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Investigation of ovotransferrin conformation and its complexation with sugar beet pectin

Zihao Wei, Pei Zhu, Qingrong Huang*

Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, United States

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ABSTRACT

Small-angle X-ray scattering (SAXS) has been used to investigate the conformation of ovotransferrin (OVT) in solutions of different pH values. OVT was found to be a spherical molecule at native state in aqueous solutions. Radius of gyration (R_g) of OVT increased by 19.6% upon transition from native to molten globular state, and R_g increased by 9.7% as OVT further unfolded to an extended conformation. Besides, OVT showed concentration scaling behaviors at molten globule state. The information about size and shape was expected to facilitate the understanding of interactions between OVT and sugar beet pectin (SBP). Increasing OVT/SBP mixing ratios altered critical pH transition points in phase diagram, and the ratio of 5:1 was close to the saturated complexation ratio between OVT and SBP. Turbidities of OVT–SBP mixtures decreased significantly in the presence of sodium chloride, indicating the domination of electrostatic interaction in OVT–SBP complexation. Atomic force microscopy data illustrated the formation, association and dissociation processes of OVT–SBP complexes. Fluorescence spectroscopy results revealed that binding constant between OVT and SBP decreased with increasing temperature. The negative ΔH and positive ΔS further indicated the strong attractive interaction between OVT and SBP, which overcame the entropy contribution. Emulsions stabilized by OVT–SBP complexes had smaller droplet sizes and better physical stability than those stabilized by either pure OVT or SBP.

1. Introduction

Ovotransferrin (OVT), which constitutes about 12–13% of egg white proteins, is an iron-binding monomeric glycoprotein consisting of 686 amino acids. As a member of transferrin family, OVT is synthesized in chick oviduct and deposited mainly in egg albumen (Wu & Acero-Lopez, 2012). In addition to its high nutritional value, OVT has various positive bioactivities such as antimicrobial, antioxidant and anti-inflammatory properties (Baron et al., 2014; Kobayashi et al., 2015). Thus, OVT has attracted a lot of attention in recent years (Acero-Lopez, Ullah, Offengenden, Jung, & Wu, 2012; Faure & Nyström, 2016).

Pectin is an abundant source of natural fiber and widely used in food industry as gelling, stabilizing or thickening agent (Voragen, Coenen, Verhoef, & Schols, 2009). Most of commercial pectin originates from citrus or apple peels, and sugar beet pectin (SBP) is a relatively new pectin with better emulsifying property and poorer gelling property than citrus and apple pectins (Kuuva, Lantto, Reinikainen, Buchert, & Autio, 2003; Mesbahi, Jamalian, & Farahnaky, 2005). Sugar beet pectin (SBP) is a polysaccharide extracted from sugar beet pulp. It contains ferulic acid, high proportion of acetyl groups and hairy regions in structure (Saulnier & Thibault, 1999). Previous studies revealed that sugar beet pectin could act as microencapsulation wall materials, stable emulsifiers and thickening agents (Drusch, 2007; Gromer, Penfold, Gunning, Kirby, & Morris, 2010; Mesbahi et al., 2005).

Small-angle X-ray scattering (SAXS) is a powerful tool to determine the conformation (i.e., size and shape) of proteins in solutions (Mertens, Jamalian, & Farahnaky, 2010). The effect of pH on the conformation of OVT still remains unclear. Since various parameters such as pH, ionic strength and protein concentration may shift conformational states of proteins (Fang, Zhang, Jiang, Jing, & Ren, 2012; Lipfert & Doniach, 2007), it is of great interest to investigate the influence of these factors on protein structures using SAXS. To the best of our knowledge, effects of pH, ionic strength and protein concentration on X-ray scattering profiles of OVT have not been reported, and related research may help us learn more about structural properties of OVT and enhance potential application of OVT in food systems.

Many food products are multi-component systems structured by a complex assembly of various ingredients such as proteins and polysaccharides, and complexations between proteins and polysaccharides are often greatly responsible for physicochemical properties of foods (Schmitt & Turgeon, 2011; Wei & Gao, 2016a). Protein–polysaccharide complexation may cause structural changes to both protein and

* Corresponding author.
E-mail address: qhuang@sebs.rutgers.edu (Q. Huang).

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polysaccharide components on the molecular level (Niu et al., 2016). Besides, in most cases, protein–polysaccharide complexes can exhibit functional properties different from proteins and polysaccharides alone (Miquelim, Lannes, & Mezzenga, 2010; Niu et al., 2016; Ru, Wang, Lee, Ding, & Huang, 2012). Therefore, complexation of proteins with polysaccharides may provide an approach to designing desirable foods with specific properties. However, relatively little is known about complexation of OVT with polysaccharides, and a thorough study on complexation of OVT with SBP may help produce novel complexes with broad applications in food industry. Atomic force microscopy (AFM) can be employed to visually characterize OVT–SBP complexation at the molecular level in the present study. AFM may systematically record the formation, association and dissociation processes of OVT–SBP complexes, which may corroborate different phase behaviors in OVT–SBP mixtures.

Accordingly, this study aims to use SAXS to investigate the influences of pH, ionic strength and protein concentration on OVT conformation first. The result might help to gain a better understanding of structural changes of OVT as well as its complexation with SBP. Various techniques, including turbidimetric titrations, quartz crystal microbalance with dissipation monitoring (QCMD), zeta potential measurements, atomic force microscopy (AFM), and fluorescence spectroscopy have been used to characterize the physical properties of OVT/SBP complexes. The impacts of OVT–SBP complexation on emulsifying properties of OVT-SBP complexes were also investigated.

