Journal of Chromatography A, 1402 (2015) 46–59

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Adsorption equilibrium and kinetics of monomer–dimer monoclonal antibody mixtures on a cation exchange resin

Jason M. Reck[a], Timothy M. Pabst[b], Alan K. Hunter[b], Xiangyang Wang[b], Giorgio Carta[a,*]

[a] Department of Chemical Engineering, University of Virginia, Charlottesville, VA, United States
[b] MedImmune, Gaithersburg, MD, United States

ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form 30 April 2015
Accepted 5 May 2015
Available online 23 May 2015

Keywords:
Ion exchange
Monoclonal antibodies
Monomer/dimer separation
Protein adsorption equilibrium and kinetics

ABSTRACT

Adsorption equilibrium and kinetics are determined for a monoclonal antibody (mAb) monomer and dimer species, individually and in mixtures, on a macroporous cation exchange resin both under the dilute limit of salt gradient elution chromatography and at high protein loads and low salt based on batch adsorption equilibrium and confocal laser scanning microscopy (CLSM) experiments. In the dilute limit and weak binding conditions, the dimer/monomer selectivity in 10 mM phosphate at pH 7 varies between 8.7 and 2.3 decreasing with salt concentration in the range of 170–230 mM NaCl. At high protein loads and strong binding conditions (0–60 mM NaCl), the selectivity in the same buffer is near unity with no NaCl added, but increases gradually with salt concentration reaching high values between 2 and 15 with 60 mM added NaCl. For these conditions, the two-component adsorption kinetics is controlled by pore diffusion and is predicted approximately by a dual shrinking core model using parameters based on single component equilibrium and kinetics measurements.

1. Introduction

The removal of aggregates and, especially, soluble dimers and other moderate molecular mass species, presents a significant challenge in the purification of monoclonal antibodies (mAbs) used for therapeutic applications. Such aggregates can be generated during cell cultivation [1], formed during downstream processing when harsh conditions (e.g. low pH and/or high concentrations of chaotropes) are used [2], or as a result of binding to certain chromatographic surfaces [3]. In addition to a potentially lower efficacy than their monomeric form [2], mAb aggregates are also more likely to generate an immunogenic response [4], and can result in less stable formulations [5], making them a key impurity attribute monitored during downstream processing. The removal of insoluble aggregates or higher-order aggregated species can typically be achieved through filtration or with relatively simple chromatographic processes, owing to the large difference in size and charge compared to the monomer. However, the chromatographic separation of antibody dimers or other soluble species with modest differences in molecular mass from the monomeric form is often challenging, due in part to low selectivity and in part to slow mass transfer. These difficult separations may require the use of operationally demanding techniques at manufacturing scale such as gradient elution and small stationary phase bead size to achieve high column efficiencies.

Currently, the separation of soluble antibody aggregates is most commonly achieved in manufacturing scale processes using hydrophobic interaction chromatography (HIC) or ion-exchange chromatography (IEX) [6,7]. SEC can be effective, but its generally low productivity and high plate number requirements make this technique unattractive. HIC is based on the fact that aggregates are often more hydrophobic than the corresponding monomers, as they are frequently formed from unfolded intermediates. While also effective, this tool depends on complex protein–surface interactions and requires the use of high concentrations of kosmotropes that reduce solubility, and stationary phases that can lead to surface-induced conformational changes and unfolding, resulting in low recoveries [8]. IEX, on the other hand, is based on the higher charge of aggregates compared to the monomer, which generally results in stronger binding. Because of the generally milder operating conditions and more predictable behavior, IEX is often preferred. In most case, mAbs have relatively high pIs, thus cation exchange chromatography (CEX) is commonly used. Ansaldi and Lester [9], for example, have described the use of salt steps and salt gradients to separate dimers and multimers from protein monomers on both weak and strong CEX resins. Zhou et al. [10] demonstrated aggregate clearance from a mAb feedstock utilizing salt gradients and hybrid pH-salt gradients with both weak and

* Corresponding author. Tel.: +1 434 924 6281; fax: +1 434 982 2658.
E-mail address: gc@virginia.edu (G. Carta).

http://dx.doi.org/10.1016/j.chroma.2015.05.007
0021-9673/

strong CEX resins. Suda et al. [11] compared and modeled mAb aggregate clearance using both gradient elution and flow-through operation on macroporous and polymer-grafted CEX resins. In practice, some form of CEX is typically included as an aggregate removal step in platform processes for mAb purification (e.g. [6,12]).

