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Salt-independent hydrophobic displacement chromatography for antibody purification using cyclodextrin as supermolecular displacer

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ABSTRACT

Hydrophobic interaction chromatography (HIC) offers an orthogonal selectivity to ion exchange chromatography and the combination of the two processes can provide a potential cost-effective alternative to protein A chromatography in industrial antibody purification. However, the application of HIC is limited by its close dependence on high concentrations of kosmotropic salts to achieve desired separation. These salts can cause antibody precipitation and induce the corrosion of manufacturing facilities. Here, we report a new strategy of salt-independent HIC, which can capture antibody at the physiological salt concentration and allow the recovery of bound proteins through cyclodextrin (CD)-based displacement elution. Hydrophobicity-intensified HIC media with different coupling amount of phenyl ligands were prepared and assessed for their antibody binding capacity and selectivity. β-CD was investigated for its supermolecular interaction with phenyl ligands and elution capacity as a displacer. The results clarified a nearly linear correlation between binding capacity of human immunoglobulin G (IgG) and phenyl coupling density in the range of 44–159 μmol/mL. The host–guest interaction between β-CD and the phenyl ligands revealed a modest binding strength ($K_a = 4.1 \times 10^3\ M^{-1}$), and 15 mM β-CD solution showed a general effectiveness as displacement eluent for these HIC media, with IgG recovery varying with the ligand density. This strategy allowed the direct purification of human IgG from serum with satisfactory purity. The whole procedure of this method, including loading and elution, can be performed under physiological conditions. We expect such a salt-independent mode of HIC could be used as a capture or intermediate step in industrial antibody purification.

1. Introduction

Cost-effective manufacturing of antibody has gained increasing importance with the growing number of monoclonal antibodies (mAbs) candidates currently in development [1–3]. Recent advances in host-feeding strategies have led to expression levels as high as 10 g/L in large batch volumes [4]. Consequently, process bottlenecks have moved downstream and protein A chromatography is generally regarded as a productivity bottleneck despite its effectiveness [5,6]. Protein A resin is highlighted as the most expensive component as it contributes a quarter of the overall consumable costs in mAb downstream processes [7]. Development of alternatives to bioaffinity chromatography is critical for commercial success of antibody industry in the foreseeable future.

For decades, several different types of low-cost biomimetic ligands have been identified to be able to capture antibodies [8–14], such as peptide mimetic designated TG19318 [9,10], A2P [11], and triazine scaffold dye-ligands [12–14]. These ligands have shown selectivity to varying extent, but the purification factors are generally lower than those obtained with protein A. Hydrophobic mixed modes are another primary focus in the search for alternatives to protein A [15–20]. A range of commercial resins based on hydrophobic mixed mode ligands have been introduced in recent years, including those based on 4-mercaptoethyl pyridine (MEP) [17–20], N-benzyl-N-methylethanolamine (Capto adhere™), hexylamine (HEA) and phenylpropylamine (PPA). While recent publications have documented impressive performance of several mixed-mode procedures, they have not demonstrated adequate contaminant clearance to support commercial applications. Therefore, it is still hard to harness synthetic ligands to offer sufficient overall benefit in a single step like protein A can achieve.

Hydrophobic interaction chromatography (HIC) is expected to play a more important role in antibody purification [21]. Since most mAbs are more hydrophobic than the common contaminants in feedstocks, such as albumin and transferrin, HIC media

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could offer relatively high selectivity toward mAbs. In one case of industrial application, a combination procedure that employed ions exchange chromatography (IEX) and HIC has been commercially applied by Abbott Laboratories for purifying a mAb named Adalimumab, which was approved in 2002 for the treatment of immune diseases [22]. This procedure is one of rare examples of industrial processes that achieve antibody purification without using protein A chromatography. However, the above procedure has not been applied widely in other cases, because HIC has an intrinsic problem that restricts its application as a capture or intermediate step. Traditional HIC media normally depend on high concentrations of salt to adsorb proteins. High salt concentrations are often needed to obtain satisfactory binding capacities, but they can also lead to antibody precipitation [1]. The resulting aggregates would reduce product recovery and pose an added burden on the following polishing step. In addition, high concentrations of salt can also induce the corrosion of stainless steel facilities and increase environmental costs. Accordingly, decreasing the dependence of HIC on high concentrations of salt might effectively improve the applicability of HIC for antibody purification [21,23].

