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Preparation and evaluation of dextran-grafted agarose resin for hydrophobic charge-induction chromatography

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ABSTRACT

Hydrophobic charge-induction chromatography (HCIC) is a new and effective technology for antibody separation. In the present work, HCIC resin MMI-B-XL was prepared with dextran-grafted agarose gel as the matrix and 2-mercapto-1-methyl-imidazole (MMI) as the functional ligand. The preparation procedures were optimized, and the maximum ligand density could reach as high as 200 μmol/g gel. The adsorption isotherms and kinetics on new resins were investigated with human immunoglobulin G (hIgG) as the model protein, which were compared with non-grafted HCIC resin MMI-B-6FF. It was found that the saturated adsorption capacity (Q_m) increased with the increase of ligand density for MMI-B-XL. Moreover, the effective diffusivity (D_e) could be dramatically enhanced with the increase of ligand density for MMI-B-XL, and the D_e for MMI-B-XL with the ligand density of 200 μmol/g gel was 18–40 times higher than that for MMI-B-6FF. The breakthrough experiments indicated that new resins with the ligand density of 200 μmol/g gel could be used for high superficial velocity and high dynamic adsorption could be obtained. The results indicated that dextran-grafted layer on the resin could increase the ligand density, enhance the mass transport in the pore, and improve the dynamic adsorption at high velocity, which showed a potential application for large-scale antibody purification.

1. Introduction

Hydrophobic charge-induction chromatography (HCIC) has been developed in recent years for improving protein separation [1,2]. The basic properties of HCIC are the pH-dependent behavior of mixed-mode ligands that combine the hydrophobic, thiophilic, hydrogen bonding and electrostatic interactions together. The target protein could be adsorbed on the uncharged ligand at the neutral pH mainly by the hydrophobic interaction, and desorbed by the electrostatic repulsion interaction between target protein and charged ligand at acidic condition. Normally, HCIC adsorbents could capture target protein over a broader ionic strength range compared to ion-exchange resins, which was called "salt-independent" or "salt-tolerance" properties [3–5]. MEP HyperCel is a typical commercial HCIC adsorbent developed by Pall Corporation. The functional ligand is 4-mercaptoethyl-pyridine (MEP) with the pK_a of 4.8, and the matrix is cellulose [6]. HCIC has been applied to the purification of antibodies and other proteins, which showed a potential as the cost-effective alternative to Protein A chromatography due to high adsorption capacity and reusability, mild elution condition and convenient Clean-In-Place process [7].

Combining several molecular interactions with specially designed ligands [8–10], HCIC resins usually show high selectivity on the adsorption of target protein compared to traditional ion-exchange chromatography (IEC) and hydrophobic interaction chromatography (HIC) [11]. However, due to the relatively weak hydrophobic interactions between target protein and ligand, the dynamic binding capacities (DBC) of HCIC resins are limited, especially for low retention time, which strongly impedes the large-scale application of HCIC for protein production. In recent years, polymer-functionalized IEC adsorbents have been developed in order to improve the performance of protein separation [12]. Dextran-grafted agarose resins such as SP Sepharose XL, Streamline Q XL and Capto S have been commercialized successfully [13,14]. Besides, a new directly grafting charged polymer poly(ethylenimine)-modified Sepharose FF has also been

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developed [15–19]. These polymer-grafted resins show significant enhancements on DBC, which reflects in both adsorption equilibrium and mass transport properties [20–26]. The improvements could be attributed to the following reasons: a solid or homogeneous diffusion and the specific architecture of the stationary phase [27], increased adsorption sites and more ligand exposed to three-dimensional binding space [28], an overall higher charge density or a shortening of the path length for diffusion [29], "bucket brigade effect" (protein passed from one polymer chain to the next) in the grafted layer [30], and the electrostatic coupling of diffusion fluxes [31]. In addition, the flexibility of the grafted polymer could improve diffusion process of protein in the pores to a great extent, as a result, polymer-grafted resins have higher uptake rates on the target protein [32]. Until now, most efforts have been made on the improvements of IEC resins. The effects of dextran layer on protein adsorption to dextran-grafted HCIC adsorbents have also been reported by Yu et al. [33]. However, the saturated adsorption capacity (Q_m) and effective pore diffusion coefficient (D_e) of dextran-grafted HCIC adsorbents did not change obviously, which might be attributed to the low ligand density of dextran-grafted adsorbents used in their work. Obviously, ligand density determines the strength of the hydrophobic and electrostatic interaction between resins and target protein, which might have a great influence on the adsorption equilibrium and adsorption kinetics [34–37]. The effects of ligand density on protein adsorption to HCIC adsorbents have also been reported by Lu et al. [38]. It was found that the Q_m and D_e increased with the increase of ligand density. In general, the polymer modification (such as dextran grafting) on the internal porous surface and the control of ligand density on the resins are two effective ways to improve the target binding and consequently the DBC for HCIC process.

