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The enrichment and characterization of ginger-derived glycoprotein using magnetic particles

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ABSTRACT

Ginger-derived glycoproteins are a widely distributed group of biological macromolecules with multiple functions. To date, the structure of ginger-derived glycoproteins has not been clarified with regard to their complexity, their sequence diversity and their uneven micro-distribution. In this study, a lectin microarray was used to evaluate 37 types of lectins and determine the optimal lectins that can conjugate with glycoproteins based on the fluorescence intensity. Subsequently, the lectins were immobilized on magnetic beads, coupled with glycoproteins to enrich ginger-derived glycoproteins, and evaluated using SDS-PAGE. Our results showed that five lectins (e.g. VVA, ConA, STL, LEL, and LCA) were selected by the lectin microarray and that VVA showed the highest fluorescence intensity. In addition, it is indicated that the structure of the carbohydrate chains might contain GlaNAc, mannose, GlcNAc, and LacNAc.

1. Introduction

Glycosylation is one of the most common and vital post-translational modification of proteins and has essential roles in a variety of biological processes, such as cell–cell communication, molecular recognition, protein folding, and immune responses (Li et al., 2015). Glycoproteins that contain glycan covalently attached to a polypeptide backbone play a vital role in biological processes (Xie, Zhong, Cai, Chen, & Chen, 2017). A number of studies have suggested that more than half of all proteins are glycosylated (Gorg, Weiss, & Dunn, 2004). Therefore, research on the bioactivities and structures of glycoproteins has attracted attention (Dai et al., 2016). However, because non-glycoproteins are more abundant than glycoproteins, the latter are prone to be disturbed and covered by non-glycoproteins. Thus, it is important to enrich the less common glycoproteins.

Recently, hydrazide chemistry, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and chromatography have been commonly used for enriching glycoproteins. Hydrazide chemistry was used to enrich the *N*-linked glycoproteins; however, in this study, a series of side reactions occurred during the reaction process (Jiang, Messing, & Ye, 2017). SDS-PAGE is used for identifying the purity of proteins, and for separating target proteins from complex samples. However, the low sensitivity of the method is limited to obtaining high-purity glycoproteins. Lectin affinity chromatography as a general method can reversibly and selectively enrich specific types of glycosylated peptides or proteins (Rowe, El Khoury, & Lowe, 2016). Lectins are carbohydrate-binding proteins (or glycoproteins) of non-immune origin that agglutinate cells and/or precipitate glycoconjugates; all plant lectins possess at least one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide (Van Damme, Peumans, Barre, & Rouge, 1998). Different structures of glycopeptides can be enriched by different lectins. ConA specifically binds mannosyl and glucosyl residues of polysaccharides and glycoproteins containing free hydroxyl groups at positions C3, C4, and C6, and can serve as a tool to capture *N*-glycosylated peptides and proteins with broad specificity (Idil et al., 2015). Wheat germ agglutinin (WGA) binds both *N*-acetylglucosamine and Nacetylneuraminic acid. The significant advantage of lectin-based technology is that, following minimal sample preparation, a simultaneous quantitative analysis of N- and O-linked glycans on intact biological structures can be performed without the need for glycan release (Fry et al., 2011). Boronate affinity chromatography is the preferred method for binding with cis-diol-containing glycoproteins and saccharides, and this method has been used in various studies (Rowe et al., 2016; Yang et al., 2011). Boronate affinity materials,

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including magnetic materials, have aroused enormous interest for the enrichment of glycoproteins (Jiang et al., 2017). Magnetic particles are preferred carriers for enriching glycoproteins (Idil et al., 2015). The use of magnetic particles in supporting the functionalization has several advantages, such as durable magnetic susceptibility, and can be combined with the advantages of a lectin-based approach, which is flexible, convenient, and reversible. In addition, it is convenient to identify the structure and bioactives of a single glycan.

Ginger-derived glycoproteins contain a variety of functional components, including specific phenolic compounds, purine compounds, and bioactive polysaccharides. Moreover, glycoproteins belong to the glycoconjugates, which combine some of the properties of polysaccharides and proteins. It has been reported that glycoproteins support various biological functions and activities, such as the maintenance of protein conformation and stability, hypolipidemic functions, and antioxidation (Ivashchenko et al., 2017).

In this study, a crude glycoprotein was evaluated using a UV spectrophotometer. A variety of lectins binding with crude ginger-derived glycoproteins were investigated by the lectin microarray technology. Finally, the enrichment of ginger-derived glycoproteins by magnetic particles was the main focus of this paper, aimed at providing a foundation for comprehending the relationship between the structure and the activities of glycoproteins.

2. Materials and methods

2.1. Chemicals and materials

Epoxy-coated magnetic particles were obtained from Shaanxi Lifegen Co. (Xi'an, China). 4-hydroxybenzhydrazide and N, *N*-dimethylformamide (DMF) were purchased from Alfa Aesar (Phentex (China) Ltd.). Phenylmethyl sulfonylfluoride (PMSF), N,N,N′,N′-tetramethylethylenediamine (TEMED), Protein Marker, and Tris were purchased from Sigma-Aldrich Company (Deisenhofen, Germany). The other chemical reagents were obtained from commercial suppliers and used without further purification. All of the solutions were prepared with ultra-pure water.

