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Research Article

Protein A Membrane Shows an Advantage Over Protein A Resin in Purifying Antibodies/Fc-Fusions Forming Noncovalent Aggregates

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ABSTRACT

Certain mAbs, bispecific antibodies (bsAbs) and Fc-fusion proteins are prone to form aggregates in cell culture. We previously showed that in these cases large aggregates do not bind to Protein A resin as their sizes prevent them from entering the pores of resin beads, leading to reduced step yield. Nevertheless, such aggregates can bind to Protein A membrane, which has larger pore size. We also showed in a previous publication that noncovalent antibody aggregates can be converted into monomers upon acidic pH or salt treatment. In the current work, with an antibody case in which noncovalent aggregates were formed in cell culture, we showed that using Protein A membrane for capture allowed for not only aggregate binding but also aggregate conversion during and after low pH elution. Thus, replacing Protein A resin with Protein A membrane can improve step yield while maintaining good product quality. This finding suggests that Protein A membrane has an advantage over Protein A resin in purifying antibodies/Fc-fusions forming noncovalent aggregates.

ABBREVIATIONS

bsAb: Bispecific Antibody; SEC-HPLC: Size-Exclusion Chromatography-High Performance Liquid Chromatography; CV: Column Volume; MV: Matrix Volume

INTRODUCTION

Certain mAbs, bispecific antibodies (bsAbs) and Fc-fusion proteins are prone to form aggregates in cell culture. In many of these cases, the aggregates do not bind to Protein A resin, leading to low step yield [1]. We previously figured that non-binding is mainly due to the large sizes of the aggregates, which prevent the aggregates from entering the resin beads to bind the Protein A ligands inside [2]. Evidence supporting this conclusion is that the aggregates, while do not bind to the Protein A resin, bind well to the Protein A membrane, which is known to have larger pore size [2,3]. In addition, we previously showed that aggregates formed through noncovalent interactions can be converted into monomers by acidic pH or salt treatment [4].

While Protein A feed treatment can convert noncovalent aggregates into monomers and improve step yield, it requires extra labor, time and cost, which will be especially evident at scale. Recently, while purifying a mAb which formed noncovalent aggregates by Protein A membrane instead of Protein A resin, we noticed that while the step yield was increased, the product quality was not compromised despite of the

binding of aggregates. We speculated that the bound aggregates were converted into monomers during low pH elution as it is known that noncovalent aggregates can disassociate when exposed to acidic pH. Thus, replacing Protein A resin with Protein A membrane can improve step yield while maintaining good product quality without the need for special treatment of the feed. This finding suggests that Protein A membrane has an advantage over Protein A resin in purifying antibodies/Fc-fusions forming noncovalent aggregates.

MATERIALS AND METHODS

MATERIALS

Ethanol, sodium acetate trihydrate, sodium chloride, sodium hydroxide and tris(hydroxymethyl)aminomethane were purchased from Merck (Darmstadt, Germany). Acetic acid was purchased from J.T. Baker (Phillipsburg, NJ, USA). Sodium phosphate monobasic was purchased from Sigma-Aldrich (Darmstadt, Germany). Sodium phosphate dibasic was purchased from Vetec (Beijing, China). MabSelect SuRe LX and HiTrap Fibro PrismA (0.4 mL) were purchased from Cytiva (Uppsala, Sweden). BioCore SEC-300 stainless steel column (5 µm, 7.8 × 300 mm) was purchased from NanoChrom (Suzhou, China). The target mAb used in this study was expressed in stably transfected CHO-K1 cells grown in HyClone ActiPro culture medium supplemented with Cell Boost 7a and 7b (the feeding medium supplements are from HyClone). The cell culture was allowed to grow for 14 days before harvest.

EQUIPMENT

An AKTA pure 150 system installed with Unicorn software version 7.3 (Cytiva, Uppsala, Sweden) was used for Protein A column and membrane chromatography. pH and conductivity were measured using SevenExcelence S470 pH/Conductivity meter (Mettler-Toledo, Columbus, OH, USA). Protein concentration was measured using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). An Agilent 1260 liquid chromatography instrument (Agilent Technologies, Santa Clara, CA, USA) was used for size-exclusion chromatography-high performance liquid chromatography (SEC-HPLC) analysis. The bioreactor system from Applikon Biotechnology (Delft, Netherlands) was used for cell cultivation.

METHODS

Protein A (resin) chromatography

MabSelect SuRe LX Protein A resin was packed in a 0.66 cm diameter column with 15.3 cm bed height. The column volume (CV) was approximately 5.2 mL. The column was loaded at 30 mg of protein per mL of resin and run at a flow rate to maintain 5 min residence time. After loading, the column was washed consecutively with 50 mM Tris-HAc, 150 mM NaCl, pH 7.4, 50 mM NaAc-HAc, 1 M NaCl, pH 5.5 and 30 mM NaAc-HAc, pH 5.5, each for 5 CV. The column was eluted with 30 mM NaAc-HAc, pH 3.5 for 4 CV.

