Journal of Chromatography A, 1400 (2015) 47–53

Contents lists available at ScienceDirect

Journal of Chromatography A

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

One-pot preparation of a sulfamethoxazole functionalized affinity monolithic column for selective isolation and purification of trypsin

Yuan Xiao[a,1], Jialiang Guo[a,1], Danni Ran[a], Qianqian Duan[a], Jacques Crommen[a,b], Zhengjin Jiang[a,*]

[a] Department of Pharmacy and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of Traditional Chinese Medicine & New Drug Research, Jinan University, Guangzhou 510632, China
[b] Laboratory of Analytical Pharmaceutical Chemistry, Department of Pharmaceutical Sciences, University of Liege, CHU B36, B-4000 Liege, Belgium

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form 8 April 2015
Accepted 22 April 2015
Available online 30 April 2015

Keywords:
Affinity monolith chromatography
Sulfamethoxazole
Sulfonamide drug
Trypsin

ABSTRACT

A facile and efficient "one-pot" copolymerization strategy was used for the preparation of sulfonamide drug (SA) functionalized monolithic columns. Two novel SA-immobilized methacrylate monolithic columns, i.e. poly(GMA-SMX-*co*-EDMA) and poly(GMA-SAA-*co*-EDMA) were prepared by one-pot *in situ* copolymerization of the drug ligand (sulfamethoxazole (SMX) or sulfanilamide (SAA)), the monomer (glycidyl methacrylate, GMA) and the cross-linker (ethylene dimethacrylate, EDMA) within 100 μm i.d. capillaries under optimized polymerization conditions. The physicochemical properties and column performance of the fabricated monolithic columns were characterized by elemental analysis, scanning electron microscopy and micro-HPLC. Satisfactory column permeability, efficiency and separation performance were obtained on the optimized poly(GMA-SMX-*co*-EDMA) monolithic column for small molecules, such as a standard test mixture and eight aromatic ketones. Notably, it was found that the poly(GMA-SMX-*co*-EDMA) monolith showed a selective affinity to trypsin, while the poly(GMA-SAA-*co*-EDMA) monolith containing sulfanilamide did not exhibit such affinity at all. This research not only provides a novel monolith for the selective isolation and purification of trypsin, but it also offers the possibility to easily prepare novel drug functionalized methacrylate monoliths through a one-pot copolymerization strategy.

1. Introduction

Affinity chromatography, which is based on highly specific interactions between two molecules, represents one of the most diverse and powerful chromatographic methods for the selective separation and analysis of biologically active molecules, or the study of specific target compounds in a sample. Although particle-based affinity columns have been well advanced and widely used [1], there has been an increasing interest in developing affinity-based separations on monolithic supports. The combined use of monolithic supports and biologically related binding agents as stationary phases has recently given rise to a novel concept known as affinity monolith chromatography (AMC) [2–7]. Owing to its advantages of high permeability, fast mass transfer, small sample volume and strong enrichment of trace biomarkers, AMC has represented a hot topic in the field of either analytical chemistry or biochemical separations [2]. Recently, most reports of AMC columns were mainly focused on the selection and application of appropriate immobilized ligands [2] and supports [3,5].

So far, most of these ligands used in affinity chromatography have been biological macromolecules such as antibodies [8,9], peptides [10–12], receptors [13], or other proteins [14,15], which are generally very expensive and sometimes unstable and easily denatured in chromatographic systems. As an alternative option, small molecule ligands, such as chelated metal ions [16], amino acids [17], bioactive compounds [18], triazine dyes [19–21] and drugs [22–24] have proved to be efficient ligands for isolating the target proteins by affinity chromatography. Most drugs produce their biological effects by interacting with specific macromolecular components of the organism. As a consequence, drug ligand based affinity columns could be an interesting and useful model for studying drug-receptor interaction and purifying targeted proteins [22–24]. Compared to macromolecule functionalized affinity columns, small drug molecule functionalized affinity columns also have many advantages such as good stability, long life, low cost,

* Corresponding author. Tel.: +86 2085223604; fax: +86 2085224766.
E-mail address: jzjjackson@hotmail.com (Z. Jiang).
[1] [1]These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.chroma.2015.04.046
0021-9673/

and easy preparation. Surprisingly, very few studies related to drug functionalized monoliths have been reported so far. Mattiasson and co-workers [22] developed sulfamethazine modified monolithic gels, which exhibited specific binding to IgG- and IgY-treated inclusion bodies. Yu and Zhang [23] developed doxorubicin functionalized polyacrylamide based monoliths in SPE cartridges using either the glutaraldehyde or the epoxy method. Both kinds of columns were used for screening T7-phage display human liver cDNA library. The excellent properties of the macroporous monolithic cryogel material make it a good matrix for drug coupling and target screening from the phage display library. Unfortunately, both methods have some shortcomings such as laborious and tedious preparation. More recently, Kang *et al.* [24] prepared two kinds of immunosuppressive drug (FK506 or CsA derivative) functionalized monoliths through a one-step copolymerization of the drug derivative with glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA). These novel AMC columns were successfully used to screen drug-binding proteins from cell lysate with HPLC-MS/MS, and the totality of the 33 FK506- and 32 CsA-binding proteins, including all target proteins for these two drugs reported in literature, were identified. However the synthesis and purification of the polymerizable FK506 and CsA derivatives could be time consuming.

