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Laccase-aided modification: Effects on structure, gel properties and antioxidant activities of α-lactalbumin

Zhanmei Jiang [a, b, *], Xiangying Yuan [a], Kun Yao [a], Xueyan Li [a], Xinliu Zhang [a], Zhishen Mu [b], Lianzhou Jiang [a], Juncai Hou [a]

[a] Key Laboratory of Dairy Science (Northeast Agricultural University), Ministry of Education, College of Food Science and Technology, Harbin 150030, PR China

[b] Inner Mongolia Mengniu Dairy Group Co. Ltd., Hohhot 011517, PR China

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ABSTRACT

Effects of laccase-catalyzed cross-linking on the structure, gel properties and antioxidant activities of α-lactalbumin (α-LA) were investigated in the presence of ferulic acid (FA). Particle size of laccase-catalysed a-LA-FA congugates became significantly larger with the increasing incubation time. Size exclusion chromatography showed that FA monomers disappeared almostly in the presence of laccase and high molecule polymers were formed with increasing incubation time. α-LA treated with laccase and FA led to the formation of oligomers and polymers by SDS-PAGE analysis. Moreover, intrinsic fluorescence intensity and surface hydrophobility of laccase-catalysed α-LA-FA conjugates remarkably increased from approximately 600 to 1200 A.U. and from 304.1 to 481.3, respectively, corresponding the incubation time from 0 to 24 h, compared with three control systems. After α-LA was treated by laccase and FA, its stronger gel strength and higher water holiding capacity were achieved, and had positive correlation with incubation time. Meanwhile, DPPH radical scavenging activity and ferrous reducing power of laccase-catalysed α-LA-FA conjugates significantly increased with the incubation time increased. Therefore, laccase catalyzed cross-linking of α-LA in the presence of FA could change α-LA structure, and enhance its gel properties and antioxidant activities.

1. Introduction

Enzymatic modification is the most innovative tools in food protein processing to optimise functional properties in food products (Lantto, Puolanne, Kruus, Buchert, & Autio, 2007; Li & Zhao, 2009). Enzymatic modification has higher specificity and typically mild characteristics of reactions. These modifications often only induce the modest changes in protein molecular conformation, which minimizes the risk of formation of possible toxic side products.

In recent years, enzyme-induced protein cross-linking has raised significant interest in production of value-added food products with novel functional and structural properties. Applications of transglutaminase (TGase) on various proteins are widely used in food industry (Bönisch, Huss, Weitl, & Kulozik, 2007; Gauche, Vieira, Ogliari, & Bordignon-Luiz, 2008). Various improvements of food protein functionality have been reported for TG-induced cross-linking, such as enhancing thermal stability, solubility and gelation properties (Chanarat & Benjakul, 2013; Hu et al., 2015). Meanwhile, another large class of cross-linking oxidative enzymes such as horseradish peroxidase, tyrosinase and laccase have also been investigated with respect to their potential for structured foods (Manu & Prasada Rao, 2011; Minamihata, Goto, & Kamiya, 2011).

Laccase (E.C.1.10.3.2), blue copper oxidases, can catalyze the oxidation of various phenolic compounds with broad specificity (Gianfreda, Xu, & Bollag, 1999). Atmospheric oxygen is used as the electon acceptor by laccase in the oxidation reaction and only a byproduct H_2O is formed (Ma, Forssell, Partanen, Buchert, & Boer, 2011). However, in laccase-catalyzed reactions, the phenolic groups in protein did not seem to be a good substrate. Small phenolic compounds facilitated laccase catalysis of protein as electron transfer mediators acted as bridging agents in

* Corresponding author. Key Laboratory of Dairy Science (Northeast Agricultural University), Ministry of Education, College of Food Science and Technology, Harbin 150030, PR China.
E-mail address: zhanmeijiang@neau.edu.cn (Z. Jiang).

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heterpolymer structures (Selinheimo, Lampila, Mattinen, & Buchert, 2008). Phenolic substances with low molecular weight such as ferulic acid, caffeic acid and chlorogenic acid, enhance laccase mediated polymerization of casein and WPI (Færgemand, Otte, & Qvist, 1998; Jung & Wicker, 2012). Laccase combinated with FA significantly accelerated wheat flour dough breakdown (Labat, Morel, & Rouau, 2000). Heated β-lactoglobulin treated with laccase and sugar beet pectin, had a more compact structure and promoted greater emulsion stability than that untreated (Jung & Wicker, 2012). Vanillic acid enhanced cross-linking of WPI by laccase and its conjugates showed improved emulsion stability (Chen, Li, Ding, & Suo, 2012; Gauche et al., 2008). Although laccase was established to be in the formation of covalent cross-linking in several food proteins, effects on bioactivities of food proteins have not been elucidated. However, a major issue that so far has not been addressed in detail, is architecture of clusters of cross-linked food proteins and changes in bioactivities and functionalities induced by enzymatic cross-linking.