Protein A membrane chromatography

The Protein A membrane used, HiTrap Fibro PrismA, has a matrix volume (MV) of approximately 0.4 mL. For all runs, the unit was loaded at 30 mg of protein per mL of matrix, which is close to the unit's dynamic binding capacity under the selected condition. For elution step the system was run at a flow rate of 40 MV/min or 0.2 MV/min (residence time: 1.5 sec and 5 min, respectively), and for all other steps the system was run at 40 MV/min. After loading, the membrane was washed consecutively with 50 mM Tris-HAc, 150 mM NaCl, pH 7.4 (for 30 MV), 50 mM NaAc-HAc, 1 M NaCl, pH 5.5 and 30 mM NaAc-HAc, pH 5.5 (each for 20 MV). The membrane was eluted with 30 mM NaAc-HAc, pH 3.5 for 30 MV.

SEC-HPLC

SEC-HPLC analysis was performed on an Agilent 1260 liquid chromatography instrument using a BioCore SEC-300 stainless steel column (7.8 × 300 mm). 100 µg of sample was injected per run. The mobile phase consisted of 50 mM sodium phosphate, 300 mM sodium chloride at pH 6.8. Each sample was eluted isocratically for 20 min at a flow rate of 1.0 mL/min. Protein elution was monitored by UV absorbance at 280 nm.

RESULTS AND DISCUSSION

Protein A (resin) chromatography

For the case under study, SEC-HPLC analysis of culture harvest (Protein A feed) suggested that the sample contained approximately 16% of aggregates (Figure 1). When the culture harvest was processed by Protein A (resin) chromatography, the step yield was 83.6% (the chromatogram is shown in Figure 2A). As suggested by SEC-HPLC analysis of column flow-through, low Protein A yield was due to failed

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binding of large aggregates (Figure 2B, orange line). We previously demonstrated that the reason for non-binding is that the aggregates cannot enter the pores of resin beads to bind the ligands inside due to their large sizes [2]. As the major portion of aggregates did not bind, SEC purity of the Protein A eluate reached 98.2% (Figure 2B, blue line). We previously showed that this low yield issue can be resolved by treating the Protein A feed with acid or salt, which converted noncovalent aggregates into monomers [4]. However, this extra step (Protein A feed treatment) significantly increases labor, time and cost at scale.

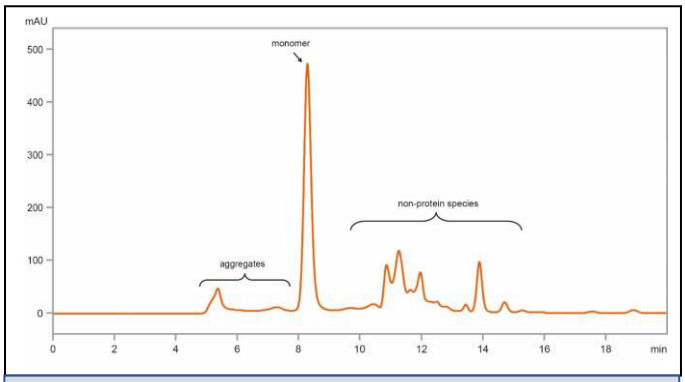

Figure 1: SEC-HPLC profile of clarified culture harvest (Protein A feed). Peaks that are due to culture media components are labelled as non-protein species.

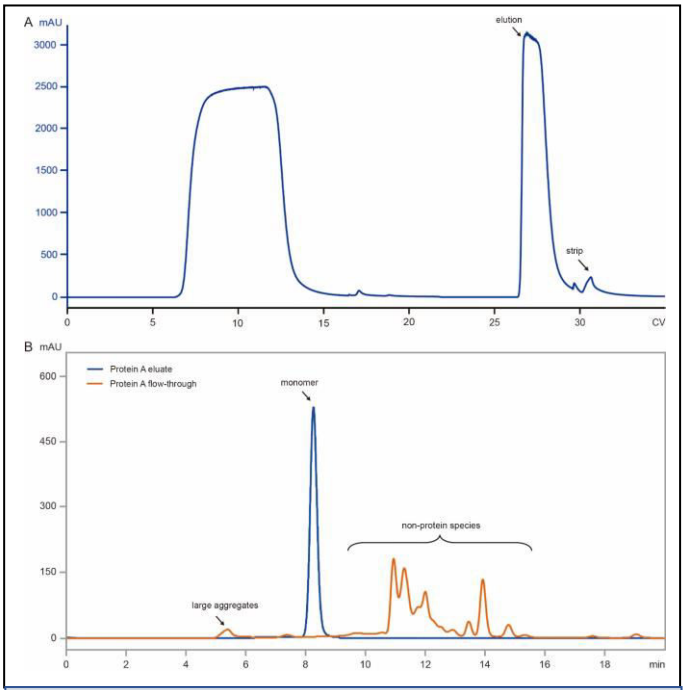

Figure 2: (A) Protein A (resin) chromatogram and (B) overlay of SEC-HPLC profiles of Protein A (resin) flow-through and eluate. In SEC profile of Protein A flow-through, peaks that are due to culture media components are labelled as non-protein species.

