Separation and Purification Technology 202 (2018) 165–173

Contents lists available at ScienceDirect

Separation and Purification Technology

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

Integration of mixed-mode chromatography and molecular imprinting technology for double recognition and selective separation of proteins

Wei Shi, Si-Qi Zhang, Kai-Bin Li, Wen-Ping Jia, De-Man Han*

School of Pharmaceutical and Chemical Engineering, Taizhou University, Taizhou 318001, China

ARTICLE INFO

Keywords:
Double-recognition resin
Mixed-mode chromatography
Molecular imprinting technology
Protein separation
Adsorption selectivity

ABSTRACT

Adsorption selectivity is one of the most important parameters for chromatography. As a promising technology for protein purification, mixed-mode chromatography (MMC) has unique advantages; however, its selectivity needs to be improved further. In this study, one strategy for improving selectivity was tested. We combined MMC with molecular imprinting technology (MIT) to gain double-recognition capabilities. Molecularly imprinted polymer (MIP) enabled the enhancement of the selectivity of MMC. Cross-linked 4% agarose beads (4FF) with tryptamine (Try) as ligands were used to prepare the double-recognition resin (4FF-Try/MIPs), with bovine serum albumin (BSA) as the imprinting template protein. After removing the template protein from the imprinted layer, 3D cavities with double-recognition abilities toward BSA were obtained, owing to the combination of hydrophobic interaction and molecularly imprinted spatial matched cavities. The static and dynamic adsorption behaviors of BSA, bovine immunoglobulin G (bIgG), and bovine hemoglobin (BHb) on resins were investigated. The results showed 4FF-Try/MIPs retained the typical adsorption properties of pH dependence and salt tolerance of MMC. High and similar dynamic binding capacities for BSA were obtained for both 4FF-Try and 4FF-Try/MIPs. However, 4FF-Try/MIPs showed lower dynamic binding capacity for the competitive impurity bIgG. When 4FF-Try/MIPs was used to separate BSA from bovine serum, the purity reached 75.1%, which was higher than 4FF-Try by ~20%. We conclude that MIT could be applied to enhance selectivity of MMC with double recognition capabilities, and this might be a straightforward strategy for improving selectivity in other modes of chromatography.

1. Introduction

In recent years, mixed-mode chromatography (MMC) has emerged as a new technology for protein purification [1–5]. It employs multiple binding modes, including ionic exchange, hydrophobic interaction, hydrogen bonding, etc. Due to its unique adsorption selectivity, high capacity, salt-tolerance and relatively low cost, attempts have been made to use MMC as an alternate or complementary technology to traditional chromatography. Thus far, many kinds of protein have been successfully purified through MMC, including bone morphogenetic protein-2 [2], recombinant human Apolipoprotein A-I [6], serum albumin [7–9], antibodies [10], and lysozyme [9] with sizes between ~15 and ~150 kDa. These results demonstrated that MMC is a promising technology for purifying protein from biological samples. However, the selectivity of MMC towards the target proteins is still not satisfied by researchers. Development of new methods to improve MMC performance is imperative. The lack of specificity might be because MMC ligands are small molecules, and only recognize small parts of the protein. For example, MMC ligand 4-mercaptoethyl-pyridine is adsorbed to the Fc fragment around Y319 and L309 [11], and 5-aminobenzimidazole is bound to His 435, Met 252 and Ile 253 on CBS region of Fc [12]. Target and impurity proteins can both be adsorbed by MMC. Therefore, combining an additional recognition mode with MMC mode might be a good solution.