2. Materials and methods

2.1. Materials

Ovotransferrin (OVT) (> 88%, purity) with a molecular weight of about 7.6×10^4 Da was purchased from Neova Technologies Inc. (Abbotsford, Canada). According to analysis report, iron-binding activity of the ovotransferrin was 1099 μg Fe/g. Sugar beet pectin (SBP) with a degree of esterification of approximately 55% and protein content of 4.5 wt % was provided by CP Kelco (Atlanta, USA), and the average molecular weight of SBP was 6.5×10^4 Da. SBP was further purified by dialysis (molecular weight cutoff 6000 Da) against distilled water to remove low molecular weight oligosaccharides and followed by freeze-drying before use. Medium chain triacylglycerols (MCT) was kindly provided by Stepan Company (Northfield, IL, USA). All other chemicals used in this study were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise stated.

2.2. Small-angle X-ray scattering (SAXS) measurements

2.2.1. Preparation of samples

To study the influence of pH on SAXS profiles, OVT (0.5 mg/mL) was slowly dispersed in 10 mM Tris solution and stirred (200 rpm) at room temperature to ensure complete dispersion and dissolution. The OVT solution was then filtered with a 0.22 μm pore-sized syringe filter (Thermo Fisher Scientific, Waltham, USA) to remove impurities and adjusted pH to corresponding values (2–8) with 0.5 M HCl.

To investigate the influence of ionic strength on SAXS profiles, OVT (0.5 mg/mL) was first dissolved in Tris solutions of different concentrations (10, 50, 100 and 200 mM), and the solution pH values were adjusted to 3.0.

In order to test effect of protein concentration on SAXS profiles, OVT solutions of different concentrations (0.2, 0.5, 1 and 2 mg/mL) were dissolved in 10 mM Tris solutions, and the solution pH values were adjusted to 3.0.

2.2.2. SAXS analysis

SAXS measurements of the sample solutions were performed at the BioCAT 18-ID beamline of Advanced Photon Sources (Argonne National Laboratory, Lemont, USA) according to the method of Li et al. (Li, Xia, Shi, & Huang, 2011). The sample solutions were filtered with 0.22 μm pore-sized syringe filters before analysis. The wavelength of X-ray radiation was set as 1.033 Å, and the scattering intensity was acquired with a detector located 3.5 m from the sample. The short exposure time was 1 s. The detector response was calibrated by subtraction of corresponding solvent background, and the final scattering data were obtained by averaging 15 measurements.

2.3. Turbidimetric titrations of the mixtures of OVT and SBP

2.3.1. Sample preparation

OVT (0.1–1 mg/mL) and SBP (0.2 mg/mL, a concentration below the overlap concentration of this SBP) solutions were prepared by dispersing OVT and SBP in distilled water, followed by stirring (200 rpm) for 12 h at room temperature and storage overnight at 4 °C. All solutions were filtered using 0.22 μm pore-sized syringe filters prior to use. Equal volumes of OVT and SBP solutions with different initial OVT/SBP mass ratios (from 1:2 to 5:1) were mixed homogeneously. The final concentration of OVT ranged from 0.05 to 0.5 mg/mL and the final concentration of SBP was fixed at 0.1 mg/mL. The pH values of mixed solutions were adjusted to 9 with 0.2 M NaOH under magnetic stirring.

In addition to the biopolymer mass ratio, influence of NaCl on turbidimetric titrations was also investigated. OVT (0.2 and 0.4 mg/mL) and SBP (0.2 mg/mL) solutions were prepared by first dispersing OVT and SBP in different NaCl solutions (0, 50, 100 and 200 mM). All other procedures were same as described above.

2.3.2. Turbidimetric titrations

pH-dependent turbidity was measured using a Brinkmann PC910 colorimeter (Metrohm, Riverview, USA) equipped with a 1-cm-path-length optical probe. The colorimeter was calibrated to read 100% transmittance (T) with distilled water, and turbidity was defined as $100 - T$% (Ru et al., 2012). The titration measurements were performed at 25 °C under magnetic stirring, and the time interval between adjacent measuring points was fixed at 1 min. To minimize the effect of dilution, HCl solutions with concentration gradients (0.05, 0.1, 0.25, 0.5 and 1 M) were used to adjust the solution pHs.

2.4. Zeta potential measurements

To determine the overall surface charge, zeta potential of OVT solutions, SBP solutions and OVT–SBP mixtures at different OVT/SBP mass ratios (0 mM NaCl) within the pH range 2–9 was measured from their electrophoretic mobility using Zetasizer Nano-ZS90 instrument (Malvern Instruments, Worcestershire, UK). Smoluchowski model was applied for analysis. All measurements were peroformed in triplicate, and the zeta potential results were expressed as mean value ± standard deviation (SD).

2.5. Atomic force microscopy (AFM) measurements

The sample preparation for AFM measurements was the same as that for turbidimetric titrations. Tapping mode AFM images of OVT–SBP mixtures (2:1 ratio, 0 mM NaCl) at different pHs were collected with NanoScope IIIA Multimode AFM (Veeco Instruments Inc., Santa Barbara, USA) equipped with a silicon-etched RTESP7 cantilever. After sample preparation in beakers, samples (10 μL) were applied to the surface of freshly cleaved mica. After 1 h of adsorption, samples were dried under a nitrogen stream and then mounted on the scanner tube. The drive frequency of the silicon tip (with spring constant of 40 N/m) was tuned to a frequency range of 300–320 kHz before height mode image collecting.

2.6. Fluorescence spectroscopy

Fluorometric experiments were carried out on a FluoroMax 3