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Evaluating antibody monomer separation from associated aggregates using mixed-mode chromatography

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ABSTRACT

Aggregate level is a key factor when assessing quality attributes of antibody product, since the aggregation might impact the biological activity of biopharmaceutical. Effective separation methods are usually essential in manufacturing processes. In this study, three mixed-mode resins, i.e. Capto adhere and two home-made resins with benzylamine and butylamine as the functional ligands (named BA and AB), were used to investigate the separation performances of antibody aggregate removal processes. In addition, two traditional resins (Q Sepharose FF and Phenyl Sepharose 6 FF) were also investigated to explore the role of molecular interaction in the aggregate removal. The results indicated that the removal efficiency was highly dependent on the mass loading. With the sample load of 50 mg/ml resin both Capto adhere and BA resins can significantly reduce the aggregate level from 20.5% to 2.6% and 2.4%, respectively, while a relatively low degree of aggregate reduction was found with the traditional resins. The research indicates that both hydrophobic interaction and electrostatic interaction are critical for the aggregate removal and the cooperativity of different molecular interactions is important for the effective aggregates removal with mixed-mode resins.

1. Introduction

Currently, the pharmaceutical and biotechnological industries are investing substantial resources in the development of antibody-based therapeutic products. By the end of 2010, a total number of 30 candidates (25 monoclonal antibodies (mAb) and 5 Fc fusion proteins) were in Phase 2/3 or Phase 3 clinical studies [1]. During biopharmaceutical manufacturing processes, such as cell culture, purification, formulation and storage, a common issue of mAb aggregation is encountered frequently, and several factors are known to intensify the aggregate formation, such as low pH, high shear rate, high concentration and thermal stress [2,3]. Aggregates may cause multiple side effects in patients. For example, it can neutralize antibodies and inhibit the efficacy of the products [4], cause severe hypersensitivity responses such as anaphylaxis [5] and break the immune tolerance [6]. Therefore, effective removal of mAb aggregates is essential during the manufacturing process.

Chromatographic separation techniques are of particular interest in the downstream processing stage as it provides high opportunity to remove aggregates [3]. Anion- and cation-exchange chromatography (AEC and CEC) have been used in industrial scale to separate mAb monomers from dimers and larger molecular weight species [7]. However, the resolution between monomer and aggregate is poor for CEC and the specification of peak collection is quite important in determining the final aggregate level. Sometimes low operating capacity of CEC may be used to improve the selectivity for aggregate removal and the optimum conditions should be often determined empirically. AEC is generally more effective in removing higher multimer aggregates, and it is also often used for removing impurities such as host cell proteins (HCP), DNA and viruses [8]. Hydrophobic interaction chromatography (HIC) is usually effective for the reduction of aggregates that are normally more hydrophobic [9]. The performance of HIC is sensitive to small changes of operating conditions and adsorbent lot to lot variability, and the power of aggregate removal appears to be modest [10]. Ceramic hydroxyapatite chromatography (CHT) had been reported to provide good capabilities for aggregate removal. As reported by Gagnon [11,12], the aggregate level greater than 60% could be reduced to less than 0.1%. However, the usage of CHT technique in industry is limited because large-scale column packing is difficult currently and the resin has a short lifetime due to dissolution and discoloration. In addition, in order to keep the resin stable without the degradation of the calcium phosphate backbone, the buffer conditions must be strictly maintained [13].

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Table 1
Properties of mixed-mode resins used.

Resins	Manufacturer	Matrix	Average particle size (μm)	Ligand group	Ligand density (μmol/ml)
Capto adhere	GE Healthcare	Agarose	75	*N*-Benzyl-*N*-methyl ethanolamine	90–120
BA	Home-made	Agarose	90	Benzylamine	~60
AB	Home-made	Agarose	90	Butylamine	~70

Mixed-mode chromatography using multimodal functional ligands can adsorb target proteins with the combination of ionic interactions, hydrogen bonds and hydrophobic interactions [14–16]. Mixed-mode resins can directly capture target proteins at relatively high conductivity of 15–30 mS/cm without dilution or other additives due to their multiple binding interactions. Currently, commercially available mixed-mode resins include Capto MMC, Capto adhere and Capto Core 700 from GE Healthcare, PPA Hypercel, HEA Hypercel and MEP Hypercel from Pall Corporation, Eshmuno HCX from Merck Millipore, Toyopearl MX-Trp-650 M from TOSOH Bioscience, and Nuvia cPrime, CHT Ceramic Hydroxyapatite, and CFT Ceramic Fluoroapatite from Bio-Rad. Different properties of these resins such as static and dynamic binding properties [17,18], adsorption kinetics [19], adsorption selectivity [20], ligand design and molecular simulation [15,21–23] and applications [24–27] have been reported in the literature. Recently mixed-mode chromatography was also used for the removal of mAb aggregates [28]. For example, Capto adhere can be operated in the flow-through mode during mAb manufacture processes. However, there are only few publications so far to investigate the usage of mixed-mode chromatography for mAb aggregates removal [29,30]. Limited understanding of chromatographic behaviors certainly impedes the application of mixed-mode chromatography for aggregates removal.

