Journal of Chromatography A, 1394 (2015) 71–80

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Thermo-responsive adsorbent for size-selective protein adsorption

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ARTICLE INFO

Article history:
Received 16 October 2014
Received in revised form 25 February 2015
Accepted 16 March 2015
Available online 21 March 2015

Keywords:
Thermo-responsive
Ion exchange chromatography
Poly(oligoethylene glycol) methacrylate (POEGMA)
Temperature-dependent
Phase transition
Lower critical solution temperature (LCST)

ABSTRACT

A thermo-responsive random copolymer, POEGMA (poly(oligoethylene glycol) methacrylate) grafted on cationized agarose adsorbent was used for size selective protein adsorption. The effects of $OEGMA_{300}$ ((oligoethylene glycol) methyl ether methacrylate, M_n = 300 g/mol) content and temperature on the adsorption of bovine serum albumin (BSA) were evaluated. Increasing the content of $OEGMA_{300}$ resulted a reduction in BSA adsorption due to the enhanced shielding effect of $OEGMA_{300}$ chains. Grafting of POEGMA chains onto cationized agarose adsorbent reduced the BSA adsorption by more than 95% at 26.5 °C, which is below the LCST (lower critical solution temperature) of POEGMA. The BSA adsorption capacities for adsorbents grafted with 10 and 20 mol% of $OEGMA_{300}$ decreased by 48% and 46% respectively at 38 °C, a temperature higher than their LCSTs. The temperature-dependent adsorption of BSA on the adsorbents was attributed to changes in the polymer conformation. The thermal transition of grafted POEGMA conformation exposed the ligand when the temperature was increased. Myoglobin (Myo), which was smaller than BSA, its adsorption behavior was less dependent on the polymer conformation. The adsorption of myoglobin onto the adsorbent with and without POEGMA showed similar percentage of reduction whereas the adsorption of BSA onto the adsorbent with POEGMA decreased by 7.6 times compared to the one without POEGMA. The packed bed of POEGMA grafted adsorbent was used for flow through separation of a protein mixture consisted of virus-like particle, Hepatitis B virus-like particle (HBVLP), BSA and insulin aspart. The recovery of HBVLP in 20 mol% of $OEGMA_{300}$ grafted adsorbent was increased by 19% compared to ungrafted adsorbent. The flow through of BSA can be reduced by increasing the operating temperature above LCST of 20 mol% of $OEGMA_{300}$ while the smaller protein, insulin aspart, remained adsorbed onto the cationized surface. Hence, this thermo-responsive adsorbent has a potential for size-selective separation of protein especially for the recovery of large biomolecule.

1. Introduction

Thermo-responsive polymers experience fast conformational change in their macromolecular structures even with minimal external heat input near their critical solution temperature. Their applications include cell culture, proteins separation, artificial tissue and in vivo sensor. The most explored candidate in biotechnology is poly(*N*-Isopropylacrylamide) (PNIPAM) due to its optimal low critical solution temperature (LCST ~32 °C) that is close to the body temperature. Okano and coworkers incorporated this concept into liquid chromatography by grafting PNIPAM onto silica, where heat-induced hydrophobic interaction was harnessed for steroids separation [1]. In addition to the phase transition of thermo-responsive polymers ranging from hydrophilic to hydrophobic, grafted thermo-responsive polymers experience coil-to-globule transition when the temperature was raised beyond its LCST. Hence, changes in the polymer physical properties should deserve consideration during the separation of biomolecules.

More recently, Lutz et al. (2006) reported on the potential application of poly(oligo[ethylene glycol] methyl ether methacrylates) (POEGMAs) as thermo-responsive materials. The LCST of POEGMA can be tuned by combining different molar proportions of oligo[ethylene glycol] methyl ether methacrylate (OEGMA) and diethylene glycol methyl ether methacrylate (MEO_2MA) [2]. The LCST of POEGMA is less sensitive to the presence of salt, molecular weight and concentration when compared to PNIPAM homopolymers [3]. Motivated by this, POEGMA was used to separate hydrophobic steroids and proteins [4,5]. In fact, POEGMA is an excellent non-biofouling material due to the hydrophilicity of the branched ethylene oxide chains [6,7]. Hydration of the tethered

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http://dx.doi.org/10.1016/j.chroma.2015.03.034
0021-9673/

polymer induces spatial restriction to protein penetration, or controlling the accessible path for protein to approach the active site beneath the polymer layer [8,9]. Therefore, the tunable hydration state of thermo-responsive polymer provides additional parameter to control and manipulate the separation of biomolecules.

Previous study has shown that the tethered POEGMA brushes experienced gradual transition in polymer conformation starting from substrate's surface towards interfacial layer of polymer and medium, rather than a sharp transition as in free polymer solution [10,11]. This progressive behavior of the POEGMA-grafted surface was employed to design and develop coatings to repel cell at temperature close to its LCST [12]. Although linear PEG (polyethylene glycol) grafted surfaces was able to provide size selectivity for proteins approaching the active site [13–15], none of the mechanisms involved the thermo-responsive properties of polymer. Conceptually, thermo-responsive polymer provides greater versatility in size selectivity by controlling the polymer conformation with a modulation in temperature. Non-thermo-responsive polymer could only provide fixed size selectivity based on the grafting density and its molecular weight.

