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Polar silica-based stationary phases. Part III- Neutral silica stationary phase with surface bound maltose for affinity chromatography at reduced non-specific interactions

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ABSTRACT

This research article reports the coating of large pore silica microparticles with a maltose layer to which bioaffinity ligands were attached *via* reductive amination reaction between the aldehyde activated maltose and the amino groups of the bioaffinity ligands. This was achieved first by the periodate oxidation of the maltose-silica (MALT-silica) yielding pairs of aldehyde groups at each monosaccharide ring. These di-aldehyde functionalities were then reacted with the primary amino groups of protein bio-affinity ligands and eventually formed Schiff bases (*i.e.*, aldimines) which were reduced using the mild reducing agent sodium cyanoborohydride to form stable amine linkages between the immobilized protein ligands and the maltose layer. Anti-human serum albumin antibody (aHSA), anti-human serum transferrin antibody (aTf) and concanavalin A (Con A) were the bio-affinity ligands immobilized onto the MALT-silica and were evaluated in high performance affinity chromatography (HPAC), namely immunoaffinity chromatography (IAC) and lectin affinity chromatography (LAC). Our initial studies reported here revealed zero or reduced nonspecific interactions with the two immunoaffinity sorbents (*i.e.*, aHSA-MALT-silica and aTf-MALT-silica) and the lectin affinity sorbent (*i.e.*, Con A-MALT-silica). The absence of nonspecific interactions is attributed to the hydrophilicity of the maltose layer and its shielding effect of the residual silanols (*i.e.*, unreacted silanols) on the silica surface. Conversely, the IAC and LAC sorbents exhibited specific interactions with the target biomolecules, namely human serum albumin (HSA) and transferrin (Tf) in the case of aHSA-MALT-silica and aTf-MALT-silica columns, respectively, and glycoproteins known for their affinity to Con A in the case of Con A-MALT-silica column.

1. Introduction

Affinity processes in general and high performance affinity chromatography (HPAC) in particular form the basis of important methods for sample preparations and for solving many separation problems in the life sciences [1–4]. Among the various HPAC methods, immunoaffinity chromatography (IAC) and lectin affinity chromatography (LAC) play important roles in proteomics/glycoproteomics profiling providing unique tools for the depletion of high abundance proteins [5], and capturing of specific glycoproteomes/glycoconjugates [6,7], respectively, from complex matrices such as human serum.

In addition to the selection of the immobilized affinity ligands in HPAC [8], the choice of the chromatographic support is equally crucial when developing a given HPAC stationary phase [1,4]. Various properties must be considered when choosing a given support for HPAC including its mechanical strength under fast flow rates, hydrophilicity, low levels of nonspecific interactions with the sample components, permanent porosity, moderate back pressure, stability to a wide range of solvents, ease of ligand immobilization, stable chemical bonding with the ligand, biocompatibility, large surface area, low cost and reusability [1,9]. Although various chromatographic supports are currently available, an ideal support, which fulfills all these requirements, is still not found yet. For instance, the matrices of some modified natural polymers such as agarose, cellulose, and dextran, have been widely used as affinity chromatographic supports over many decades due to their distinct advantages including biocompatibility, low nonspe-

Abbreviations: aHSA, anti-human serum albumin; aTf, anti-transferrin; BSA, bovine serum albumin; Con A, concanavalin A; DMF, *N,N*-dimethylformamide; GPTMS, γ-glycidoxypropyltrimethoxysilane; HSA, human serum albumin; MALT, maltose; Me-α-D-Man, methyl-α-D-mannopyranoside; PNP, *p*-nitrophenyl; Tf, transferrin; TRIS, tris(hydroxymethyl)aminomethane.

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cific interactions and stability over a wide pH range [10,11]. Also, the carbohydrate based stationary phases are inexpensive and they contain high amounts of hydroxyl groups for the activation and ligand coupling under mild conditions. However, the poor mechanical stability, severe shrinkage and compressibility of these low performance supports limit their applications in HPAC, which operates at high flow rates and pressure. In contrast, inorganic matrices such as silica have been successfully used as chromatographic supports for immobilizing affinity ligands in the field of HPAC [12]. Silica supports are known for their availability in a broad range of particle and pore sizes and for their excellent mechanical stability. Silica microparticles can be also easily derivatized to introduce functional groups *via* organo-silane coupling agents. However, the acidic silanol groups on the silica surface could establish various nonspecific interactions such as electrostatic interactions with proteins, peptides and other biomolecules. It is well known that nonspecific adsorption of the affinity matrix severely decrease the selectivity and performance of the separation [8] and this factor limits the applicability of silica supports in affinity chromatography separations. Moreover, silica supports are unstable at extreme pH conditions [13,14] and this further limits the usage of certain buffers as the mobile phase in HPAC.