In this work, three mixed-mode resins with different ligands were investigated for aggregate separation under flow-through operation mode using a monoclonal antibody as the model target. Two traditional single-mode resins, an anion exchanger and a hydrophobic interaction resin were also evaluated for comparison. Moreover, the effect of different types of interaction forces, such as the electrostatic interaction, hydrophobic interaction or their combination on the aggregate separation was studied and the removal mechanism was discussed.

2. Materials and methods

2.1. Materials

The IgG1 monoclonal antibody used was recombinantly expressed using Chinese Hamster Ovary (CHO) cell lines and purified by rProtein A Sepharose FF affinity resin. Three mixed-mode resins used in this study were Capto adhere (CA) from GE Healthcare (Piscataway, NJ, USA), BA with benzylamine as the functional ligand and AB with butylamine as the ligand. BA and AB resins were prepared using the method published previously [31]. The ligand structures and some properties of the mixed-mode resins are shown in Table 1 and Fig. 1. Q Sepharose FF (QFF) and Phenyl Sepharose 6 FF_HS (PHE) were purchased from GE Healthcare (Piscataway, NJ, USA). Chemicals for the preparation of buffers were of analytical grade and purchased from local suppliers.

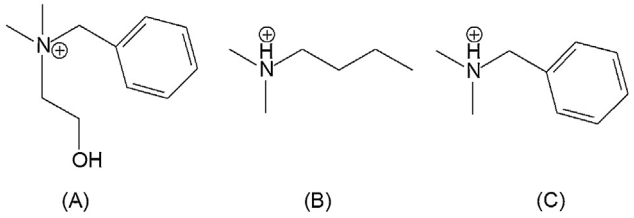

Fig. 1. Ligand structure of three mixed-mode resins used. (A) CA, (B) AB, and (C) BA.

2.2. Chromatographic methods

The sample loading for mixed-mode chromatography was firstly purified with rProtein A Sepharose FF and then buffer exchanged with 50 mM phosphate buffer (pH 7.5) using Sephadex G 25. The experiments of aggregate removal were conducted in the flow-through mode using an ÄKTA Explorer 100 (GE Healthcare). Tricorn 5/50 column (GE Healthcare) was used and packed with resins to a bed height of 5 cm (equivalent to a column volume of 1 ml). The column was first equilibrated with equilibration buffer, i.e. 50 mM phosphate buffer (pH 7.5), and loaded with mAb samples (~5.8 mg/ml). Then the column was washed with the equilibration buffer until all proteins passed through. The flow-through and wash fractions were pooled. The adsorbed components (mainly mAb aggregates and other impurities) were eluted by 50 mM citrate buffer (pH 2.6) for further analysis. The superficial velocity of the mobile phase was fixed during the experiments at 60 cm/h (residence time of 5 min) and all chromatographic processes were monitored online by UV detector at 280 nm.

2.3. Assays

SEC-HPLC was used to determine the concentration of monomeric and aggregated forms of mAb and conducted with a Tosoh Bioscience G3000SWXL column (7.8 mm × 30 mm) on the Agilent 1200 HPLC system (Palo Alto, CA). The mobile phase consisted of 50 mM sodium phosphate (pH 7.0) and 200 mM NaCl and the flow rate was 0.5 ml/min. 30–60 μl of samples were injected and analyzed by UV detector at 280 nm. This method was used for the analysis of both flow-through fractions and the effluents.

The Host Cell Protein (HCP) ELISA was performed using the CHO HCP ELISA kit F015 from Cygnus Technologies (Southport, NC, USA). Standard and test samples were measured following the manufacturer's instructions.

3. Results and discussion

3.1. mAb aggregates in the elution pool from Protein A capture

Protein A affinity chromatography is a powerful purification technique used in the production of mAbs. High affinity of mAbs to the resin usually requires acidic conditions for the elution. However, exposure to these acidic conditions often results in the formation of mAb aggregates. Fig. 2 shows the result of SEC-HPLC analysis of mAb fraction eluted from rProtein A Sepharose FF affinity chromatography. The results show that several minor peaks existed prior to the main peak (monomer mAb) at 17.5 min. These peaks were the high molecular weight aggregate species and defined as HMW-A and HMW-B. After the integration of the peaks in the chromatogram, it was found that the percentages of the monomer mAb, HMW-A and HMW-B were approximately 79.0, 8.4 and 12.1%, respectively. The concentration of the aggregates was relatively high and the removal process should be challengeable.