In our previous research, a facile "one-pot" copolymerization approach was developed for the preparation of a β-CD functionalized monolith [25]. This proposed one-pot process involved the synthesis of the monomer glycidyl methacrylate-ethylenediamine-β-CD (GMA-EDA-β-CD) *via* a ring-opening reaction between the epoxy groups of GMA and the amino groups of ethylenediamine-β-CD (EDA-β-CD), and the subsequent copolymerization with the cross-linker EDMA in the same vial. This approach has proved to be able to simplify the fabrication, improve the reproducibility, and avoid the time-consuming post-polymerization derivatization of the traditional two-step strategy. This study opened up possibilities for preparing other monolithic columns that involve the ring opening reaction between epoxy and primary amino groups. In theory, drugs containing nucleophilic substitution groups (e.g. amino groups) could also be suitable for this one-pot strategy. For example, antimicrobial sulfonamide drugs (SAs) contain a primary aromatic amino group. Some of them have been successfully used as pseudo-bioaffinity ligands for the purification of proteins [22,26–28], such as sulfamethoxazole for the selective isolation of trypsin [26]. They could therefore be utilized as model ligands for proof of concept in the preparation of drug-functionalized affinity monoliths by the one-pot strategy.

In this work, two classical sulfonamide drugs, sulfamethoxazole (SMX) and sulfanilamide (SAA) were selected as model drugs to investigate the applicability of the one-pot strategy to the preparation of the corresponding AMC columns. The SA functionalized affinity monolithic columns poly(GMA-SMX-*co*-EDMA) and poly(GMA-SAA-*co*-EDMA) were prepared through one-pot *in situ* copolymerization of the drug ligand (SMX or SAA), the monomer (GMA), and the cross-linker (EDMA) within 100 μm i.d. capillaries under carefully optimized conditions. The characteristics of the monolithic columns were systematically evaluated by elemental analysis, scanning electron microscopy (SEM) and micro-HPLC. The selective affinities of two SA functionalized monoliths toward six proteins were also comparatively studied. The molecular docking method was used to study the interaction mechanism between trypsin and the SMX-immobilized monolith.

2. Experimental

2.1. Chemicals and materials

SMX, SAA, 3-(trimethoxysilyl)-propyl methacrylate (γ-MAPS), GMA, 2,2'-azobisisobutyronitrile (AIBN), acetonitrile (ACN), acetone, methanol (MeOH), thiourea, toluene, dimethylphthalate, anisole, naphthalene, acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, decanophenone, tris (hydroxymethyl) aminomethane and hydrochloric acid (HCl) were purchased from Aladdin Chemicals (Shanghai, China). EDMA was purchased from Alfa Aesar Chemicals (Tianjin, China). Trypsin and bovine serum albumin (BSA) were purchased from asegene (Guangzhou, Guangdong, China). Cytochrome *c*, lysozyme, myoglobin and chymotrypsin were purchased from Sigma-Aldrich (Shanghai, China). The fused-silica capillaries (375 μm o.d. × 100 μm i.d.) were purchased from Ruifeng Chromatography Ltd. (Yongnian, Hebei, China).

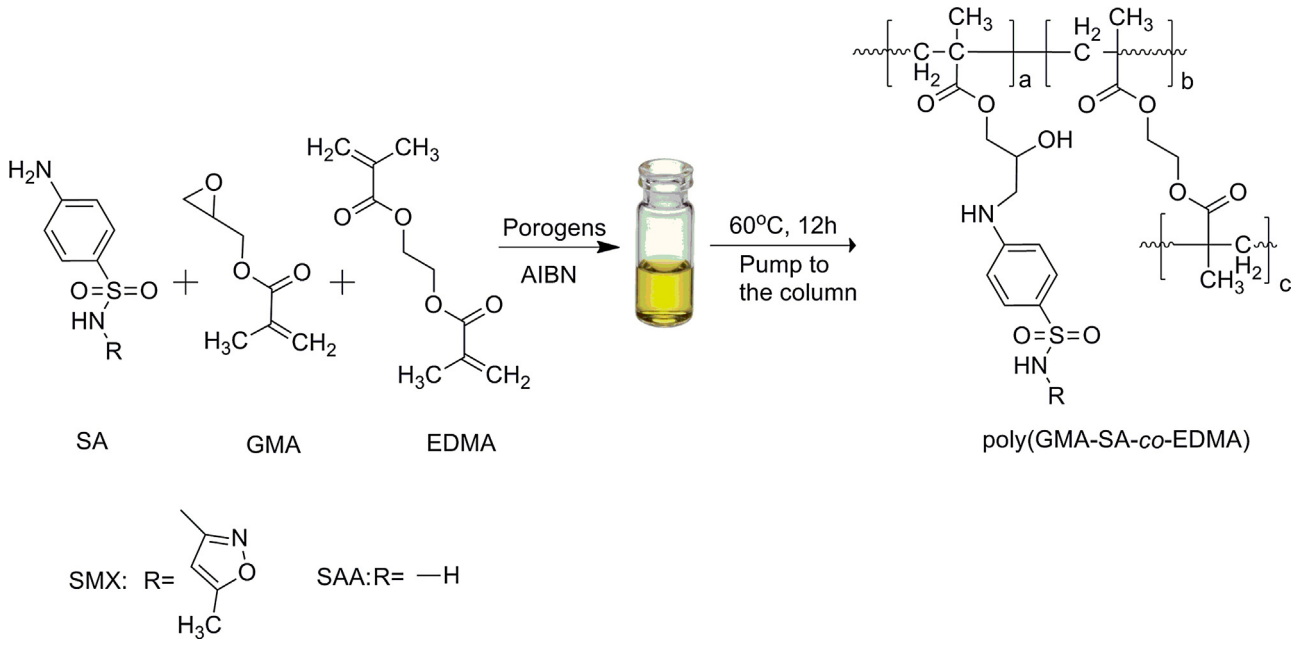

Fig. 1. Scheme for the preparation of the poly(GMA-SA-*co*-EDMA) monolithic columns.