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β-Lactoglobulin conformation and mixed sugar beet pectin gel matrix is changed by laccase

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ABSTRACT

Sugar beet pectin (SBP) and β-lactoglobulin (BLG) contain ferulic acid and tyrosine, respectively, potential substrates for laccase. Dispersions of BLG were treated with laccase and/or heat and SBP to assess potential for ferulic acid in SBP to influence laccase conjugation of BLG. Changes were investigated by size exclusion chromatography combined with multi angle laser light scattering (HPSEC-MALLS), refractive index (RI) and UV detector, particle size and ζ-potential analysis. Molecular weight (MW) of BLG decreased from 3.7×10^4 to 2.9×10^4 and root mean square (RMS) decreased from 41 to 36 after laccase treatment. The slope of a conformation plot increased from 0.35 to 0.72, indicating a change in shape in laccase treated BLG to more random coil. While laccase did not affect MW of BLG, MW of BLG increased after sequential heat and laccase treatment to 2.9×10^6 and 4.4×10^6, respectively. Free thiol (SH) increased by heat and laccase treatment of BLG; tyrosine in BLG increased only by heat. An increased MW and decreased ferulic acid in SBP was observed in the presence of BLG and laccase. The increase in MW was attributed to entrapment of BLG in SBP cross-linked matrix catalyzed by laccase.

1. Introduction

Whey protein isolate (WPI) is widely used as an ingredient in the food industry, due to high nutritional value and physical functionality, such as gelling, foaming, emulsifying and stabilizing abilities. β-lactoglobulin (BLG), the most abundant protein in whey protein, exists as a dimer at neutral pH with a molecular weight (MW) of ~ 36 kDa, two disulfide groups, and one free, buried cysteine residue (Walstra, Wouters, & Geurts, 2006). The dimer dissociates into a monomer and hydrophobic groups are exposed by partial unfolding if heated to temperatures over 70 °C (Qi et al., 1997; Relkin, 1998).

Laccase (p-diphenol oxidase, E.C.1.10.3.2) oxidizes phenolic compounds with broad specificity compared to polyphenol oxidase or peroxidase. Laccase enhances functional properties of proteins and polysaccharides, such as the mixing properties of wheat flour dough (Labat, Morel, & Rouau, 2000) and protects embedded protein against pepsin proteolysis in cross-linked feruloylated arabinoxylan (Vansteenkiste, Babot, Rouau, & Micard, 2004). Laccase improves the gelling ability of SBP via cross-linked ferulic acid (Micard & Thibault, 1999; Oosterveld, Beldman, Schols, & Voragen, 1996). Laccase cross-linked SBP has a more compact, branched structure and promotes greater emulsion stability than control SBP (Jung & Wicker, 2012a).

Cross-linking of proteins by laccase is often limited by the accessibility of reactive phenolic groups. While tyrosine, cysteine and tryptophan residues are potential cross-linking sites for laccase in protein, the reactive amino acids are inaccessible to laccase in the 3-dimensional structure of bovine serum albumin (BSA) and BLG, even though BSA and BLG have sufficient reactive amino acid residues (Mattinen et al., 2006; Uhrinova et al., 2000).

Phenolic compounds facilitate laccase catalysis of WPI or BLG. Low molecular weight phenolic substances such as ferulic acid, p-coumaric acid and hydrolyzed oat spelt xylan, enhance laccase mediated polymerization of casein and WPI (Faergemand, Otte, & Qvist, 1998; Selinheimo, Lampila, Mattinen, & Buchert, 2008). Moreover, laccase cross-linked BLG conjugates were formed in the presence of sour cherry phenolics; conjugates were less allergenic and more digestible (Tantoush et al., 2011). Vanillic acid enhanced cross-linking of WPI by laccase and conjugates showed improved emulsion stability (Ma, Forssell, Partanen, Buchert, & Boer, 2011). Laccase reinforced physical texture of the complex coacervation between SBP and WPI formed by electrostatic interaction and showed potential as a delivery system (Chen, Li, Ding, & Suo, 2012).

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Sugar beet pectin (SBP) is unique from other major commercial pectins, such as citrus or apple, due to ferulic acid and acetyl groups, which makes SBP a poor gelling pectin (Voragen, Pilnik, Thibault, Axelos, Renard, 1995), but rich in protein which contains tyrosine (Levigne, Ralet, & Thibault, 2002; Siew & Williams, 2008). Laccase cross-linked SBP forms layer by layer stabilized emulsions, with greater stability at high ionic strength with BLG (Littoz & McClements, 2008) and fish gelatin (Zeeb, Fischer, & Weiss, 2011). Further, BLG cross-linked with SBP has improved solubility near the pI of 5.1 for BLG (Jung & Wicker, 2012b). New applications of whey protein are likely to be facilitated by the controlled modification of BLG structure. The aim of this research was to examine the modification on BLG by laccase, heat and ferulic acid rich SBP.

2. Materials and methods

2.1. Material

Non-denatured β-lactoglobulin (BLG) (Lot no. JE 003-6-922) was obtained from fresh whey, prepared by ion exchange chromatography (Davisco Foods International, Le Sueur, MN). Total protein was 93.6 g/100 g and BLG represented 97.8 g/100 g of protein content as stated by the manufacturer. Sugar beet pectin (SBP) was donated by Herbstreith & Fox KG (Lot no. 0 05 03 024, Elmsford, NY). Based on manufacturer's specifications, SBP had 73% galacturonic acid and 59% degree of esterification. Molecular weight (MW) was estimated to be 212,200 Da by HPSEC-MALLS; polydispersity and RMS values (root mean square) were 1.368 and 33 nm, respectively. Laccase (*Rhus vernicifera*, EC.1.10.3.2, p-diphenol oxidase) and syringaldazine were purchased from Sigma–Aldrich Co. (St. Louis, MO).