To promote the application of HIC in antibody purification, we propose a strategy to conduct salt-independent HIC using a hydrophobicity-intensified medium and cyclodextrin (CD)-based displacement eluent (Fig. 1). Most proteins can retain on a hydrophobic stationary phase material if the material is sufficiently hydrophobic. The coating process in ELISA serves as a good example, in which antibodies can be coupled on the hydrophobic surface of polystyrene stably without additional salts. In the case of HIC medium, the extent of hydrophobicity is mainly depended on its ligand density [24]. Thus, a resin with relatively high density of phenyl ligands is expected to be capable to capture IgG at physiological salt concentration. On the other hand, increasing ligand density would threaten the elution performance of bound protein. In this salt-independent HIC, a displacement elution mode based on host–guest interaction is conceived to release bound protein. The balance between hydrophobicity and solubility is the prime principle for the design of HIC displacers [25–27]. Most low-molecular weight displacers reported effective in HIC consist of charged molecules with several short alkyl chains and/or aromatic units [26,27]. Previous work of our laboratory proved the efficiency of CD-based displacement eluents for hydrophobic mixed mode chromatography [20]. The cylinder-shaped structure of CDs and their unique apolar cavities provide a perfect balance between hydrophobicity and solubility, making them as suitable supermolecular hosts for a range of nonpolar groups [28,29]. Host–guest interactions between β-CD and phenyl groups have been well studied for their self-assembly behaviors [30], but the application of β-CD as a displacement eluent in HIC has not been described to our best knowledge.

The intention of this study was to establish the method of salt-independent hydrophobic displacement chromatography and apply it to antibody purification. A range of hydrophobic media with different coupling density of phenyl ligands were prepared. Their binding capacities and selectivity for human immunoglobulin G (IgG) were compared at physiological salt concentration, and the elution performance with β-CD was evaluated. We specially focused on characterizing the efficacy of this mode of HIC in purifying human IgG from complex feedstock like serum.

2. Materials and methods

2.1. Materials

N,N′-carbonyl diimidazole (CDI), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl), and benzoic acid were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Sepharose CL-4B was supplied by GE Healthcare (Uppsala, Sweden). β-CD was supplied by Alfa Aesar (USA). 3,3′-Diaminodipropylamine (DADPA) and other reagents were purchased from J&K Chemical (Beijing, China) and used as received unless otherwise stated. Human IgG and human serum albumin (HSA) were supplied by RAAS Blood Products Co., Ltd. (Shanghai, China) with the purity more than 96% according to the manufacturing introduction. Human serum was obtained from Blood Bank of Dalian (Dalian, China). Deionized (DI) water was obtained from a Milli-Q ultrapure water purification system (Millipore; Billerica, USA).

2.2. Preparation of hydrophobic media

Agarose beads were activated with CDI in acetone; the amount of active groups was controlled by adjusting the amount of CDI used. Dosages of 0.01, 0.02, 0.04, and 0.1 g CDI/mL moist gel were used to synthesize a series of resins with different density of active points. DADPA was employed as spacer molecule. After intensive wash with acetone, CDI-activated gel (10 mL) was added to DADPA solution (2 mL DADPA in 10 mL acetone) and stirred at 30 °C for 3 h. Subsequently, the gel was washed with 100 mL acetone. Coupling of benzoic acid was through the reaction of EDC in MES buffer at pH 4.6. For each sample of 10 mL gel, 0.61 g benzoic acid and 0.6 g EDC·HCl were used, and the reaction was carried out at 30 °C for 4 h. At last, all the above four resins were tested with photometric ninhydrin and proved no primary amines left after the coupling reaction. Thus, it is reasonable to determine the density of phenyl ligand by evaluating the coupling amount of DADPA spacer. Measurements of the ligand density of the resins were performed by detecting the N content of each unit of dried matter using a Vario EL III Elementar Analyzer (Elementar, Germany).

2.3. Measurement of dynamic binding capacity and recovery

Determination of dynamic binding capacity (DBC) was performed using a glass column (0.66 cm in diameter) packed with 0.6 mL of resin. A slurry of 50% agarose beads in DI water flowed through the column, and the settled beads got a bed height of 1.7 cm for each packed column. Peak detection was performed with a Jingke UV-detector (type HD-9705) at 280 nm. For each measurement, the column was first equilibrated with 10 mM phosphate buffered saline (PBS, pH 7.4, containing 0.15 M NaCl) or other buffers indicated in the text. Protein sample that was dissolved in the same buffer at the concentration of 2 mg/mL was loaded at a linear flow rate of 75 cm/h. Calculations of DBC were made at 10% breakthrough. The column was washed with equilibration buffer until the absorbance of breakthrough returned to baseline, and then eluted with β-CD elution buffer (15 mM in PBS, pH 7.4) at a flow rate of 100 cm/h. Measurements of IgG or HSA concentration were carried out by determining UV absorbance at 280 nm. The recovery was expressed as the ratio of eluted protein to the total bound protein. The DBC and recovery values given are the average over three independent measurements with three unused columns.

2.4. ITC characterization of molecular interaction

ITC experiments were performed using an ITC200 Microcalorimeter (GE Healthcare, USA) by titrating 10 mM benzoic acid solution into 1 mM β-CD solution. During the titration, 39 μL of benzoic acid solution was injected into the β-CD solution dropwise (2 μL for each drop) at an interval of 2 min while stirring at 1000 rpm. CD-IgG interaction was detected in the same method by titrating 2 mM β-CD solution into 0.2 mM human IgG