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Selective capture of glycoproteins using lectin-modified nanoporous gold monolith

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ABSTRACT

The surface of nanoporous gold (np-Au) monoliths was modified via a flow method with the lectin Concanavalin A (Con A) to develop a substrate for separation and extraction of glycoproteins. Self-assembled monolayers (SAMs) of α-lipoic acid (LA) on the np-Au monoliths were prepared followed by activation of the terminal carboxyl groups to create amine reactive esters that were utilized in the immobilization of Con A. Thermogravimetric analysis (TGA) was used to determine the surface coverages of LA and Con A on np-Au monoliths which were found to be 1.31×10^{18} and 1.85×10^{15} molecules m^{-2}, respectively. An in situ solution depletion method was developed that enabled surface coverage characterization without damaging the substrate and suggesting the possibility of regeneration. Using this method, the surface coverages of LA and Con A were found to be 0.989×10^{18} and 1.32×10^{15} molecules m^{-2}, respectively. The selectivity of the Con A-modified np-Au monolith for the high mannose-containing glycoprotein ovalbumin (OVA) versus negative control non-glycosylated bovine serum albumin (BSA) was demonstrated by the difference in the ratio of the captured molecules to the immobilized Con A molecules, with OVA:Con A = 2.3 and BSA:Con A = 0.33. Extraction of OVA from a 1:3 mole ratio mixture with BSA was demonstrated by the greater amount of depletion of OVA concentration during the circulation with the developed substrate. A significant amount of captured OVA was eluted using α-methyl mannopyranoside as a competitive ligand. This work is motivated by the need to develop new materials for chromatographic separation and extraction substrates for use in preparative and analytical procedures in glycomics.

1. Introduction

Glycoproteins have attached oligosaccharide units called glycans, and belong to the collective group known as glycoconjugates. They are formed as a result of the post-translational modification of proteins in which glycans are covalently attached to an amino acid sequence, with N-linked or O-linked glycans being the most prevalent. Glycans on the cell surface serve as the recognition armies of cells, a function that is essential in cell-to-cell adhesion and recognition [1,2]. Another significant role of glycans is their role in the conformational changes that glycoproteins must undergo in order to perform specific biological functions [3]. Aberrant glycosylation, i.e., changes in oligosaccharide structure upon biosynthesis can be related to diseases, such as cancer [4]; therefore, glycoproteins are now used as cancer biomarkers [5,6].

The study of glycans and their structures and functions is called glycomics. Unlike proteins or polynucleotides, the structures of glycans are complex, heterogeneous and isobaric. This poses a challenge in glycomics where elucidation and identification of glycan structure and the glycosylation sites of glycopeptides are required in determining their respective biological functions [7]. Various methods are used in glycan analysis such as mass spectrometry (MS) [8–10], nuclear magnetic resonance (NMR) [11], electrochemistry [12], UV detection of derivatized glycans [13] and fluorescence imaging [14]. Comprehensive glycan analysis using microarrays of lectins [15] and antibodies [16] has also been developed. Prior to analysis, several preparative methods are usually needed that may include separation, isolation and enrichment in order to increase the abundance of glycoproteins, glycopeptides and glycans in complex samples such as serum, plant and cell tissue extracts and cultured cells. Preparative methods in glycomics include sodium dodecyl sulfate polyacrylamide gel electrophoresis

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(SDS-PAGE) [17], capillary electrophoresis (CE) [18] and chromatographic separation methods such as liquid chromatography (LC) [19] and capillary electrochromatography (CEC) [20].

One major factor that determines the success and efficiency of chromatographic separation is the optimization of the stationary phases in which different ligands are attached to a matrix or substrate that can selectively capture and isolate target analytes. In separation of intact glycoproteins, a popular and widely used method is lectin affinity chromatography (LAC). Lectins are proteins that contain carbohydrate-binding domains that bind with specificity to glycan structures of glycoproteins [21]. The binding is reversible and ligands can be displaced by competition or by changing the conditions of the mobile phase, such as pH and ionic strength. Lectins are classified by their sequence similarity and structural organization. Lis and Sharon proposed five groups according to the monosaccharide for which the lectin exhibits the highest affinity: (1) mannose (2) galactose/*N*-acetylgalactosamine (3) *N*-acetylglucosamine (4) fucose, and (5) *N*-acetylneuraminic acid [22]. Lectin–carbohydrate interactions are due primarily to cooperative hydrogen-bonding wherein a hydroxyl group (OH) acts simultaneously as H-bond donor and acceptor. Water molecules may also mediate H-bonding between sugar and proteins. Polar groups of both protein amino acid residues and of sugar units also provide electrostatic stabilization while non-polar patches of sugars formed by aliphatic protons and carbons stack with tryptophan and phenylalanine residues of lectins to create non-polar interactions [23].