Molecularly imprinting technology (MIT) can effectively construct tailor-made materials. Due to their complementarity with the template molecules in shape, size and functional groups, molecularly imprinted polymers have good recognition capacity and high selectivity, and have been much employed in various fields [13–22]. This technology has thus been recognized as a promising tool for protein recognition. In fact, some relevant progress has been made in recent years. For example, surface imprinting [23–29], epitope imprinting [30–32], metal-chelating imprinting [33–35], etc. were developed and applied successfully in protein recognition and purification. Some researchers have tried combining MIT with other technologies. Luo et al. [36] combined boronate affinity with molecularly imprinted spatial matched cavities,

* Corresponding author.
E-mail address: hdmtzc@126.com (D.-M. Han).

https://doi.org/10.1016/j.seppur.2018.03.057
Received 7 December 2017; Received in revised form 19 March 2018; Accepted 25 March 2018
Available online 27 March 2018
1383-5866/

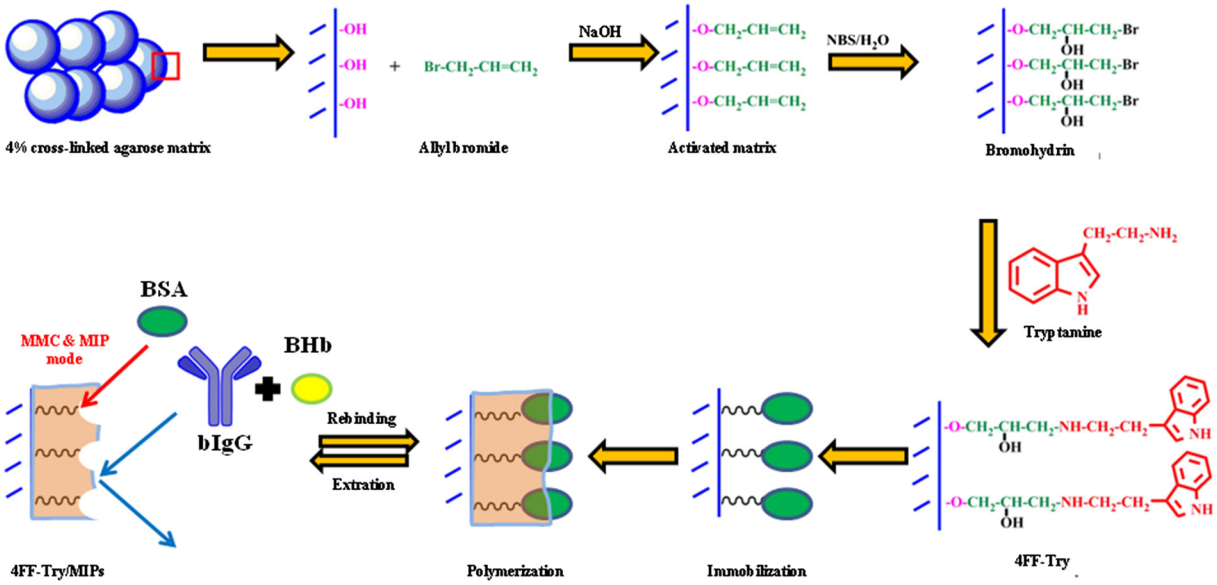

Fig. 1. Schematic illustration of the synthesis of 4FF-Try/MIPs.

applied to recognition and separation of glycoproteins. These results indicate that MIT has good potential to support other recognition technologies. Based on this point, it will be worthwhile to test MIT as an additional recognition mode to improve the selectivity of MMC. To the best of our knowledge, the combination of MIT and MMC has not been reported before. Therefore, the effect of the imprinted layer on the adsorption properties of MMC is given emphasis in this study, and it is expected that MIT will enhance selectivity in the combined method.

To sum up, MMC and surface imprinting will be integrated and steric hindrance will be applied as a second recognition mode to improve the adsorption selectivity of MMC. This new kind of double-recognition resin (4FF-Try/MIPs) was prepared using tryptamine functionalized agarose beads (4FF-Try) as supporting materials. Therefore, it possesses hydrophobic interaction, and steric recognition arises from the molecularly imprinted cavities. In order to investigate the effect of the imprinted layer on MMC, adsorption capacity, adsorption kinetics, and selectivity of the resultant imprinted resins with respect to the template protein were investigated. The effects of pH and salt were also studied. Finally, we used the challenge of purifying BSA from bovine serum in which bIgG was the main impurity, to assess the capabilities of the 4FF-Try/MIPs.

