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Multimodal charge-induction chromatography for antibody purification

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ABSTRACT

Hydrophobic charge-induction chromatography (HCIC) has advantages of high capacity, salt-tolerance and convenient pH-controlled elution. However, the binding specificity might be improved with multimodal molecular interactions. New ligand W-ABI that combining tryptophan and 5-amino-benzimidazole was designed with the concept of mutimodal charge-induction chromatography (MCIC). The indole and benzimidazole groups of the ligand could provide orientated mutimodal binding to target IgG under neutral pH, while the imidazole groups could induce the electrostatic repulsion forces for efficient elution under acidic pH. W-ABI ligand was coupled successfully onto agarose gel, and IgG adsorption behaviors were investigated. High affinity to IgG was found with the saturated adsorption capacity of 70.4 mg/ml at pH 7, and the flow rate of mobile phase showed little impact on the dynamic binding capacity. In addition, efficient elution could be achieved at mild acidic pH with high recovery. Two separation cases (IgG separation from albumin containing feedstock and monoclonal antibody purification from cell culture supernatant) were verified with high purity and recovery. In general, MCIC with the specially-designed ligand is an expanding of HCIC with improved adsorption selectivity, which would be a potential alternative to Protein A-based capture for the cost-effective purification of antibodies.

1. Introduction

The increasing market potential of monoclonal antibodies (mAbs) has promoted the revolution of antibody production techniques [1,2]. With the rapid advancement of upstream processing, antibody titers have been improved to 5–13 g/L [3,4], which leads the downstream processing to be a bottleneck of mAb production [5]. The development of more economical bioseparation technologies with high efficiency has been one of research hotspots.

Protein A affinity chromatography is the most widespread technique to capture antibody for large-scale production [6]. However, there are still some limitations, such as high cost, ligand leakage, and harsh elution conditions [7]. Researchers tried to develop new ligands with low cost and high affinity as alternatives to Protein A. Naik et al [8]. immobilized tryptophan to separate IgG, but the addition of PEG 600 in the buffer was required to improve adsorption selectivity. Histidine was used as a ligand to bind IgG at neutral pH and elute by salt addition [9–11]. However, due to the relatively simple structure, the binding specificity was limited. Moreover, some biomimetic synthetic ligands with more complex structure were developed, such as triazine ligands [12–15], peptide mimetic ligands [16–19] and new synthetic ligands based on the multi-component Ugi reaction [20–22]. A series of linear Fc-binding hexamer peptides and cyclic peptides were also reported [23–27]. D_2AAG ligand composed by amino acids and a synthetic aromatic acid was designed to improve mAb binding selectivity [28]. The specificities of these peptide ligands are generally comparable with Protein A, but they are also expensive and complicated to synthesize. Furthermore, high affinity leads to the difficulty on elution that often needs harsh pH or special additives. Therefore, novel ligands with both perfect binding and mild elution are still under development.

Hydrophobic charge-induction chromatography (HCIC) was introduced in 1998 as a novel technology for protein purification, especially for antibody [29]. MEP HyperCel with 4-mercaptoethyl-

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pyridine (MEP) as ligand is the typical HCIC resin, which has been used for antibody separation [30–32]. Other HCIC ligands, such as mercapto-methyl-imidazole, mercapto-benzimidazole, 2-mercaptoimidazole, histamine, and 5-aminoindole, have also been reported in literatures [33–36]. Generally, HCIC resins could bind target proteins through hydrophobic interactions at neutral pH, and elute proteins effectively by electrostatic repulsion between protein and charged ligands at acidic pH. In addition, the salt-tolerant adsorption property can offer the flexibility in feedstock preparation without the need of dilution or salt addition [31]. However, due to simple chemical structure with only one functional group, the binding selectivity of these ligands was limited. Therefore, better ligand design with multimodal molecular interactions to improve the specificity and expand HCIC to multimodal charge-induction chromatography (MCIC) is necessary.

The ideal MCIC ligand should be highly specific with high capacity, and the elution process could be achieved easily under weak acidic conditions. In our previous work [37], the recognition mode between IgG-Fc domain and some natural Fc-specific ligands (*e.g.* Protein A, Protein G, and Fc receptor) were evaluated with molecular simulation. The results revealed that Met252, Ile253, Asn434, His435, and Tyr436 are the key residues of Fc and two binding modes based on tryptophan or tyrosine were constructed. Therefore, tryptophan was introduced in this work as a functional group of new ligand to enhance IgG-Fc affinity. 5-amino-benzimidazole (ABI) was selected as the charge-induced group to facilitate protein elution. Novel resin with tryptophan-ABI (W-ABI) ligand was then prepared. The static adsorption behaviors, dynamic binding capacity and elution properties were investigated. Moreover, MCIC with W-ABI resin was applied for IgG separation from albumin containing feedstock and mAb purification from cell culture supernatant.