Lectins are polyvalent and in vitro studies show that due to their polyvalency lectins have the ability to cross-link between cells resulting in cell-agglutination. Lectin polyvalency can be inhibited by the carbohydrate for which it is specific [24]. 3D structures confirm that members of each lectin group have conserved residues at the core of its carbohydrate binding site that provide H-bonding to sugars. This core is flanked by two variable loops that provide additional *van der Waals* and H-bond interactions [25]. Due to the high selectivity of lectins to specific glycan structures, lectins are now used as binding ligands of affinity matrices in purification of glycoproteins and glycopeptides and also in cell separations. To create the stationary phase, lectins are commonly covalently immobilized to the surface [26]. Due to the selectivity of lectins and improved immobilization techniques, LAC is the most useful and efficient mode of separation of glycans and glycoproteins. For example, multi-lectin affinity columns were developed using different lectins for comprehensive capture of serum glycoproteins [27,28].

The current approach in glycomics is the development of more sensitive, efficient, and faster methods of glycan separation and analysis. One specific strategy is the development of new materials to be used in designing separation columns and extraction media. The conventional packed columns with uniform size porous particles have been traditionally used in these chromatographic separations. A new generation of separation media called monolithic materials has become an interesting option due to their design that allows faster, more efficient and versatile separations of glycans, glycopeptides and glycoproteins [29]. Monolithic columns are usually prepared in situ fused with silica capillary tubes by co-polymerization of cross-linking and functional monomers together with porogens and initiators. Other monoliths are silica-based and prepared via sol–gel synthesis. The applications of these monoliths are exclusive depending on their morphology and structure. They also have respective disadvantages; for example, organic polymer-based monoliths swell in organic solvents whereas silica-based monoliths are limited by their effective pH ranges. Therefore, rather than choosing the material to use in designing chromatographic separation and extraction media, it is important to optimize the nature of ligands bound to the substrate for an efficient, stable and selective capture of target analytes.

Recently, a number of efforts to modify porous polymer monoliths with gold nanoparticles (GNPs) have appeared. The GNPs are either formed in situ or by flowing a GNP dispersion through the monolith whose surface presents amine or thiol groups for binding the GNPs. Porous polymer monoliths modified with GNPs were used for the capture and separation of cysteine containing peptides [30]. These monoliths were then modified with carboxylic acid, hydroxyl, or amine terminated alkanethiols and applied to separate short peptides by CEC [31]. The surface chemistries were shown to be exchangeable by removal using an excess of 2-mercaptoethanol. The monoliths were also shown to separate a mixture of three proteins by nano-HPLC in either reverse phase or ion exchange mode. GNP immobilization onto amine-terminated grafted polymer chains was shown to provide a dense and homogenous coverage [32]. A polymer monolith was decorated with 20 nm gold nanoparticles onto which 3,3′-dithiodipropionic acid di(N-hydroxysuccinimide ester) (DTSP) was assembled and used to immobilize *Erythrina cristagalli* lectin (ECL) for extraction of glycoproteins with terminal galactose units on their glycans [33]. GNP-modified polymer monoliths modified with cysteine were used to separate a mixture of nucleosides in hydrophilic interaction chromatography (HILIC) mode and their modification with polyethyleneimine was used to separate a mixture of di- and tripeptides [34]. GNP decorated monoliths were found most effective for a particle size of 15, 20, or 30 nm when modified with octanethiol or octadecanethiol and used in reverse phase separation of a mixture of three proteins [35]. Strategies using photomasking have been used to create monolith columns with specific segments being GNP modified [36,37]. Application of GNP decorated polymer monoliths for mixed modes of separation by modifying the GNPs with mixture of alkanethiols, ω-mercaptoalkanoic acids, and amine-terminated alkanethiols was demonstrated for a three-protein mixture in reverse phase, cation exchange, anion exchange and mixed modes of separation [38]. GNP decorated polymer monoliths have also been applied in Au driven catalysis [39] and to create a lipase flow through reactor [40].

Nanoporous gold has been of growing interest in analytical and biosensing research due to its composition-dependent tunable porosity, sizes and morphologies. As commonly prepared by dealloying in acid or electrochemically, np-Au typically has pore dimensions in the tens of nanometers range [41]. These features make np-Au promising in electrochemical and optical detection and also in catalysis [42]. Nanoporous gold surfaces can also accommodate various chemical-surface modification and conjugation, mostly through Au-S chemistry, that can be utilized in separation methods of biomolecules [43]. One popular and simple way of fabricating np-Au is through selective dissolution (leaching or dealloying) of a gold alloy, wherein the less noble constituents are removed via oxidization when immersed in concentrated nitric acid solution [41]. The three-dimensional, interconnected nanoporous structure, as well as biocompatibility and good chemical stability make np-Au monoliths suitable for affinity separations [44].

In this work, we prepared free-standing monolithic np-Au plates referred to herein as np-Au monoliths that underwent surface chemical modifications to develop Concanavalin A (Con A)-modified np-Au monolith and showed its potential to selectively capture glycoprotein. np-Au as a material can add to and complement the possibilities described using GNP decorated monoliths, and np-Au is relatively easy to prepare. The surface coverage of SAM and protein molecules prepared by a flow method onto the np-Au monolith was characterized using thermogravimetric analysis (TGA) and an in situ solution depletion method monitored by UV detection. The demonstrated selectivity of the developed