2.2. Stock preparation

SBP and BLG dispersions were prepared by dispersing 30 mg/mL dry powder in 100 mmol/L sodium phosphate buffer (pH 6.5), stirring at room temperature at least 2 h and storing overnight at 4 °C for complete hydration. Both dispersions were centrifuged for 30 min at 5000 × *g*, 4 °C to remove insoluble materials (Sorvall RC 6Plus, Thermo Scientific Products), observed in SBP, but not BLG.

2.2.1. Laccase mediated SBP & heat treated BLG conjugation preparation

The procedure of sample preparation is presented in Fig. 1, which was adapted from the procedure described to crosslink SBP with laccase (Jung & Wicker, 2012a). The centrifuged SBP and BLG dispersions were equilibrated with and without laccase in a water bath (30 °C) for 20 h with mild agitation, setting: 2.5, about 93 rpm, on an orbital shaker (PR 70 Red Rotar Hoffer Scientific, MO). An aliquot of 1 unit laccase/mg BLG solids was added to each dispersion. The activity of the laccase was verified just before addition. An aliquot of water was added to the controls. Aliquots of 2 mL of BLG and laccase treated BLG were heated at 80 °C for 15 min in a water bath and heated BLG and heated BLG with laccase were denoted as HBLG and HLBLG, respectively Laccase and non-laccase treated BLG dispersions (LBLG and BLG) were mixed in equal volumes with laccase treated SBP (LS) and without laccase treated SBP (S) at the same ratio. These mixtures were equilibrated for another 20 h at ambient temperature. Mixed samples were named BLG-S, LBLG-S and LBLG-LS, following individual sample abbreviation. For further characterization and analysis, the samples were used immediately or were diluted 10 fold to slow laccase activity. Negligible laccase activity in diluted samples was confirmed by enzyme assay.

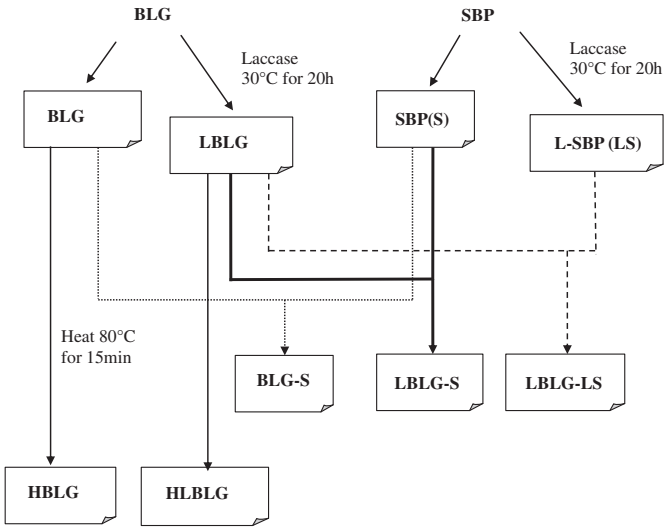

Fig. 1. Flow diagram of sugar beet pectin (SBP) and β-lactoglobulin (BLG) sample preparation upon laccase and heat treatment. BLG : β-lactoglobulin, LBLG : Laccase treated BLG, HBLG : Heat treated BLG at 80 °C for 15 min, HLBLG : Heat treated LBLG at 80 °C for 15 min, S : Sugar beet pectin, LS : Laccase treated Sugar beet pectin, BLG-S : Mixed sample with BLG and S, LBLG-S : Mixed sample with LBLG and S, LBLG-LS : Mixed sample with LBLG and LS.

2.2.2. Laccase activity measurement

Laccase activity was measured by modified procedure of a previously described method using syringaldazine as substrate at 30 °C (Ride, 1980). Laccase was dissolved in cold, deionized water (0.5 mg/mL), stirred for 10 min, and filtered through 5 μm PVDF (polyvinylidene fluoride) syringe filters (Millipore, Bedford, MA). An aliquot of 2.2 mL sodium phosphate buffer (100 mmol/L, pH 6.5) was mixed with 0.5 mL of diluted laccase solution, 0.3 mL syringaldazine (0.216 mmol/L in methanol) was added and measurement promptly followed. The changes in absorbance monitored at UV_{530} per min were used to assess activity. The activity of laccase was analyzed for each treatment and an aliquot of 1 unit laccase/mg BLG solids was added to each dispersion.

2.2.3. Particle size measurement

Particle size was performed using a Particle Size Analyzer with the BI-Zeta option (90 Plus, Brookhaven Instruments Corporation, Holtsville, NY) with a 50 mV diode laser (90° angle) and a BI-9000AT correlator. The samples were diluted to 0.5 mg/mL with deionized water prior to analysis. In preliminary trials, filtration through 0.5 μm filters yielded similar results to unfiltered samples and filtration was discontinued. The dust cut off option on the Brookhaven was employed. The signal intensity at 0.5 mg/mL unheated BLG was too low to obtain reliable values. Preliminary trials at 30 mg/mL BLG and acceptable sample quality resulted in similar particle sizes. Volume and weight diameters were reported as individual particle size and calculated as: $\sum nd^4/\sum nd^3$ with diameter d and number of particles n. Particle size measurements were performed in duplicate with 5 measurements in each run. Average volume diameter was reported from triplicate particle size measurements.

2.2.4. ζ-potential measurement

Each sample was diluted to 0.5 mg/mL with deionized water prior to analysis. The ζ-potential was performed after the particle size determination for all samples. The ζ-potential was determined in duplicate with 5 runs each and calculated from the average of ten readings.