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Structural characterization and formation mechanism of zein-propylene glycol alginate binary complex induced by calcium ions

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ABSTRACT

Effect of calcium ions (Ca^{2+}) on characteristics of zein-propylene glycol alginate (PGA) binary complex was studied in this work. Ca^{2+} induced the formation of zein aggregates with decreased fluorescence intensity and a significant α-helix loss of zein. Zein-PGA binary complex with Ca^{2+} showed the decreased dimension and the minimum size was observed at 50.0 mM Ca^{2+}. Ca^{2+} resulted in the formation of strong hydrogen bonds between zein and PGA, strengthened their hydrophobic interactions, and induced a new peak at the diffraction angle of 30° in the pattern of Zein-PGA binary complex. PGA fortified with Ca^{2+} exhibited an overall plane-like structure, also an interwoven flat profile appeared in Zein-PGA binary complex with Ca^{2+}. Three potential mechanisms were proposed to explain the morphological changes of samples after Ca^{2+} addition: (i) particle-particle collision and aggregation of particles; (ii) chain-chain association and further cross-linking of associated chains; (iii) simultaneous cross-linking coupled with aggregation.

1. Introduction

Calcium ions (Ca^{2+}), as a kind of bivalent cations, show a variety of functions in biological systems, such as biomineralization in bones, teeth, and shells, and play a complex role as an intracellular messenger (Kretsinger & Wasserman, 1980). In recent years, Ca^{2+} is particularly applied to modulate the conformational and structural properties of water soluble proteins, such as carp muscle protein (Coffee & Bradshaw, 1973), bovine plasma protein (Amphlett, Kisiel, & Castellino, 1981), wheat germ protein hydrolysates (Liu, Wang, Wang, & Chen, 2013), whey protein concentrate (Clare, Lillard, Ramsey, Amato, & Daubert, 2007), bovine serum albumin (Donato, Garnier, Doublier, & Nicolai, 2005), and soy protein isolate (Zhang, Liang, Tian, Chen, & Subirade, 2012). Compared to native proteins, calcium-binding proteins, for example muscle calcium-binding parvalbumin, exhibited a unique characteristic conformation, consisting of a helix, calcium-binding loop, and second helix (Kretsinger & Wasserman, 1980). What's more, proteins exhibited enhanced thermal stability and resistance to proteolytic degradation due to the binding of Ca^{2+} (Gifford, Walsh, & Vogel, 2007).

Despite water soluble proteins, alcohol soluble proteins are becoming attractive research topics. Zein, as a typical kind of prolamins, shows its inherent amphiphilic feature, and can be converted into spherical colloidal nanoparticles by the anti-solvent precipitation method, which has been regarded as a popular delivery system for active compounds and micronutrients in food and biotechnological industries (Zhong, Jin, Davidson, & Zivanovic, 2009). Previous studies reported the acidic or alkaline deamidation or enzymatic hydrolysis (Funatsu & Shibata, 1998) to modify the functional properties of zein. To the best of our knowledge, little information is available about the influence of Ca^{2+} on structural modification of alcohol soluble proteins. On the base of the fact that zein contains three-fourths no-polar hydrophobic amino acids such as alanine and one-fourth hydrophilic amino acids like glutamic acid (Magoshi, Nakamura, & Murakami, 1992; Shukla & Cheryan, 2001), a hypothesis was proposed that potential interaction may take place between zein and Ca^{2+}, and the incorporation of Ca^{2+} will modify the physicochemical properties of zein, which may be adapted to the requirement of processes and broaden its application fields.

In addition to proteins, Ca^{2+} is commonly used to fabricate hydrogel beads on the base of food-grade hydrophilic polysaccharides, such as starch (Islam & Azemi, 1997), pectin (Fraeye, Duvetter, Doungla, Van Loey, & Hendrickx, 2010), carrageenan (Janaswamy & Chandrasekaran, 2002) and especially alginate (Leong et al., 2016) due to the formation of cationic bridges between the guluronic rich entities along the biopolymer backbone (Li, Hu, Du, Xiao, & McClements, 2011), which have been broadly used as delivery systems of bioactive compounds (Hua, Ma, Li, Yang, & Wang, 2010). Propylene glycol alginate (PGA), as a high molecular weight linear polysaccharides, is the reaction derivative

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between propylene oxide and alginic acid, which is composed of 1, 4 linked-D-mannuronic acid (31%–65%) and L-guluronic acid (69%–35%) (Sarker & Wilde, 1999). In comparison with non-esterified alginates, PGA possesses unique surface-active properties, and is much less sensitive to a low pH condition attributed to the existence of propylene glycol groups, which has been approved to be utilized as the stabilizer, emulsifier as well as thickener in a variety of food products (Martinez, Pizones Ruiz-Henestrosa, Carrera Sanchez, Rodríguez Patino, & Pilosof, 2012). Previous report uncovered the gelling potential of PGA in the presence of Ca^{2+} (Nilsen-Nygaard, Hattrem, & Draget, 2016). However, the investigation into the effect of Ca^{2+} on the structure and morphology of PGA is still limited. According to our previous study, PGA exhibited a great solubility in aqueous ethanol solution (Sun, Dai, & Gao, 2016). It may be interesting to explore the characteristics of PGA induced by Ca^{2+} under the condition of aqueous ethanol solution.

It should be noted that PGA at low and intermediate degrees of esterification was able to form gels with relative strength (Nilsen-Nygaard et al., 2016), whereas gelling property of PGA was not the research emphasis in the present work. Therefore, to avoid the occurrence of gel formation, PGA with a high degree of esterification (87.9%) was selected since it showed a poor gelling ability due to the lower fraction of protonizeable carboxyl groups.