In the present work, with 2-mercapto-1-methyl-imidazole (MMI) as the typical HCIC ligand developed in our previous work [39], new resin based on the dextran-grafted agarose gel would be prepared to improve the protein binding and DBC. The matrix was activated by allyl bromide (AB), brominated by N-bromosuccinimide (NBS) and then coupled with MMI ligand to prepare new dextran-grafted HCIC resins, named as MMI-B-XL. The reaction conditions of activation, bromination and ligand coupling would be optimized, and a series of new resins MMI-B-XL with different ligand densities would be prepared. With human immunoglobulin G (hIgG) as the model protein, the adsorption isotherms and adsorption kinetics would be investigated with series of MMI-B-XL resins and non dextran-grafted HCIC resins MMI-B-6FF based on 6% crosslinked agarose gel would be used as the comparison. The influences of dextran-grafted layer and ligand density on the Q_m, the dissociation constant (K_d), D_e and DBC would be discussed.

2. Materials and methods

2.1. Materials

The dextran-grafted agarose gel prepared with 6% agarose and grafted with dextran (Mw = 140,000), named Bestarose XL, was purchased from Bestchrom Bio-Technology Co., Ltd. (Shanghai, China). Non-grafted agarose gel with 6% agarose, named Bestarose 6FF, was also purchased from Bestchrom Bio-Technology Co., Ltd. (Shanghai, China) as the comparison. Allyl bromide (AB) and mercaptoacetic acid (MMA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 2-Mercapto-1-methylimidazole (MMI) and N-bromosuccinimide (NBS) were purchased from Aladdin (Shanghai, China). Human γ-globulin which is human normal immunoglobulin G (IgG > 98%), was obtained from Merck KGaA (Darmstadt, Germany). hIgG for intravenous injection (IgG > 96%), was purchased from RAAS Blood Products Co., Ltd. (Shanghai, China). Other reagents were of analytical reagent grade and purchased locally.

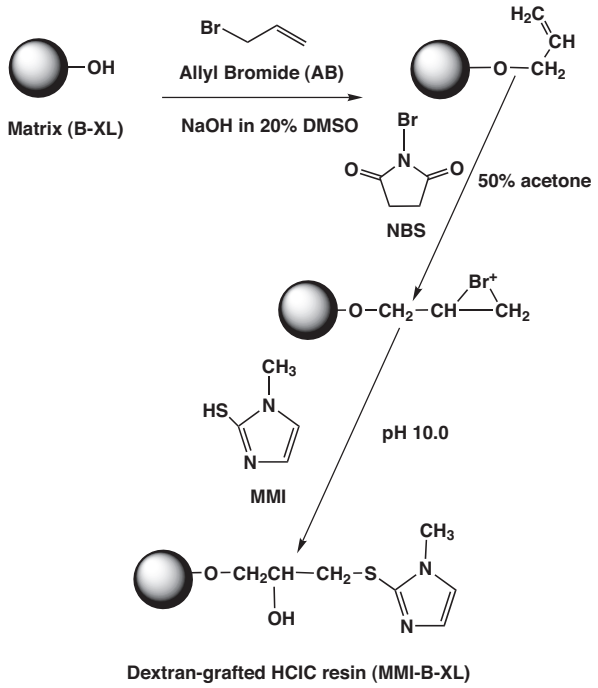

Fig. 1. Preparation scheme of the dextran-grafted HCIC resins, MMI-B-XL.

2.2. Preparation of HCIC resins

The preparation routes of HCIC resins were based on the previously reported methods [39,40] with some modifications, and the scheme is shown in Fig. 1. The dextran-grafted agarose gel was activated by allyl bromide (AB). Then the activated matrix was brominated by N-bromosuccinimide (NBS). Finally the brominated matrix was coupled with MMI ligand. Typically, 2 g drained agarose gel was mixed with AB (0.8 ml, 9.3 mM) and sodium hydroxide (0.41 g, 10.3 mM) in 20% dimethyl sulfoxide (DMSO) solution in a 25 ml triangular flask. The reaction was continuously agitated at 180 rpm and 30 °C for 24 h. After being washed with ethanol and deionized water, the allyl-activated gel was obtained and named as AB-B-XL, and then AB-B-XL was brominated with 3 molar excess of NBS over allyl groups in 50% acetone solution at 180 rpm for 1 h at 30 °C. Finally, the brominated gel was mixed with 3 molar excess of MMI over allyl groups in 1 M carbonate buffer (pH 10.0) at 180 rpm and 30 °C for 24 h. The resin was washed with deionized water and stored in 20% (v/v) ethanol. The dextran-grafted HCIC resins prepared were named as MMI-B-XL. As the comparison, the HCIC resins prepared with non-grafted agarose gel, Bestarose 6FF, using similar procedure were named as MMI-B-6FF.

The activated density on AB-B-XL and the ligand density on MMI-B-XL were determined by the titration method [2,39]. To measure the activated density, 1 g drained activated resin was mixed with 120 μl mercaptoacetic acid (MMA), 1 ml deionized water and 25 mg ammonium persulfate, then incubated at 60 °C for 8 h with a water bath shaker (180 rpm). After that, the resin was washed with deionized water, 0.1 M NaOH and 0.1 M HCl solution, then drained and transferred to a vial. Thereafter, 5 ml 0.5 M NaCl solution was added into the vial, and the mixture was titrated with 0.1 M Tris solution to pH 6.4. For the measurement of ligand density, 1 g drained MMI-B-XL was washed with 0.1 M NaOH solution and deionized water, then drained and transferred to a vial. Thereafter