Food Hydrocolloids 60 (2016) 29–37

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Emulsifying properties of ovalbumin: Improvement and mechanism by phosphorylation in the presence of sodium tripolyphosphate

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ARTICLE INFO

Article history:
Received 30 October 2015
Received in revised form
28 February 2016
Accepted 2 March 2016
Available online 10 March 2016

Keywords:
Ovalbumin
Phosphorylation
Emulsifying properties
^{31}P NMR
XPS
Mechanism

ABSTRACT

In this work, ovalbumin (OVA) was phosphorylated by dry-heating at three different pH values (5.0, 7.0 and 9.0) and 45 °C for 12 h in the presence of sodium tripolyphosphate (STP). The results indicated that, when compared with native OVA (N-OVA), the zeta potential absolute value of phosphorylated OVA (PP-OVA) increased from 2.13 mv (N-OVA) to 13.57(PP-OVA-pH5.0), 16.00(PP-OVA-pH 7.0) and 11.20 mv (PP-OVA-pH 9.0); the volume-weighted mean diameter (D [4,3])of emulsions stabilized by the N- and PP-OVA decreased from 70.22 μm (N-OVA) to 37.16(PP-OVA-pH 5.0), 16.21(PP-OVA-pH 7.0) and 43.52 μm (PP-OVA-pH 9.0); and PP-OVA emulsions exhibited a more narrow monomodal size distribution and a smaller particle size than those of N-OVA, with the corresponding emulsifying activities index (EAI) increased from 92.71(N-OVA) to 142.51(PP-OVA-pH5.0), 224.88(PP-OVA-pH 7.0), and 150.97(PP-OVA-pH 9.0), respectively. The microscopic observation showed that the emulsion droplets of PP-OVA were more densely and uniformly distributed than those of N-OVA. Furthermore, characterization of PP-OVA by Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and ^{31}P nuclear magnetic resonance (^{31}P NMR) revealed that phosphate groups were bound to the -OH on serine and threonine and the -NH_2 on lysine and arginine, with covalent interactions (C—O—P and C-N-P bounds) formed between OVA and STP. This study demonstrated that the introduction of phosphate groups could increase the amount of negative charge and intermolecular repulsion, decrease the particle size of droplets and improve the emulsifying ability of PP-OVA.

1. Introduction

Egg white protein (EWP) is not only used as a good source of protein but also as a functional food material in food processing (Matsudomi, Nakano, Soma, & Ochi, 2002). Ovalbumin (OVA), the main constituent of egg white protein with a molecular weight of 45 kDa and a pI of 4.5, consists of 385 amino acids residues and has a single carbohydrate chain linked to Asn-292 and an acetyl group at the N-terminus (Enomoto, Ishimaru, Li, Hayashi, Matsudomi, 2010; Huntington & Stein, 2001). Additionally, OVA contains both hydrophobic and hydrophilic groups and always acts as emulsifier, foaming agent, gels, etc. in food due to its good emulsifiability, foamability and gelation ability (Le Floch-Fouéré, Pezennec, Lechevalie, Beaufils, Desbat, 2009; Pezennec, Gauthier, Alonso, Graner, Croguennec, 2000).

It is expected that the improvement in the functional properties of food proteins could maximize their effective use in the food industry. Previous studies have confirmed phosphorylation has been recognized as an efficient method for improving the functional properties of food proteins such as soybean, egg white, and buffalo milk proteins in terms of heat stability, emulsification, foamability, gelation, water- and oil-binding capacity, the calcium phosphate-solubilising abilities and so on (Krupa, Preethi, & Srinivasan, 2004; Li, Ibrahim, Sugimoto, Hatta, & Aoki, 2004; Miedzianka & Pęksa, 2013; Nayak, Arora, Sindhu, & Sangwan, 2006).

Food proteins can be phosphorylated by enzymatic and chemical methods. Despite the most desirable method for phosphorylating food proteins with respect to food safety (Feeney & Whitaker, 1985), enzymatic modification brings in few phosphate groups for the specificity of the substrate. Such a low level of phosphorylation is not sufficient to improve the functional properties of food proteins. Additionally, this method is not suitable to fulfill the needs of industrial-scale production due to the high cost of enzymes (Campbell, Shih, & Marshall, 1992). Comparatively, chemical

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http://dx.doi.org/10.1016/j.foodhyd.2016.03.007
0268-005X/

modification is a more economical and effective method, ensuring wide application of proteins as food ingredients and the effective use of unutilized proteins in the food (Enomoto, Li, Morizane, Ibrahim, Sugimoto, 2008; Wang & Chi, 2012).

Phosphorylation in food proteins can increase the electronegativity and decrease the pI of phosphorylated proteins, thereby changing the functional properties, especially near the pI of the original proteins (Zhang, Li, & Ren, 2007). Extending the application of proteins by phosphorylation is very significant. Several studies have been carried out on OVA phosphorylation. For example, (Hayashi, Nagano, Enomoto, Li, Sugimoto, 2009). analyzed the effects of phosphorylation on the foaming property of egg white protein at pH 4.0 and 85 °C through dry-heating in the presence of pyrophosphate, and found that the foaming stability of phosphorylated egg white protein was much higher than that of the untreated egg white protein. Lv and Chi (2012) investigated the structural and functional properties of phosphorylated OVA and found that phosphorylation definitely improved its heat-induced insolubility, emulsifying properties, water- and oil-binding capacity, and calcium phosphate-solubilizing ability.