2. Experimental section

2.1. Materials

Cross-linked 4% agarose beads were obtained from Bestchrom (Shanghai, China) Biosciences Co. Ltd. Tryptamine, tetramethoxysilane (TMOS), and phenyltriethoxysilane (PTEOS) were purchased from J&K technology Co., Ltd (China). Bovine serum γ-globulin (bIgG, electrophoresis purity > 98%) was purchased from Merck KGaA (Darmstadt, Germany). Bovine serum albumin (BSA, Mw 66.4 kDa, pI 4.9) and bovine hemoglobin (BHb; Mw 64 kDa, pI 6.8) were obtained from Sigma (Milwaukee, WI, USA). Bovine serum was purchased from Pingruei biotechnology (Beijing, China) Co., Ltd. Other reagents were of analytical grade and were provided by local suppliers.

2.2. Analytical methods for structure characterization and concentration determination

Fourier transform infrared (FTIR) spectroscopy measurements were performed with an FTIR spectrometer (Nicolet 5700, Thermo Fisher Scientific). UV–vis spectroscopy measurements were carried out with a UV-2450 (SHIMADZU CORPORATION). A Hitachi S-4800 field-emission scanning electron microscope was used for scanning electron microscopy (SEM) measurements. Nitrogen adsorption–desorption isotherms were determined using a GEMINI-V (MICROMERITICS INSTRUMENT CORPORATION). The analytical SEC-HPLC was performed on LC3000 system (Beijing Chuangxintongheng Science & Technology Co., Ltd., Beijing, China) with TSK G3000SWXL column (7.8 mm × 30.0 cm, TOSOH, Japan). The mobile phase was 0.1 M sodium phosphate buffer containing 0.1 M Na_2SO_4 (pH 6.7). The buffer was filtered with 0.22 μm membrane and degassed before use. The flow rate was 0.5 mL·min^{-1}. The purities of BSA were defined as the percentage ratio of the peak area of monomers to the total integrated peak areas. The recoveries of BSA were calculated as the percentage ratio of monomer in the elution fraction to that in the loading sample during the separation process. The samples and fractions collected during the separation process were analyzed by non-reducing SDS-PAGE. The concentrations of the resolving gel and condensing gel were 10% and 5%, respectively. Protein migration was performed under 180 V for 60 min. The gel was stained with Coomassie Blue R-250 and destained. Tanon 3500 gel imaging system (Tanon Science & Technology Co., Ltd.) was used to image the protein gel.

2.3. Preparation of 4FF-Try/MIPs

The general scheme for the synthesis of 4FF-Try/MIPs is illustrated in Fig. 1. 4FF-Try resin was prepared according to a previously established protocol [37]. Cross-linked agarose beads were activated with allyl bromide, then the allyl-activated matrices were brominated by *N*-bromosuccinimide. Finally, tryptamine was coupled onto the brominated matrices. The ligand density was 70 μmol·mL^{-1}.

The imprinting procedure of 4FF-Try/MIPs was similar to that published by Luo et al. [36]. BSA was used as the template and was immobilized onto 4FF-Try surface via the formation of hydrophobic interactions between tryptamine and BSA at the specifically chosen pH value of 5.0. Then the resins were prepared by the sol-gel polymerization of organic silanes (TMOS and PTEOS) on the surface of 4FF-Try. Typically, 0.5 g drained 4FF-Try was dispersed in 20 mL PBS buffer (pH 6.0, 20 mM) and 300 mg of BSA was added, after which the solution was continuously agitated for the immobilization of BSA on 4FF-Try surface at room temperature for 5 h. Next, TMOS and PTEOS were added in a ratio of 1–3, and the pH of mixture was regulated to ~9.3