2.2. Preparation of ginger-derived glycoprotein

The ginger was cut into slices, washed under flowing water (Sparbier, Wenzel, & Kostrzewa, 2006), dried in a hot air oven at a temperature of 60 °C for 24 h, and screened through a 100-mesh sieve. Two grams of the powdered ginger were added to a beaker containing a 100-ml buffer (0.1 mol/l NaCL solution with potassium phosphate). The ginger-derived glycoprotein was extracted at 50 °C, with a liquid–solid ratio of 35.5:1 at a pH of 7.5 for 3 h and was subsequently concentrated. The extract was precipitated with four times the volume of 95% (v/v) ethanol stored at 4 °C in a refrigerator in order to improve the purity of the ginger-derived glycoproteins. The supernatant was subjected to centrifugation at 4000 rpm for 10 min. The precipitate was treated with an appropriate amount of a water solution and was subjected to dialysis at 4 °C. The crude glycoprotein of the ginger was obtained by freeze-drying. In addition, ultraviolet absorption was performed at full wave at room temperature.

2.3. Detection of carbohydrate chains by lectin microarray method

2.3.1. Preparation of lectin microarrays

The preparation procedure for the lectin microarrays was performed according to the methods described by Qin et al. (2013) with some modifications. Firstly, the lectins were dissolved in a manufacturer-recommended buffer by diluting to a concentration of 1 mM with Stealth micro spotting pins (SMP-10B) (TeleChem), using a Capital smart microarrayer (CapitalBio, Beijing) spotted on homemade epoxy-coated slides according to the protocol (Qin et al., 2012). Each lectin was

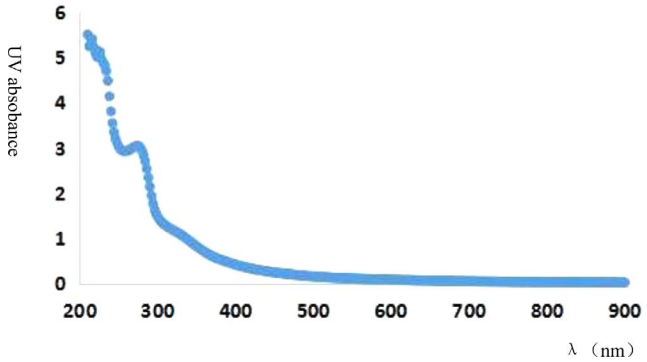

Fig. 1. Ultraviolet absorption performed at full wave at room temperature.

divided into three portions and every portion was placed in one block on the slide. The slide was incubated at 50% humidity overnight prior to immobilizing the lectin for 3 h in a vacuum dryer. Finally, the slide was submerged in PBS blocking buffer with 2% BSA for 1 h then washed twice for 5 min each. One buffer was a 1 × PBST buffer (0.2% Tween-20 in 0.01 mol/l phosphate buffer containing 0.15 mol/l NaCl, pH 7.4), the other was a PBS buffer.

2.3.2. Ginger-derived glycoprotein labeling and incubation

This method has been previously described (Qin et al., 2013). The extracted glycoproteins were labeled with Cy3 fluorescence dye (GE Healthcare, Buckinghamshire, UK) and the excess fluorescence dye was removed by Sephadex G-25 columns. Subsequently, 5 μg of Cy3-labeled glycoproteins diluted with a 0.5-ml incubation buffer (2% BSA, 500 mM glycine and 0.1% Tween-20 in PBS, pH 7.4) were applied to the blocks of the lectin microarrays. The slide was incubated at 37 °C in the chamber for 3 h prior to rinsing with a PBST buffer for 5 min and washing with a PBS buffer for 5 min. The last rinse was collected and analyzed using a laser scanning confocal microscope FV 1000.

2.4. The enrichment of ginger-derived glycoproteins by magnetic microsphere technology

This method has been previously described (Sun et al., 2009). The magnetic particles (1 mg) were hydroxyl functionalized mixed with a 500-μl coupling buffer (90% boric acid buffer, 10% borax buffer, pH = 7.4) under gentle shaking. The particles were collected from the coupling buffer using a magnetic separation device operating for 3 min. The test was performed in triplicate. Several types of lectin (300 μg) were dissolved in a 600-μl coupling buffer under gentle shaking at 180 rpm for 6 h at 25 °C; subsequently, the magnetic particles were added. The separation of the magnetic particles was similar to the last step. Unbound or weakly–bound components were removed from the conjugates. The lectin-particles were blocked with a 1-ml blocking buffer (2% ethanolamine, 0.1% BSA, pH 9.0) under gentle shaking at 180 rpm for 1 h at 25 °C and the supernatant was discarded. The magnetic particle-lectin conjugates were washed 3 times with 1 ml of binding fluid (0.1 mol/l Tris-HCl, 1 mmol/l $MnCl_2$, 1 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 0.15 mol/l NaCl, pH 7.4); subsequently, the ginger-derived glycoprotein solution (2 mg ginger-derived glycoproteins, 10 μl PMSF, binding buffer) was added under gentle shaking at 120 rpm for 3 h at 25 °C and the unbound supernatant was collected. The beads were rinsed five or six times with the washing buffer (0.1 mol/l Tris-HCl, 1 mmol/l $MnCl_2$, 1 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 0.15 mol/l NaCl, 0.1% Tween-20, pH 7.2) until there were no more glycoproteins in the supernatant. The last washing supernatant was collected. Finally, the glycoproteins bound to the lectins were eluted with a 0.1% SDS solution under gentle shaking at 120 rpm for 1 h at 25 °C. The eluting supernatant was also collected and all the collected supernatants were subjected to SDS-PAGE analysis.