Protein A (membrane) chromatography

Recently, several vendors launched Protein A membrane [3]. A major difference between Protein A membrane and Protein A resin is that the former has larger pore size. Consistently, we previously found that aggregates which failed to bind to Protein A resin bound to Protein A membrane. In the current case, when Protein A membrane was used to replace Protein A resin for product capture, the step yield reached ~100%, which confirmed the previous observation. The corresponding chromatogram for Protein A membrane chromatography is shown in Figure 3A. Binding of aggregates was also indicated by SEC-HPLC analysis of Protein A membrane flow-through, which showed that the aggregates presented in column flow-through was largely absent (Figure 3B, orange line). SEC-HPLC analysis of membrane eluate suggested that the sample's SEC purity was 92.6% when the eluate was immediately neutralized after collection (Figure 3B, blue line). This number is higher than that of the load (i.e., 84.0%) despite of the binding of most aggregates.

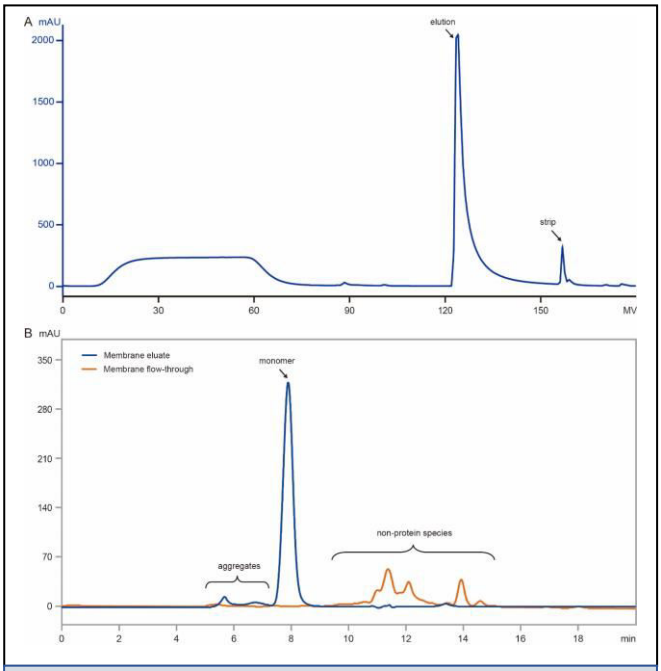

Figure 3. (A) Protein A membrane chromatogram and (B) overlay of SEC-HPLC profiles of Protein A membrane flow-through and eluate. In SEC profile of Protein A flow-through, peaks that are due to culture media components are labelled as non-protein species.

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As we know that noncovalent aggregates can be converted into monomers under acidic pH or salt treatment [4], the bound aggregates may partially disassociate during the low pH elution. However, the conversion from aggregates to monomers is likely far from complete in this case as the duration of elution is relatively short (the residence time for Protein A membrane is only 1.5 sec). Thus, we also studied the impact of membrane eluate hold time (under low pH condition) on aggregate conversion. Individual aliquots of Protein A membrane eluate were neutralized when the intended hold time (i.e., 0, 0.5, 1.0 or 2.0 h) was reached and the neutralized samples were analyzed by SEC-HPLC.

Table 1: Monomer content in Protein A membrane eluate as a function of hold time under acidic condition.

Hold time (h)	0	0.5	1	2
Monomer (%)*	92.6	96.7	96.9	97.7

*Measured by SEC-HPLC.

The data, which are summarized in Table 1, suggest that extending the hold time increased monomer content, confirming that aggregates can be converted into monomers under low pH. We also tested the impact of longer residence time on aggregate conversion. When the elution residence time was increased from 1.5 sec to 5 min, SEC purity of the eluate increased to 96.2% despite of immediate neutralization. This further confirms that aggregates can be converted into monomers during low pH elution. Increasing the residence time is not absolutely necessary. As holding the membrane eluate under acidic condition for half an hour can achieve an SEC purity comparable to that of column eluate (96.7% vs. 98.2%, respectively), this should be an effective and convenient approach at scale.

CONCLUSION

For certain mAbs, bsAbs and Fc-fusion proteins, they form relatively high percentage of aggregates in cell culture. Most of these aggregates are formed through noncovalent interactions. In a series of papers published recently [1-2,4], we demonstrated the following: (1) large-size aggregates do not bind to Protein A resin as their sizes prevent them from entering the pores of resin beads, which leads to low step yield; (2) aggregates which do not bind to Protein A resin can bind to Protein A membrane, which has larger pore size; (3) acidic pH or salt treatment can convert noncovalent aggregates into monomers, which regain Protein A binding capability.

The last finding suggests that for antibodies/Fc-fusions forming noncovalent aggregates, Protein A step yield can be improved by treating the column feed with acid or salt prior to loading. In the current work, we demonstrated that, when Protein A membrane instead of Protein A resin was used for product capture, aggregates not only bound but also were converted into monomers during and after low pH elution. Thus, using Protein A membrane in this case improved step yield while maintaining good product quality without the need for special treatment of the feed. This finding suggests that Protein A membrane has an advantage over Protein A resin in purifying antibodies/Fc-fusions forming noncovalent aggregates.

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