In general, the interaction of proteins with porous CEX resins in one-component systems is relatively well understood with regards to both equilibrium (e.g. [13–17]) and kinetics (e.g. [18–23]). Less is known, however, when multiple proteins bind competitively. A few studies have investigated competitive binding of model proteins. Weinbrenner and Etzel [24], for example, showed the occurrence of concentration overshoots during the frontal loading of a strong cation exchange membrane with either mixtures of BSA and α-lactalbumin or mixtures of BSA monomer and dimer. In both cases, the overshoot was attributed to competitive binding. Gadam et al. [25] investigated the displacement-based separation of mixtures of α-chymotrypsinogen and cytochrome c using a sulfated dextran displacer with a strong CEX resin. Martin et al. [26] investigated the competitive binding kinetics of lysozyme and cytochrome c using finite bath and shallow bed methods. The studies above demonstrated that the competitive binding of these relatively small, but also very different, proteins occurred over time scales that are shorter than those associated with intraparticle diffusion. Linden et al. [27] used confocal laser scanning microscopy (CLSM) to visualize the simultaneous adsorption of polyclonal IgG and BSA on individual CEX resin beads. As shown by these authors, relatively sharp fronts were established within the resin suggesting that competitive binding of these two species occurred on time scales shorter than intraparticle diffusion. More recently, Tao et al. [28–30] studied the competitive binding of a deamidated mAb mixture on both macroporous and polymer-grafted CEX resins in both batch and column experiments. Competitive binding of native and deamidated mAb species was shown to take place at a rate limited only by intraparticle diffusion for both resin types. However, while the rate was essentially independent of the direction of transport for the macroporous resin, the rates observed for the polymer-grafted resin were different dependent on whether native and deamidated isoforms were adsorbed simultaneously or in a consecutive manner, with the native species displacing the deamidated isoforms. Tao et al. [31], Perez et al. [32], and Zhu and Carta [33] also used CLSM to study the evolution of intraparticle concentration profiles during adsorption of protein mixtures in CEX and AEX resins for, respectively, deamidated mAb mixtures, multiple mAbs, and mixtures of native and PEGylated proteins. These authors considered both macroporous and polymer-grafted resins. For all three protein systems, simultaneous adsorption resulted in the formation of distinct bands within the resin bead in the case of the macroporous materials, consistent with rapid displacement, within the particle, of the weaker binding species by the stronger binding one. Smooth profiles and slow rates for consecutive binding were seen however for adsorption in the polymer-grafted materials indicating that transport and mechanisms are fundamentally different in these matrices. A detailed review addressing many of the underlying phenomena of protein adsorption and transport in polymer-grafted resins can be found in Lenhoff [34].

Several models have been developed to describe competitive protein binding equilibrium and kinetics for model systems in both macroporous materials and in polymer-grafted resins [26,30,34–36]. Descriptions of column performance based on the general rate model of chromatography have also been introduced to quantify the separation of mAb aggregates ([11,37]). Although useful as a design tool and to help define the critical parameter space, absent a more fundamental understanding of the underlying phenomena, such models have required parameterization though extensive experimental data. By probing and understanding the fundamentals of adsorption of monomers and their aggregates, it may be possible to reduce the development burden that these separations demand.

This work has three primary objectives. The first is to determine equilibrium and kinetics of adsorption on a macroporous CEX resin of a mAb monomer in comparison with those of its soluble dimer, which was obtained from an actual process stream, with the goal of understanding how the increased size and charge of the dimer affect interactions with the stationary phase. The second objective is to understand the competitive binding equilibrium of mAb monomer/dimer mixtures and the influence of salt concentration on selectivity. The final objective is to characterize the kinetics of simultaneous and sequential binding of mAb monomer and dimer using CLSM with the goal of comparing the kinetics of single component adsorption kinetics with those of the two-component system over a range of salt concentrations.

2. Experimental

2.1. Materials

The resin used in this work is Nuvia HR-S from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). The resin possesses a macroporous backbone based on acrylamido and vinyl copolymers functionalized with sulfonic acid groups. Its structure is similar to that of UNOsphere S and Rapid S, which have apparent pore sizes around 120 nm based on inverse size exclusion chromatography [38]. The particle size distribution of the Nuvia HR-S sample used in this work was obtained from micrographs and gave a volume-average particle diameter $d_p = 50.8\ \mu m$ with a range of $\pm 5.5\ \mu m$. Two samples of an IgG1 antibody were provided by MedImmune (Gaithersburg, MD, USA). The first was a purified sample containing minimal levels of higher molecular weight species (>97% monomer by SEC). The second sample was a fraction enriched in soluble aggregates assumed to be primarily dimers (68% monomer, 32% aggregated species by SEC) derived from a Protein A eluate following separation with a hydroxyapatite chromatography column. All buffering species and salts were purchased from Fisher Scientific (Waltham, MA, USA) and Sigma–Aldrich (St. Louis, MO, USA). Rhodamine Red™-X and Rhodamine Green™-X succinimidyl ester amine-reactive dyes were purchased from Life Technologies (Waltham, MA, USA). All experiments were performed at room temperature ($22 \pm 2\ ^\circ C$).

2.2. Methods

2.2.1. Analytical SEC and isolation of antibody dimer

Analytical high performance SEC was performed using a Waters e2695 Alliance HPLC unit (Milford, MA, USA) equipped with an in-line dynamic light scattering (DLS) module (DynaPro NanoStar and miniDAWN TREOS, Wyatt Technologies, Santa Barbara, CA, USA) using a TSKgel 3000SW$_{XL}$ column (Tosoh Bioscience LLC, King of Prussia, PA, USA) and a mobile phase of 100 mM Na_2HPO_4, 300 mM NaCl, pH 6.8 at 1 mL/min. The same mass-based extinction coefficient value of 1.33 mL/(mg cm), determined from a protein assay, was used for both monomer and dimer to convert the UV absorbance at 280 nm to mass concentrations in g/L. The resulting chromatograms were fitted using exponentially modified Gaussian functions [39] to determine the ratio of monomer to dimer. A cumulant fit of the autocorrelation function of the DLS signal assuming a single exponential decay [40,41] was used to determine the hydrodynamic radius of the eluted fractions.

Preparative size exclusion chromatography (SEC) was used to separate the antibody dimer from the aggregate-enriched feedstock with a Superdex 200 10/300 GL column from GE Healthcare