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A rapid and simple approach for glycoform analysis

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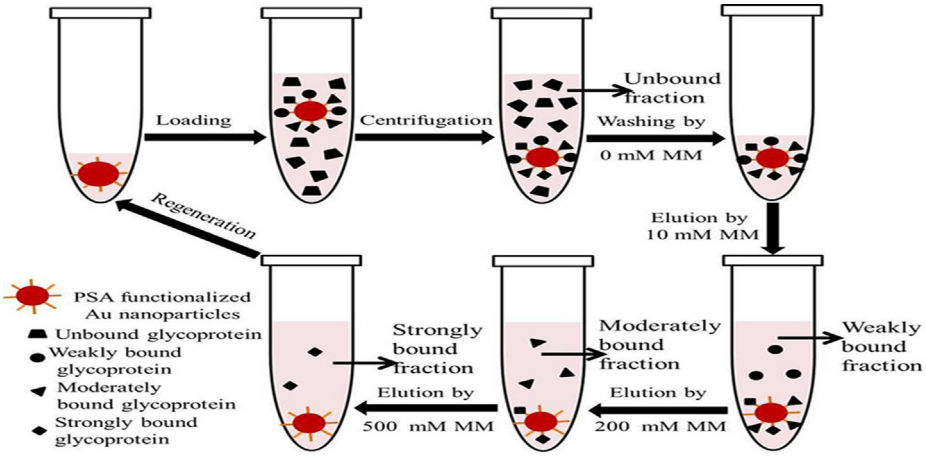
HIGHLIGHTS

- Au nanoparticle was firstly used as lectin affinity chromatography (LAC) support.
- Reproducibility of LAC is easily achieved by monitoring Au nanoparticle's LSPR.
- Our method has the potential use in on-site quality control of glycoprotein.

GRAPHICAL ABSTRACT



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ABSTRACT

Fast glycoform analysis is important for quality control of glycoproteins that account for over 40% of the approved biopharmaceuticals. Herein, we realized an Au nanoparticle-based lectin affinity chromatography (LAC) using simple standard laboratory equipment for fast glycoform analysis. *Pisum sativum* agglutinin (PA), a lectin derived from *P. sativum*, was covalently conjugated to Au nanoparticles via naturally formed carboxylic groups onto the surface of Au nanoparticles and amino groups of PA. Each model glycoprotein was separated into several fractions including the unbound, weakly bound, modestly bound, and strongly bound glycoforms based on affinity strength of the glycoform toward PA. A single run of Au nanoparticle-based LAC was finished within 18 min, which could be further decreased by centrifuging the mixture of the PA functionalized Au nanoparticles and the glycoproteins at a higher speed. To our knowledge, we are the first to use Au nanoparticles as LAC matrix.

1. Introduction

Over 40% of the approved biopharmaceuticals are glycoproteins that contain oligosaccharide chains (glycans) covalently attached to polypeptide side-chains [1]. Changes in glycosylation sites, and composition and structure of glycans lead to populations of glycosylated variants of a single protein that are usually referred to

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as glycoform [2]. Glycoform affects pharmacodynamic and pharmacokinetic behavior of therapeutic glycoprotein [3,4]. For example, the glycosylated human recombinant erythro-proteins have prolonged life in blood compared with those without glycans [5]. During biosynthesis, glycoprotein glycoforms are affected by host organism, production cell line, and/or culture conditions [6–8]. Therefore, fast glycoform analysis is required to ensure glycoform consistency and thus activity of the replicated biopharmaceuticals.

Lectin affinity chromatography (LAC) is a powerful tool for glycoform analysis [9–11]. Lectins are a family of proteins that have at least one domain with specific glycan binding affinity, which gives lectin ability of recognizing glycans in different glycoproteins. In general, lectin is immobilized onto LAC matrix. Various materials have been used as LAC matrix, including those with non-permanent porosity such as agarose and cellulose, and those with permanent porosity such as silica and polymeric media [12–18]. Each material has its own disadvantages. For example, agarose-based systems suffer from low mechanic stability, whereas polymeric monolithic surfaces exhibit hydrophobicity. Substantial effort has been directed toward finding a new matrix for LAC. In this study, we tested the possibility of Au nanoparticles as LAC matrix because of the following considerations: First, the Au nanoparticles can be easily modified with carboxylic groups. The hydrophilic layer benefits inhibiting nonspecific binding and lectin immobilization; second, the high reproducibility in lectin density of matrix can be easily achieved by monitoring a unique optical property of Au nanoparticles known as localized surface plasmon resonance (LSPR) simply with spectroscopy during lectin immobilization; third, nanoparticles have a big immobilization capacity for lectin resulting from high ratio of surface area to volume (SA/VOL). For example, the SA/VOL of our used Au nanoparticles of 113.0 nm in diameter is around 28 times of the commercial carboxylic magnet beads of 2.8 μm in diameter. Moreover, Au nanoparticle-based LAC can be conducted in a simple way, centrifuge, which significantly speeds up the entire LAC process. Using *Pisum sativum* agglutinin (PA), a lectin derived from *P. sativum*, and glycoprotein (albumin and ovomucoid from chicken egg white) as model systems, the performance of our Au nanoparticle-based LAC are demonstrated. Each model glycoprotein was separated into the unbound, weakly bound, modestly bound, and strongly bound glycoforms based on their affinity toward the immobilized PA. The relative amounts of glycoforms in the model glycoproteins exhibited a trend similar to that of reported studies [19–21].