The architecture of tethered thermo-responsive polymer has an impact on the efficiency of screening protein that approaches the active site of the surface. The longer the hydrophilic chain, the greater is the exclusion effect on the proteins [16]. Compared to known thermo-responsive polymers, POEGMA offers greater flexibility in the design and construction of separation medium due to the availability of different lengths of OEGMAs that allows us to tune the LCST. However, the selection of appropriate OEGMA monomers should be considered in terms of targeted LCST for bioseparation. However, to the best of our knowledge, there is no reported study on the application of POEGMA on the size selectivity of proteins and the role of the phase transition of POEGMA on the behavior of protein adsorption. Additionally, we could deploy POEGMA in devising a pre-screen layer on the separation material with embedded active site as another selective agent for a two dimensional separation material.

The objective of the present study is to investigate the feasibility of using POEGMA for size-selective adsorption of protein by manipulating temperature. Cross-linked agarose bead was cationized with quaternary ammonium ligand to provide the active site for ion exchange of protein. The cationized agarose bead was further grafted with POEGMA copolymer as size-selective layer. Free radical copolymerization was used to graft POEGMA on cross-linked agarose adsorbent by introducing free radicals on alkoxide groups through thermal decomposition of ammonium persulfate. Similar technique was used previously by Brook et al. [17] to graft the polymer chains on latex surface, and they observed that the polymer chain length needs not be homogeneous for size exclusion. Different molar proportions of MEO_2MA and $OEGMA_{300}$ were explored to tune the LCST of copolymer chains. The effects of the composition of $OEGMA_{300}$ and temperature on the adsorption of model protein, bovine serum albumin (BSA) and a smaller protein, myoglobin (Myo), were investigated. We further demonstrated the flow through chromatography of virus-like particle, Hepatitis B virus-like particle (HBVLP), which was mixed with BSA and insulin aspart protein mixture of similar isoelectric points (pI) but different sizes. The designed adsorbent could be an alternative to primary flow through purification of large biomolecule, such as virus-like particle.

2. Experimental

2.1. Chemicals and materials

Sample of 6% cross-linked unmodified agarose adsorbent (50–150 μm in diameter) was kindly provided by BioToolomics Ltd. (UK) in a suspension of 20% ethanol solution. Disposable SepFast™ column (0.33 ml) with 6.2 mm in diameter and 10.9 mm in length was also obtained from BioToolomics Ltd. (UK). Glycidyl trimethylammonium chloride (GTMAC), di(ethylene glycol) methyl ether methacrylate (MEO_2MA) and poly(ethylene glycol) methyl ether methacrylate M_n = 300 g/mol ($OEGMA_{300}$) were purchased from Sigma–Aldrich (Singapore). The monomers were purified with neutral alumina column. Ammonium persulfate (APS) and silver nitrate ($AgNO_3$) were used as received from Acros Organics (Geel, Belgium). Bovine serum albumin (BSA), myoglobin (Myo) and hydrochloric acid (HCl; 35%, v/v) were obtained from Nacalai Tesque, Inc. (Japan). Insulin aspart in NovoRapid® FlexPen® was obtained from Novo Nordisk (Brazil). Pure HBVLP was kindly provided by Prof. Wen Siang Tan from University Putra Malaysia. Tris(hydroxymethyl)aminomethane (Tris-base, Ultrapure grade) was obtained from 1st Base Pte Ltd (Malaysia). Sodium hydroxide pellet (NaOH, A.R. grade) was obtained from R&M Chemicals (Malaysia). Tetrahydrofuran (THF) was obtained from Merck (USA). Protein assay dye reagent concentrate from Biorad (Singapore) was diluted 5 times with deionized water and filtered prior to use.

2.2. Instruments and apparatus

Eutech Instrument PC700 (USA) was used to measure the conductivity. Thermoscientific Nicolet™ iS10 FT-IR spectrometer (USA) was used for attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy to access the functional groups on the adsorbent. Malvern Zetasizer Nano Zs (UK) (He-Ne laser 633 nm) was used to characterize the size change of free polymer and proteins with temperature. Non-Invasive Back Scatter technology (NIBS) was incorporated in the measurements. On the same instrument, the zeta potential of the proteins was characterized using M3-Phase analysis Light Scattering technique. Batch adsorption of protein was conducted using an incubator shaker (Labwit ZHWY-100D, China) with a rotational shaker and temperature controller. The thermo-responsive chromatography was performed using AKTA Purifier 10 (GE Healthcare, UK) and the absorbance of protein at 280 nm was recorded. Mini-PROTEAN® Tetra cell (Biorad, USA) was used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Gel Doc™ XR+ System (Biorad, USA) was used to obtain the images of stained gels from SDS-PAGE and Image Lab™ was used for densitometric analysis of the images.

2.3. Fabrication of adsorbent

2.3.1. Cationization of adsorbent

The cationization of crosslinked agarose adsorbent was performed according to Wang et al. [18] with some modifications. Briefly, 5 g of suction dried 6% cross-linked agarose adsorbent was mixed with 4 ml of 30 mmol GTMAC and 0.5 ml of 4 M NaOH. The mixture was stirred for 4 h at 65 °C. The quaternary ammonium cationized agarose adsorbent (Q adsorbent) was washed with distilled water and filtered until the collected washed solution attained the pH of distilled water (pH 6.8). The Q adsorbent was kept in 20% (w/v) ethanol solution until further analysis as described in Section 2.3.

2.3.2. Grafting of P(MEO_2MA-r-$OEGMA_{300}$) on blank and Q adsorbent

The grafting of random copolymer on the blank and Q adsorbents was conducted using free radical polymerization. A suspension of 10% (w/v) of filtered dried Q adsorbent in 50% (v/v) ethanol solution was purged with nitrogen for 30 min. It was then added with 1 ml of 0.1 M ammonium persulfate solution (pre-purged with nitrogen). The temperature of the mixture was raised to 60 °C and incubated for 15 min to initiate free radical at the