactions in order to produce BNPs for photosensitive compound delivery. The research especially emphasized on the production of nanosized particles with high colloidal stability in aqueous formulations. In concordance with previous studies, a particular set of materials was employed: native ovalbumin (OVA), OVA nanosized aggregate (OVAn) formerly characterized in our lab, retinol (RET) as a model of photosensitive lipophilic compound, and high methoxyl pectin (HMP) as anionic polysaccharide. OVA is a monomeric protein of 43 KDa, and it has 4 sulfhydryl groups (−SH) and one disulfide bond (S−S) per monomer (Weijers & Visschers, 2002). It is constituted of 385 aminoacids, from which a half is hydrophobic and mainly buried into the protein structure and a third are charged aminoacids (Nisbet, Saundry, Moir, Fothergill, & Fothergill, 1981). The OVA properties for binding hydrophobic ligand have been poorly studied, so this globular protein could be evaluated in innovative binding studies. OVAn is a nanosized heat-induced aggregate with a great surface hydrophobicity, which it could enhance the ability for binding hydrophobic ligands (Sponton, Perez, Carrara, & Santiago, 2015b). Finally, as it was recently reported, HMP is able to produce an electrostatic deposition (cover) onto the surface of protein-ligand complexes leading a great protection against injurious factors (Perez et al., 2015). Moreover, it is worthy to remark that this contribution is continued from a previous work, in which OVA and OVAn were assayed for vehiculization and photochemical protection of RET (Visentini et al., 2016). Results highlighted that RET photochemical stability depended on aqueous medium pH and protein conformational state (native vs aggregated). Nevertheless, the hypothesis if a polysaccharide electrostatic cover could enhance RET photochemical stability via favorable interactions with OVA and OVAn, remained to be confirmed. The present paper will address this hypothesis.

2. Materials and methods

2.1. Materials

Native ovalbumin (OVA, product A5503, purity 98% according to agarose gel electrophoresis) was purchased from Sigma (USA). Ovalbumin nanosized aggregate (OVAn) was produced according to Sponton et al. (2015b). Briefly, OVA dispersion was prepared at 10 g/L, 50 mM NaCl and pH 7.5. Then, 2 mL aliquots were dispensed in glass tubes and were heated in a water bath at 85 °C for 5 min. Subsequently, tubes were removed and immediately cooled in an ice bath. Tubes containing OVAn were kept at 4 °C until further analysis. Retinol (RET; product 17772, purity ≥95.0% according to HPLC) was also obtained from Sigma (USA). RET was kept in darkness under N_2 atmosphere at −20 °C according to manufacturer advice. High methoxyl pectin (HMP) was kindly supplied by Cargill (Argentina) and had 68.0 ± 2.0% degree of esterification (DE). According to Cargill information, composition (wt.%), was: 87.0% carbohydrate, 11.0% moisture, and 2.0% ash (Na^+ 480 mg/100 g and K^+ 160 mg/100 g, Ca^{+2} 200 mg/100 g, Mg^{+2} 30 mg/100 g and Fe^{+2} 2 mg/100 g).

2.2. Production of protein-retinol nanocomplexes

The obtention of OVA-RET and OVAn-RET nanocomplexes was performed according to Visentini et al. (2016). In summary, OVA and OVAn dispersions (23 μM) were prepared in phosphate buffer (pH 7, 50 mM). On the other hand, a 250 mM RET stock solution was prepared in ethanol. Then, protein (OVA or OVAn) dispersion and ethanolic RET solution were mixed so as to produce the saturation of protein binding sites. It is important to remark that final ethanol concentration in mixed systems was lower than 1 vol%; therefore, it could be assumed that no protein structural modifications were induced (Cogan, Kopelman, & Shinitzky, 1976). Protein-RET nanocomplexes dispersions were stored in darkness for 2 h for reaching equilibrium.

2.3. Obtention of protein-polysaccharide nanoparticles

Strategy used for biopolymer nanoparticles (BNPs) obtention involved the combination of two functional properties: (i) proteins (OVA and OVAn) ability for binding RET, in order to obtain protein-RET nanocomplexes, and (ii) HMP deposition onto the protein-RET nanocomplexes for obtaining BNPs. For this, a set for complementary techniques was applied. These ones are described as follows.

2.3.1. Biopolymer phase behaviour

In order to get information about protein-polysaccharide interactions promoting colloidally stable BNPs formation, a study of biopolymer phase behaviour was firstly performed. Biopolymer mixed systems (OVA-HMP and OVAn-HMP systems) were prepared at protein-polysaccharide concentration ratio ($R_{Prot:Ps}$) 2:1, according to Perez et al. (2015). For this, proteins (OVA and OVAn) and HMP dispersions were prepared in phosphate buffer pH 7.0. HMP dispersion was previously heated at 70 °C for 15 min for promoting the adequate polysaccharide hydration. In all systems protein concentration was 23 μM (0.1 wt.%, protein concentration used for RET-protein complexes obtention) and HMP concentration was 0.05 wt.%. Mixed systems were obtained by mixing the appropriate volume of each double concentrated biopolymer solution up to the final required bulk concentration. The phase behaviour in aqueous dispersions was evaluated considering two experimental approaches: (i) absorbance (ABS) measurements at 400 nm, and (ii) visual appearance of biopolymer systems at 24 h after preparation. For this, biopolymers mixed systems were prepared at different pH values from 7.0 to 2.5. The pH was adjusted by using 1 M HCl solution. ABS was determined as soon as systems were prepared, using a Jenway 7305 spectrophotometer (UK). ABS values were interpreted as a measure of biopolymer molecular size and/or the number of biopolymer absorbent entities (Perez et al., 2015). Subsequently, the biopolymer mixed systems at different pH values were kept in repose at room temperature (25 °C) for 24 h, and their visual appearances were registered by means of a photo camera (Cyber-shot 12.1 mpx, Sony, USA). According the phase behaviour