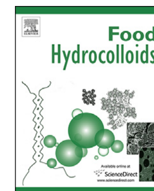
Food Hydrocolloids 39 (2014) 104–112

Contents lists available at ScienceDirect

Food Hydrocolloids

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



Improved the emulsion stability of phosvitin from hen egg yolk against different pH by the covalent attachment with dextran

Haiying Chen [a,b], Yamei Jin [b], Xiangli Ding [b], Fengfeng Wu [b], Mohanad Bashari [b], Feng Chen [c], Zhengwei Cui [a], Xueming Xu [b,*]

[a] *School of Mechanical Engineering, Jiangsu Key Laboratory of Advanced Food Manufacturing Equipment and Technology, Jiangnan University, Wuxi 214122, China*

[b] *The State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Wuxi 214122, China*

[c] *Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC 29634-0316, USA*

A R T I C L E I N F O

Article history:
Received 24 June 2013
Accepted 30 December 2013

Keywords:
Maillard
Dextran
Zeta-potential
Conjugation
Emulsion stability

A B S T R A C T

Phosvitin (Pv) from hen egg yolk was conjugated to dextran (Dex) through the initial stage of Maillard reaction in an aqueous system (Pv:Dex, 1:4 (w/w); Mw of Dex, 40 kDa) at 100 °C for 6 h. The red shift of λ_{max} occurred to Pv after the conjugation reaction from 355 to 362 nm and from 510 to 520 nm of intrinsic and extrinsic fluorescence emission spectra respectively. Circular dichroism spectra demonstrated that partial unordered secondary structure of Pv transformed into α-helix and β-turn after conjugating with Dex. The solubility of Pv–Dex conjugates represented an increase from 53.0% to 79.3% at pH 4.0. The zeta-potential of all tested emulsions decreased with lowering pH from 7.0 to 4.0. The interfacial thickness of Pv (1.0 mg/ml) absorbed onto latex particles at pH 4.0 was prominently increased by conjugation with Dex. Both the highest ESI and the slight fluctuation of D[4,3] of Pv–Dex conjugates illustrated that conjugation of Pv with Dex would be an effective method to improve its emulsion stability against more acidic pH environment.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Proteins are amphiphilic molecules that are commonly used as effective emulsifying agents in food products such as beverages, yoghurt, mayonnaise and ice creams, etc (Castano, Villamiel, & Fandino, 2007; Wooster & Augustin, 2006; Zhang, Qi, et al., 2012; Zhang, Wu, Yang, He, & Wang, 2012; Zhu, Damodaran, & Lucey, 2010). They impart excellent colloidal stability to emulsion droplets through a combination of electrostatic and steric mechanisms (Dickinson, 2008). However in the practical application, the emulsions stabilized by proteins tend to form aggregation, flocculation or coalescence when they are exposed to unfavorable environment (such as acidic food systems, particularly around the isoelectric point of the protein, the presence of electrolytes, or heat treatment) due to the loss of electrostatic charge of the protein (Day, Xu, Lundin, & Wooster, 2009) and the denaturation of the protein (Diftis & Kiosseoglou, 2006).

Modification of proteins by conjugation with a hydrophilic polymer is an effective approach to improve the solubility of proteins and further the stabilizing ability under these unfavorable conditions (Akhtar & Dickinson, 2003; Aminlari, Ramezani, & Jadidi, 2005; Castano, Fandiño, Olano, & Villamiel, 2005; Castano et al., 2007; Dickinson, 2008; Dunlap & Te, 2005; Liu, Zhao, Zhao, Ren, & Yang, 2012; Wang & Ismail, 2012; Wong, Day, & Augustin, 2011; Zhu et al., 2010). The convenient way to achieve covalent conjugates from the mixture of proteins and polysaccharides is through Maillard reaction (non-enzymatic browning), the condensation reaction of the carbonyl group of the reducing sugar with the available ε-amino groups of the protein (Ames, 1992). The Maillard reaction can be divided into three stages: initial, intermediate and advanced. The initial stage has been shown to be sufficient for conjugation between the protein and the polysaccharide (Dickinson & Euston, 1991).