Several researchers also focused on the mechanism of protein phosphorylation. Enomoto et al., (2010). investigated the effects of the introduction of phosphate groups into the carbohydrate chain on the structure and the heat stability of OVA phosphorylated by dry-heating in the presence of pyrophosphate. They found that the introduction played an important role in both the structure and the heat stability of OVA. Additionally, the basic proteins showed more tendency towards phosphorylation than acidic proteins (Li, Hayashi, Enomoto, Hu, Sawano, 2009). However, the mechanisms of the phosphorylation of OVA (such as whether the phosphate linkages, including N—P, N—PP and N—PPP, were introduced to OVA) are not fully explained because of the complex interactions among the different amino acids as well as the occurrence of denaturation for OVA in acidic proteins by dry-heating in the presence of pyrophosphate.

The objectives of the present study were to phosphorylate OVA with sodium tripolyphosphate, a food additive commonly used in food industry, at three different pH values (5.0, 7.0 and 9.0), and analyze the effects of phosphorylation on zeta potential, particle distribution and emulsifying properties of OVA. Furthermore, the phosphorylated OVA was characterized by Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and ^{31}P nuclear magnetic resonance (^{31}P NMR), and the mechanism of the improvement of its emulsifying ability was also explored. The results of this study will provide the theoretical foundation and practical references for applications of OVA in hydrocolloids, protein beverages, and cakes.

2. Materials and methods

2.1. Materials

Polyethylene glycol 8000 (PEG-8000) was made by Merck Chemicals Co., Ltd. (Shanghai China). Q Sepharose Fast Flow was supplied by RuiDaHengHui S & T Co. Ltd. (Beijing, China). Corn oil was purchased in the local market. Sodium tripoly phosphate (STP) was purchased from Aladdin Industrial Co. Ltd. (Shanghai, China). All other chemicals used in the experiment were produced by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and were of analytical grade. Ovalbumin (OVA) was purified by the following two steps (Geng, Huang, Wu, Ren, Shan, 2012): (1) Egg whites were well mixed with 3 volumes of 50 mM NaCl solution by stirring for 2 h. The pH of the solution was adjusted to 6.0 with 2 M HCl, and PEG-8000 was added while stirring (final w/w = 10%). The dispersion was allowed to settle for 2 h, followed by centrifuging the homogenate at 15,000 g at 4 °C for 10 min and collecting the supernatant. (2)The supernatant was applied to a Q Sepharose Fast Flow column (60 mm × 16 mm, Automatic low pressure liquid chromatography system (JiaPeng Technology Co., Ltd., Shanghai, China). The flow-through fraction was eluted using Tris—HCl buffer (pH 8.0, 20 mM), followed by isocratic elution using 20 mM Tris—HCl buffer (pH 8.0) successively containing 0.08, 0.18 and 0.30 M NaCl, at a flow rate of 2 mL/min. The peak of 20 mM Tris—HCl buffer (pH 8.0) containing 0.18 M NaCl was pooled and dialyzed against distilled water for 48 h and then lyophilized. The purity of OVA obtained from anion-exchange chromatography was measured to be 97.2% by reverse phase HPLC.

2.2. Preparation of phosphorylated OVA

The phosphorylated OVA (PP-OVA) was prepared as previously reported (Lv, et al., 2012) with modifications. Briefly, OVA was dissolved at 20 g/L in 0.1 M sodium tripolyphosphate at pH 5.0, 7.0, and 9.0 by adjusting the pH with 1 N HCl or NaOH, and then the solution was lyophilized by Alpha1-4 vacuum freeze dryer (Christ, Germany) for 48 h. Next, the lyophilized samples were incubated at 45 °C for 12 h. Finally, the dry-heated samples were dissolved and dialyzed to remove free pyrophosphate for 2 days against deionized water and then lyophilized for 48 h to get the dry PP-OVA powder. The dry PP-OVA powder was stored at −80 °C.

2.3. Determination of phosphorylation degree of N-and PP-OVA

The phosphorus content of samples was determined as previously reported (Chen, Toribara, &Warner, 1956). Briefly, protein samples were digested in perchloric acid, and the phosphorus in the digest measured by UV/Vis spectrophotometer (DU700, Beckman Coulter, Inc.,USA) was regarded as the total phosphorus of protein. For the determination of inorganic phosphorus (Pi), 5 ml of 10% trichloroacetic acid was added to the same volume of 10 g/l sample solution, which was centrifuged at 1000 g for 20 min. The phosphorus content in the supernatant was regarded as Pi. The amount of phosphorus bound to proteins was estimated by the difference between the total phosphorus and Pi content.

2.4. Determination of zeta potential of N- and PP-OVA

The zeta potentials of the N- and PP-OVA were measured using a Zetasizer Nano-ZS instrument (Malvern Instruments, Worcestershire, UK) according to a previous paper (Wang & Chi, 2012). Briefly, a defined amount of NaCl (0.01 mol/l) was added into 0.02 mol/l, pH 7.4, Tris—HCl buffer. Then, OVA and PP-OVA were added after degassing the solution at a concentration of 1 mg/mL. After filtration through a 0.45 μm micro porous membrane, the diluted samples were injected directly into the chamber of a Nano-ZS particle electrophoresis instrument prior to zeta potential analysis at 25 °C.

2.5. Measurements of particle size and distribution of emulsions for N- and PP-OVA

The emulsion particle sizes were determined by using a static laser light scattering analyzer at ambient temperature (Malvern MasterSizer 2000, Malvern Instruments, Malvern, UK). Phosphate buffer solution (0.1 mol/l pH 7.4) was used to prepare OVA and PP-OVA solutions (1 mg/mL). Briefly, 5 ml of corn oil was added separately into 15 ml of OVA and PP-OVA sample solutions, and the mixtures were then emulsified with a high-shear dispersion homogenizer (JRJ300-S,Shanghai Specimen And Model Factory, China) at 25 °C for 1 min at 10,000 rpm (Wang & Chi, 2012).