Lactalbumin (a-LA) is one of major constituents of whey proteins and is extensively utilized in the food processing. It plays important roles in the functional properties and physiological function of whey proteins (Thalmann & Lötzbeyer, 2002). During recent years, increasing attention has been directed towards the utilization of enzyme-induced protein cross-linking to improve structure and functional properties of a-LA. Horseradish peroxidase (HRP) catalyzes the formation of radicals on the phenol groups of the tyrosine side chains in the presence of H_2O_2 and two radicals may contribute to form a dityrosine cross-link (Manu & Prasada Rao, 2011). Nanostructures of α-LA were developed by HRP (Saricay, Wierenga, & de Vries, 2013). Furthermore, size distribution of cross-linking α-LA mediated by HRP could be directed toward the protein oligomers (Heijnis, Wierenga, van Berkel, & Gruppen, 2010). In the meantime, more dimers or oligomers of α-LA at low ionic strength or pH 5.9 are formed than those at high ionic strength or pH 6.8 (Lantto, Puolanne, Kalkkinen, Buchert, & Autio, 2005). Laccase-catalyzed oligomerization of proteins is reported to proceed through oxidation of tyrosine, cysteine or tryptophan residues (Figueroa-Espinoza, Morel, & Rouau, 1998; Labat et al., 2000). α-LA is rich in tyrosine and tryptophan residues in protein, so it is a good substrate for laccase. However, laccase has not been applied to improved the biological activities and functional properties of α-LA. Furthermore, little work has been conducted on functional properties and antioxidant activity of laccase-catalysed congugates between a-LA and phenolic acids. Therefore, in this study, it is the first time that impacts of incubation time on kinetic process of a-LA modified by laccase, are examined in the presence and absence of ferulic acid. In addition to the extent of polymerization, effects of laccase treatment on physicochemical properties, gel properties and antioxidant activities of a-LA are also systematically investigated.

2. Materials and methods

2.1. Materials

A commercial a-lactalbumin powder (91.6% protein content) were purchased from Davisco Foods International, Eden Prairie, MN, USA. Laccase enzyme (from *Aspergillus,* activity 2000 U/g) and ferulic acid (FA) were purchased from Shanghai Gold Wheat Biological Technology company (Shanghai, China). 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) and 8-Anilino-1-naphthalenesulfonic acid (ANS) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Yuanye company (Shanghai, China). Molecular weight markers (14–120 kDa) were donated by Novoprotein company (Shanghai, China). All other chemicals were of analytical grade.

2.2. Laccase-catalyzed cross-linking of α-LA

α-LA was dissolved in deionized water to a concentration of 100 mg/mL and was adjusted to pH 6.0 by adding1 mol/L HCl. The reaction was initiated by addition of laccase at 40 °C in the presence of 15 mmol/L ferulic acid and the enzyme concentration was fixed to 80 U/g (U/w protein). Samples were taken after incubating for 0, 2, 4, 6, 8, 12, and 24 h and immediately heated at 70 °C for 15 min to inactivate the enzyme. Subsequently, samples were cooled in the ice water, and lyophilised for further experiments. Three control experiments with only α-LA, α-LA incubated with laccase or α-LA incubated with FA were also carried out.

2.3. Particle size distribution

The particle sizes of α-LA incubated with FA and laccase and three control samples were performed using a particle size analyzer (Zetasizer Nano-ZS90, Malvern Ltd., UK). Samples were diluted to 1 mg/mL with deionized water and filtered through 0.45 μm filters. Particle size was reported as the size distribution by volume.

2.4. Molecular weight distribution

The Molecular weight distribution profiles of α-LA incubated with FA and laccase and three control samples were estimated by size exclusion chromatography (SEC) using a series connection column with TSK G 3000 SW XL (7.5 mm ID × 30 cm L, 5 μm, Japan) and TSK G 2000 SW XL (7.5 mm ID × 7.5 cm L, 5 μm, Japan). Samples were filtered through a 0.45 μm membrane and injected at a concentration of 2.5 mg/mL. The column was equilibrated and eluted with 50 mM Tris-HCl buffer (pH 7.0) at a flow rate of 0.6 mL/min. Eluates were monitored with a UV detector at a wavelength of 280 nm. The method was calibrated using native α-LA and β-LG standards.

2.5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Molecular mass distributions of α-LA incubated with FA and laccase and three control samples were analyzed by SDS-PAGE. In the experiments, a stacking gel of 4% acrylamide and a running gel of 12.6% acrylamide were used. Sample solutions were diluted to the concentration of 2.5 mg/mL, and mixed with SDS reducing buffer containing 62.5 mmol/L Tris-HCl (pH 6.8), 20% glycerol, 2% SDS, 0.5% bromphenol blue and 5% β-mercaptoethnol. The solution mixtures were heated in boiling water for 5 min and stored at 4 °C. Protein bands were stained by Coomassie Brilliant Blue R250.

2.6. Measurement of intrinsic fluorescence spectrum

The intrinsic emission fluorescence spectra of α-LA incubated with FA and laccase and three control samples was obtained by a Fluorphotometer (F4500, Hitachi, Tokyo, Japan). Samples were diluted with 50 mmol/L sodium phosphate buffer (pH 7.0) to final concentrations of 1 mg/mL. The sample solutions were excited at 280 nm, and emission was scanned from 290 to 420 nm. Both excitation and emission slits were set at 5 nm, and the scan rate was 240 nm/s.

2.7. Surface hydrophobicity measurement

Surface hydrophobicity (H_0) values of α-LA incubated with FA