The polysaccharide molecule attached to the protein provide an additional steric barrier between the emulsion droplets when the protein–polysaccharide complex adsorb at emulsion interfaces (Dickinson, 2008) and then stabilize them against creaming, flocculation and coalescence (Yadav, Parris, Johnston, Onwulata, & Hicks, 2010). At the same time, polysaccharides have the ability

* Corresponding author. Tel./fax: +86 51085917100.
E-mail addresses: fly88honey@126.com (H. Chen), xmxu@jiangnan.edu.cn (X. Xu).

0268-005X/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2013.12.031

to enhance the long-term stability by controlling the rheology and network structure of the continuous phase, and hence retarding phase separation and gravity-induced creaming (Dickinson, 2008). Dextran, the typical neutral polysaccharide, has been conjugated to improve the solubility at 4.0–6.0 and the emulsifying and foaming properties of peanut protein isolate (Liu et al., 2012) and whey protein isolate (WPI) (Sun, Yu, Zeng, Yang, & Jia, 2011). A whey protein isolate–dextran (WPI–Dex) conjugate purified by anion exchange chromatography and affinity chromatography remained soluble over a range of pH from 3.2 to 7.5 and ionic strengths from 0.05 to 0.2 M in contrast to native WPI (Zhu et al., 2010). The dextran molecules conjugated to the soy protein (protein to dextran, 1:9, w/w) were also demonstrated to effectively enhance the hydrophilicity and steric repulsion of the oil droplets, and therefore stabilize the emulsions against heat treatment (90 °C, 30 min), long-term storage (4 °C, 4 months), and the changes of pH (from 1 to 7) and ionic strength (0.05, 0.10, and 0.20 M NaCl) (Xu & Yao, 2009).

Phosvitin (Pv) was a phosphoprotein with an average molecular weight of 35 kDa and represented about 11% of hen egg yolk proteins (Anton, 1998). From the amino acid sequences, Pv can be represented like an elongated core of negatively charged phosphoserines, with a C-terminal part of about 15 residues relatively rich in hydrophobic amino acids (Byrne et al., 1984). The amphiphilic composition determined that Pv had good emulsifying properties, particularly the emulsion stability (Sattar Khan, Babiker, Azakami, & Kato, 1998). At the same time, Pv could act as a better emulsifier (both higher emulsifying activity and emulsion stability) than bovine serum albumin at the neutral pH values (Chung & Ferrier, 1991). Furthermore, non-aggregated Pv had higher emulsifying activity than aggregated Pv, but aggregated Pv better stabilized emulsions against coalescence (Castellani, Briand, Dubiard, & Anton, 2005). In addition, the conjugation of Pv with galactomannan (GA) by Maillard reaction (60 °C, in 79% relative humidity for 1 week) had dramatically improved both emulsifying activity and emulsion stability (Sattar Khan et al., 1998; Sattar Khan, Babiker, Azakami, & Kato, 1999). The loss of the improved emulsifying properties after protease digestion of Pv–GA demonstrated that the N- and C-terminal regions were both essential for the excellent emulsifying properties of Pv or Pv–GA conjugate (Sattar Khan et al., 1999).

In our previous research, Pv has been conjugated with dextran under high temperature in an aqueous solution via the initial stage of Maillard reaction (Chen, Wang, et al., 2013). To achieve the highest conjugation degree with the smallest browning and denaturation of protein, the optimum conjugation condition chosen was per Pv (5 mg/ml) conjugated with 4-fold dextran (Mw, 40 kDa) at 100 °C for 6 h. The content of free amino groups in the conjugated Pv decreased to 77.4% after reaction for 6 h. Meanwhile, the secondary structure of Pv showed significant changes after the Maillard reaction. However, the physicochemical properties of the conjugates, especially at different pH values, have not been characterized yet. So, the aim of this study is to analyze the effect of pH on the solubility, surface hydrophobicity, secondary structure, zeta-potential, interfacial layer thickness, and emulsion fat globule size of the Pv–Dex conjugates.