2. Experimental

2.1. Chemicals and materials

Manganese chloride tetrahydrate and calcium chloride anhydrous were purchased from Tianjin Bodi Chemical Engineering Co., Ltd. (Tianjin, China) and First Reagent Manufacturer (Shanghai, China), respectively. Sodium chloride, sodium citrate dehydrate, and trimethylamine hydrochloride were obtained from Sinopharm Chemical Reagent Co., Ltd. (Bejing, China). Albumin from chicken egg white, ovomucoid from chicken egg white, bovine serum albumin (BSA), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) were from Sigma–Aldrich (St. Louis, MO, USA). 2-Mercaptosuccinic acid (MSA), methyl α-D-mannopyranoside (MM), and chloroauric acid were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Heown Biochem Technologies (Alpharetta, GA, USA), and Shanghai Reagent Manufacturer (Shanghai, China), respectively. PSA was purchased from Shanghai Dongfeng Biotechnology Company (Shanghai, China).

2.2. Quantification of protein

Extinction spectrum of Au nanoparticles and quantitation of proteins were conducted on TU-1901 UV–vis spectrophotometer acquired from Beijing Purkinje General Instrument Co., Ltd. (Bejing, China). 7DL-5 centrifuge was from Sichuan Shuke Instrument Co., Ltd. (Chengdu, China).

2.3. Synthesis and characterization of Au nanoparticles

Spherical Au nanoparticles were obtained following the method reported by Niu et al. [22]. In brief, Au nanoparticle seeds were prepared by heating 50.0 mL of 0.25 mM $HAuCl_4$ solution to boiling followed by the addition of 1.75 mL of 1% (w/v) sodium citrate solution. After the color of the solution turned into wine red, the reaction was allowed to continue for 30 min under constant stirring and boiling. The seed solution was then cooled to room temperature. To grow the seeds, 0.4 mL of 5 mM $HAuCl_4$ solution and 50 μL of the seed solution were mixed and diluted to 50 mL. Under constant stirring, 240 μL of 1 mM MSA was added into the mixture, and the growing process proceeded for 120 min. The Au nanoparticles are characterized using transmission electron microscope FEI Tecnai G2 T12(USA).

2.4. Immobilization of PSA onto Au nanoparticles

PSA immobilization was conducted according to Liu's method with little modification [23]. The Au nanoparticles were precipitated by centrifugation and resuspended in 1 mL of 20 mM phosphate buffer (pH 5.5). This washing step was repeated twice. Up to 1.5 mL of 1 mg mL^{-1} PSA in the same phosphate buffer was added into the Au nanoparticle solution and mixed for 30 min. Up to 30 μL of freshly prepared 100 mg mL^{-1} EDC in the cold phosphate buffer was dropped into the mixture. The interaction between the protein and the Au nanoparticles proceeded for 2 h at room temperature or overnight at 4 °C. After the reaction was completed, the PSA functionalized Au nanoparticles were washed using 1 mL of 20 mM Tris buffer at 1000 × *g* centrifugation for 5 min twice. Finally, the PSA functionalized Au nanoparticles were stored in the Tris buffer at 4 °C until use.

2.5. Operation of Au nanoparticle based LCA

Fig. 1 describes the process of the Au nanoparticle-based LAC operation. The process starts from the PSA functionalized Au nanoparticle pellet obtained by precipitating 0.5 mL of the PA functionalized Au nanoparticle solution by centrifugation and removal of the supernatant. The PA functionalized Au nanoparticles are resuspended with a 130 μL mixture of heterogeneous glycoprotein in the binding buffer with 20 mM Tris buffer containing 0.1 mM manganese chloride and 0.1 mM calcium chloride (pH 7.2). The combined mixture was mixed for 2 min. The glycoforms with binding affinity toward PA are adsorbed onto the PA functionalized Au nanoparticles, whereas those having no binding affinity toward PA are recovered in the supernatant by centrifugation at 3000 rpm. After the Au nanoparticles are washed with the binding buffer twice, the glycoforms that are absorbed onto Au nanoparticles are consecutively desorbed using 130 μL of the binding buffer containing 10, 200, and 500 mM competitor MM. The corresponding glycoforms are then collected in the supernatant by centrifugation. The PA functionalized Au nanoparticles are regenerated with 20 mM phosphate buffer at pH 3.5. The concentration of the desorbed glycoform is determined by the absorbance value at 206 nm with a TU-1901 UV–vis spectrophotometer.