2. Materials and methods

2.1. Materials

Crossed-linked 4% agarose gel (Bestarose 4FF) was obtained from Bestchrom Bio-Technology Co., Ltd. (Shanghai, China). MEP HyperCel was purchased from Pall Life science (East Hills, NY, USA), and HiTrap rProtein A FF from GE Healthcare (Uppsala, Sweden). ABI was purchased from J&K Scientific Ltd. (Beijing, China), and tryptophan from Aladdin (Shanghai, China). Bovine serum γ-globulin (bIgG, electrophoresis purity 99.0%) was purchased from Merck KGaA (Darmstadt, Germany). Bovine serum albumin (BSA) was obtained from Sigma (Milwaukee, WI, USA). Human immunoglobulin G for intravenous injection was purchased from Boya Biopharmaceutical Ltd. (Jiangxi, China). CHO cell culture supernatant (CCS) containing 9.6 mg/ml mAb-1 or 0.85 mg/ml mAb-2 was provided by a local biotechnology company. Other chemicals are of analytical grade.

2.2. Molecular simulation

The crystal structure of binding complex between Fc portion of IgG and B domain of Protein A was obtained from Protein Data Bank (PDB ID: 1FC2, http://www.rcsb.org/pdb/) and used as the model for studying the interactions between W-ABI ligand and Fc. Molecular docking with Autodock vina 1.1.2 (http://vina.scripps.edu/) was employed for preliminary search of binding pose [38]. Polar hydrogen and Kollman United Atomic Charges were added to the protein structure, and Gasteiger charges were added to W-ABI ligand. According to the docking affinity energy, the best docked complex was chosen for further molecular dynamics (MD) simulation.

MD simulations were performed using Amber 11 software with amber ff10 force field at pH 7. The geometric structure of W-ABI was optimized with Firefly v.8.0.1 [39,40]. The atomic charges of W-ABI were obtained with the RESP formalism using RED-vIII.5 tools [41]. The temperature was controlled at 300 K with a Langevin dynamics algorithm and a collision frequency of 2 ps^{-1}. The pressure was controlled by a weak coupling Berendsen scheme. The SHAKE algorithm was used for all covalent bonds involving hydrogen using a 2 fs time step. The non-bonded cutoff was set as 12 Å and long-range electrostatic interactions were evaluated using the Particle Mesh Ewald (PME). Detailed MD procedures could refer to our previous work [37].

2.3. Preparation of W-ABI resin

10 g Bestarose 4FF gel beads were mixed with 5 ml allyl bromide and 2.5 g sodium hydroxide in 10 ml 20% (v/v) dimethyl sulfoxide solution, and the mixture was agitated at 150 rpm and 25 °C for 24 h. The allyl-activated gels were brominated with 1.2 molar excess of *N*-bromosuccinimide over the allyl groups in 50% acetone at 150 rpm and 25 °C for 1 h. After reaction, the gels were washed with deionized water. Three molar excess of tryptophan molecules over the allyl groups were added into the brominated gels in 1 M carbonate buffer (pH 10) at 150 rpm and 25 °C for 8 h. The gels were successively washed with 30% (v/v), 70% (v/v), 100% (v/v) acetone and dioxane. 10 ml dioxane, 1.2 g *N*-hydroxysuccinimide (NHS) and 1.2 g N, *N*-Dicyclohexylcarbodiimide (DCC) were added to the gels, which was followed by shaking at 150 rpm and 25 °C for 8 h to activate the carboxyl group of tryptophan. The NHS-activated gels were washed sequentially using 70% (v/v), 30% (v/v) acetone and deionized water. 10 ml deionized water and 200 mg ABI was added into the NHS-activated gels, and the reaction was carried out at 150 rpm and 25 °C for 8 h. The remaining epoxy groups were blocked by 10 ml 50% (v/v) ethanolamine at 25 °C for 2 h. Finally, the gels were washed extensively with deionized water and stored in 20% (v/v) ethanol at 4 °C.

2.4. Adsorption equilibrium experiments

The adsorption isotherms of human IgG on W-ABI resin were measured using the procedure described in our previous work [42]. The effects of pH at the range of 4.0–8.9 were focused. 20 mM acetate buffer (pH 4.0 and pH 5.0), 20 mM sodium phosphate buffer (pH 6.0, pH 7.0 and pH 8.0) and Tris–HCl buffer (pH 8.5 and pH 8.9) were used as the liquid phases. In addition, for MEP HyperCel the adsorption isotherms at pH 7–8.5 was also studied, and rProtein A FF was tested at pH 7–8 as recommended in the product instruction. The Langmuir equation was used to fit the experimental data with two parameters, the saturated adsorption capacity Q_m and the apparent dissociation constant K_d.

2.5. Dynamic binding capacity

The IgG dynamic binding capacity of W-ABI at various linear flowrates was determined with Tricon 5/100 column (GE Healthcare, Uppsala, Sweden) through frontal breakthrough experiments. 3.5 mg/ml human IgG for intravenous injection (pH 7) was used as the feedstock. The protein concentration was monitored on-line at 280 nm with an UV detector (WellChrom fast scanning spectrophotometer K-2600, KNAUER, Berlin, Germany). The dynamic binding capacity for MEP HyperCel and rProtein A FF at different pH and flowrate was also studied. The dynamic adsorption capacity at 10% breakthrough ($Q_{10\%}$) was calculated following the method published previously [43].

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