2. Materials and methods

2.1. Materials

Hen eggs (three or four days old) and refined rapeseed oil were purchased from a local supermarket in Wuxi City (Jiangsu, China). Pv used as the reactant was isolated from hen egg yolk according to the method described by Castellani et al. (2005), while Pv used as the standard was obtained from Sigma (St. Louis, MO, USA). Pv was stored in a hermetically sealed container at 4 °C until used. Dex (the linear polysaccharide) from *Leuconostoc mesenteroides*, 1, 8-anilinonaphthalenesulfonate (ANS), and polystyrene latex aqueous suspension (with solid content of 10%, w/v), were also purchased from Sigma (St. Louis, MO, USA). All other reagents used in this study were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Preparation of Pv–Dex conjugates

Pv–Dex conjugates were prepared following the method described by Chen, Wang, et al. (2013) with a slight modification. The ratio of Pv and Dex was 1:4 (w/w), and the reaction temperature was set at 100 °C. As a blank, the protein solution without dextran was disposed under the same condition. Samples were then taken out after incubation of 6 h and immediately cooled in an ice water bath to stop the reaction. The conjugated degree was calculated from the reduced content of free amino groups and was 22.6% (Chen, Wang, et al., 2013). The conjugated system was not separated from the unreacted protein and polysaccharide in this study and defined as Pv–Dex conjugates to distinguish with the blank system. Triplicates were carried out for each experiment.

2.3. Measurement of the solubility as a function of pH

The solubility of Pv, Pv–Dex mixture and Pv–Dex conjugate as a function of pH was determined according to the method of O'Regan and Mulvihill (2009) with a slight modification. Samples (5 mg protein/ml, w/v) were dissolved in Milli-Q water at the ambient temperature under moderate magnetic stirring conditions for 1 h. The pH was adjusted to values in the range of 2.0–7.0 (1.0 unit intervals) using 0.1 N HCl or 0.1 N NaOH as required.

2.4. Measurement of intrinsic and extrinsic fluorescence emission spectra

Intrinsic fluorescence emission spectra were measured with a Hitachi F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Sample (5 mg protein/ml, w/v) solutions were prepared in 10 mM phosphate buffer solution (pH 7.0). The excitation wavelength (slit width, 5 nm) was set at 295 nm (the peak of the prescanning spectrum) and the emission wavelength range was from 200 to 500 nm, with a scanning speed of 1200 nm/min. The phosphate buffer solution (10 mM, pH 7.0) was used as the blank for all samples.

Extrinsic fluorescence emission spectra were determined using ANS as a fluorescent probe according to the method of Kato and Nakai (1980) with some modifications. Stock solutions of 8 mM ANS and 10 mg/ml protein were prepared in 10 mM phosphate buffer (pH 7.0), and then 4 ml of protein-containing buffer and 20 μl of ANS stock solution were mixed. The samples were shaken on a vortex mixer for about 5 s. Fluorescence intensity (FI) was measured at the excitation wavelengths of 310 nm at 20 °C with the emission wavelength scanning from 200 to 600 nm.

2.5. Preparation of oil-in-water emulsions

The Pv, Pv–Dex mixture and Pv–Dex conjugate solutions were prepared in 10 mM sodium phosphate buffer solution (pH 7.0). Oil-in-water emulsions were prepared by mixing 2 ml of rapeseed oil with 6 ml of aqueous protein dispersions (5 mg protein/ml, w/v) at the ambient temperature. Each emulsion was prepared by adding the oil phase into the aqueous phase and homogenizing using a

Download English Version:

https://daneshyari.com/en/article/6988233

Download Persian Version:

https://daneshyari.com/article/6988233

Daneshyari.com