Overall, novel functionalities can be fabricated by formation of protein-polysaccharide complexes. Contrasting with water soluble proteins and polysaccharides, studies on complexes of alcohol-soluble proteins and polysaccharides are very limited. Our previous studies revealed that the non-covalent complexation of PGA and zein after anti-solvent co-precipitation at both pH 4.0 (Sun, Dai, & Gao, 2017a) and 7.0 (Sun, Dai, & Gao, 2017b) resulted in the formation of zein-PGA binary complexes, which was further confirmed to be an excellent delivery system for quercetagetin (Q) (Sun et al., 2016). While Ca^{2+} is commonly utilized to modify the properties of individual water soluble proteins (Zhang et al., 2012) or hydrophilic polysaccharides (Janaswamy & Chandrasekaran, 2002). Little information is available about the influence of calcium ions on structural modification of alcohol soluble proteins and their complexes with polysaccharides.

Our previous study reported the effect of calcium ions at relatively low levels of 1.5625, 3.1250, 6.2500, and 12.5000 mM on Zein-PGA binary complexes, as well as Q-loaded Zein-PGA ternary composites, and the results revealed that the incorporation of Ca^{2+} led to obvious conformational, secondary and tertiary structural changes of zein, and improved its thermal stability. In addition, the conclusion was obtained that specific characteristic changes were exactly dependent on the different concentrations of Ca^{2+} (Sun, Wei, Li, Dai, & Gao, 2017). On the base of the findings from our previous studies, we are extremely fortunate to have an access to explore the influence of calcium ions at relatively high concentrations (25.0, 50.0, 100.0 and 200.0 mM) on the structural and morphological characteristics of individual zein, PGA, and Zein-PGA binary complex in the present work. Besides, the potential mechanism was proposed to explain these characteristic changes of individual biopolymers and their complex. Fourier-transform infrared and X-ray diffraction spectroscopy were applied to determine the possible interaction between zein and PGA after induced by calcium ions. Far-UV circular dichroism spectroscopy was utilized to probe the secondary changes of zein. Field emission scanning electron microscopy was introduced to observe the micro-morphology of samples in the presence of calcium ions. Findings from this work will provide a theoretical basis for the application of calcium ions induction on the characteristic modification of individual or complex biopolymers, especially for alcohol soluble proteins, and may bring a new insight into the development of potential carriers for bioactive compounds.

2. Materials and methods

2.1. Materials

Zein with a protein content of 94.7% (w/w) was purchased from Sigma-Aldrich (USA). Food grade propylene glycol alginate (PGA) with esterified carboxyl groups of 87.9% was kindly provided by Hanjun Sugar Industry Co. Ltd. (Shanghai, China). Absolute ethanol (99.99%) and other reagents were acquired from Eshowbokoo Biological Technology Co., Ltd. (Beijing, China).

2.2. Preparation of Zein-PGA binary complex in the presence of Ca^{2+}

Zein-PGA binary complex was prepared by the anti-solvent co-precipitation method coupled with calcium ions induction. Briefly, zein (0.40 g) and PGA (0.08 g) were dissolved in 40 mL of aqueous ethanol solution (70%, v/v), stirring vigorously until completely dissolved. The mixed solutions were injected by a syringe with a controlled speed of 20 mL/min to the beaker containing 120 mL aqueous solutions with 25.0, 50.0, 100.0 and 200.0 mM $CaCl_2$. To acquire aqueous dispersions, approximately three quarters of ethanol were removed under reduced pressure (-0.1 MPa) by rotary evaporation at 45 °C for 35 min. Finally, Zein-PGA binary complex colloidal dispersions in the presence of Ca^{2+} were stored in the refrigerator at 4 °C for further analysis in the form of liquid, and parts of the dispersions were frozen and dried for 48 h with Alpha 1-2 D Plus freeze-drying apparatus (Marin Christ, Germany) to obtain dry particles for solid state characterization analysis. The individual zein and PGA colloidal dispersions with various levels of Ca^{2+} were obtained by the aforementioned process. At the same time, all samples in the absence of Ca^{2+} were prepared by the aforementioned method and used as the reference.

In this work, samples of zein, PGA and Zein-PGA binary complex in the presence of different Ca^{2+} concentrations (25.0, 50.0100.0 and 200.0 mM) were divided into three types and termed as (i) Zein-25, Zein-50, Zein-100 and Zein-200; (ii) PGA-25, PGA-50, PGA-100 and PGA-200; (iii) Zein-PGA-25, Zein-PGA-50, Zein-PGA-100 and Zein-PGA-200.

2.3. Determination of particle size and zeta-potential

Particle size and zeta-potential of colloidal dispersions in the presence and absence of Ca^{2+} were determined using a combined dynamic light scattering (DLS) and particle electrophoresis instrument (Zetasizer Nano-ZS90, Malvern Instruments Ltd., Worcestershire, UK) based on the description in our previous report (Sun et al., 2016). Before measurement, samples were centrifuged at 4000*g* for 10 min to remove large insoluble aggregates and then proper dilution to the instrumental range using distilled water. The particle size data were reported as a cumulative mean diameter (size, nm), which was calculated by the intensity weighted using the Stokes-Einstein equation (Edward, 1970). Zeta-potential of samples was determined by measuring the direction and velocity of particle movement in a well-defined electric field, and the data were obtained by the instrument using the Smoluchowski model. All measurements were carried out at room temperature (25 °C) and each sample was analyzed in triplicate.

2.4. Fluorescence spectroscopy

Fluorimetric experiments were carried out on a fluorescence spectrophotometer (F-7000, Hitachi, Japan). The excitation wavelength was set at 280 nm and the emission spectra were collected between 290 nm