Based on above considerations, the high mechanical stability of silica can be coupled with the chemical inertness, good stability and biocompatibility of carbohydrates to form sugar-silica composite chromatographic matrices for HPAC. These types of stationary phases may provide better shielding for the surface silanol groups, thus protecting analytes from the nonspecific interactions with the silica surface. In addition, these composite materials protect the silica matrix against chemical attacks by aggressive mobile phases, and consequently increasing the lifespan of the column [15]. So far, some attempts have been made for the coating of silica supports with different carbohydrates including agarose [16], dextran [17–20], cellulose [21], chitosan [22,23], glucose [24] and maltose [25] (see also the preceding paper in this issue). In most of these reported works, carbohydrate layers were coated on the silica surface by using non-covalent approaches leading to leaching out of the sugar layer with the attached affinity ligands, and in turn compromising the reusability of the HPAC stationary phases. Furthermore, the original porous structure of the silica gel might be altered after some of the above-mentioned coating steps involving large molecular weight polysaccharides, which may hinder the accessibility of affinity ligands to the porous surface and also increase the mass transfer resistances of the analytes. In addition, it was reported that substantial proportion of silanol groups remains unreacted even after these coating steps indicating the requirement of novel alternative pathways for the functionalization of silica with different carbohydrates.

In this work, we are reporting a novel synthetic pathway for the functionalization of silica microparticles with the disaccharide maltose (MALT), which proved effective in shielding the surface silanols as demonstrated in the preceding paper in this issue [26]. While the Zorbax silica in the preceding paper has an average pore size of 75 Å which was ideal for small molecules, the Zorbax silica used in this investigation has an average pore size of 300 Å simply because larger average pore diameter is required in affinity chromatography to provide proper accessibility to the high molecular weight affinity ligands, as well as to facilitate unhindered diffusion of relatively large sample molecules such as proteins. The polar MALT-silica stationary phase was further functionalized by converting it first to aldehyde-activated MALT-silica *via* the treatment with sodium periodate. The aldehyde–activated sorbent thus obtained served as the initial and starting hydrophilic support for immobilizing two antibodies, namely anti-human serum albumin (aHSA), anti-transferrin (aTf) and one lectin affinity ligand, *e.g.*, concanavalin A (Con A). These affinity stationary phases, which comprise a neutral polar maltose sublayer ensured minimum nonspecific interactions with proteins other than the target antigens or glycoproteins.

2. Material and methods

2.1. Reagents and materials

Zorbax silica gel having 5 μm average particle diameter, 300 Å average pore diameter was obtained from E.I. du Pont de Nemours and Company, Inc. (Wilmington, DE, USA). Human serum albumin (HSA), bovine serum albumin (BSA), horse skeletal muscle myoglobin, human transferrin (Tf), bovine pancreas ribonuclease A, bovine pancreas ribonuclease B, ovalbumin, fetal calf serum asialofetuin, anti-albumin antibody (aHSA) produced in rabbit, anti-transferrin antibody (aTf) produced in goat, concanavalin A (Con A) from *Canavalia ensiformis,* peroxidase from *Arthromyces ramosus*, *p*-nitrophenyl (PNP)-α-D-galactopyranoside, methyl-α-D-mannopyranoside (Me-α-D-Man), and calcium chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). γ-Glycidoxypropyltrimethoxysilane (GPTMS), tris(hydroxymethyl)aminomethane (TRIS) and manganese chloride were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Maltose, sodium periodate, sodium acetate, sodium azide, and urea were purchased from Fisher Scientific (Fair Lawn, NJ, USA). *N,N*-Dimethylformamide (DMF), sodium chloride, sodium monohydrogen phosphate and acetic acid were purchased from EMD Chemical Inc. (Gibbstown, NJ, USA). PNP-*a*-D-glucopyranoside and PNP-*a*-D-mannopyranoside were purchased from EMD Biosciences Inc. (La Jolla, CA, USA). BF_3·etherate was obtained from TCI America (Portland, OR, USA). Sodium cyanoborohydride was purchased from Across Organics (New Jersey, USA).

2.2. Instrumentation

Most HPAC separations were performed on a Waters Alliance 2690 Separation module (Milford, MA, USA), equipped with an in-line degasser, a quaternary solvent pump, an auto sampler and a thermostated column compartment. Detection was performed using a PDA detector (Model W2996). The sample and the column compartments were maintained at ambient temperature for all chromatographic separations. In addition, an HPLC system consisting of a quaternary solvent delivery system Q-grad pump from Lab Alliance (State College, PA, USA), a Model 3100 UV–vis variable wavelength detector from Milton Roy, LDC Division (Riviera Beach, FL, USA) and a Rheodyne injector Model 7010 from IDEX Health & Science LLC (Rohnert Park, CA, USA) equipped with a 20 μL loop was occasionally used. Data acquisitions were carried out using Empower 2 (Build 2154) software (Waters chromatography) or using the clarity version, 3.0.06.589 advanced chromatographic software from Data Apex (Prague, Czech Republic). A constant pressure pump from Shandon Southern Products Ltd. (Cheshire, UK) was used for the slurry packing of columns. A syringe pump from Cole-Parmer (Vernon Hills, IL, USA) was used to pump immobilizing chemical solutions (*e.g.*, periodate, TRIS/HCl) and protein solutions for immobilization through the column at appropriate flow rates.

The coating of maltose on the bare silica surface was verified by thermogravimetric analysis (TGA) using a TA instruments Q-50 thermogravimetric analyzer (TA instruments, New Castle, DE, USA). Samples were heated from 20 °C to 700 °C at a heating rate of 20 °C/min using a continuous airflow at 40 mL/min.

2.3. Preparation of epoxy activated silica and MALT-silica

The epoxy activated silica and MALT-silica were prepared following the same modification procedure of silica surface described in the preceding article in this issue [26], see Fig. 1.