Development of a single-step, protein A chromatography process for bispecific antibodies in early screening

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Abstract

A single-step downstream process using protein A chromatography to purify a bispecific antibody (BsAb) in early screening has been successfully developed to replace an existing-three step process. The goal was to separate BsAb monomer from fragments such as the individual heavy and light chain pairs while achieving high purity and yield of BsAb. MabSelect™ PrismA protein A resin was selected for the capture step. Most fragments could be removed as early as the capture step using gradient elution from pH 5 to 3.5, without the need for additional purification steps. BsAb constructs were efficiently purified to give more than 90% purity and 85% yield in the early screening downstream process.

Introduction

BsAb have gained increasing interest over recent years with their wide range of applications such as potential candidates for cancer immunotherapy or as drug delivery vectors (1, 2). All BsAbs share two main characteristics: (1). BsAbs are derived from monoclonal antibodies (mAbs); (2) BsAbs have at least two different sites that specifically target different antigens.

The dual specificity means that BsAbs can, for example, bind to target cells using one antigen-binding site and recruit other cells or molecules with the second antigen-binding site (Fig 1). Numerous BsAb constructs are shown in Figure 2.

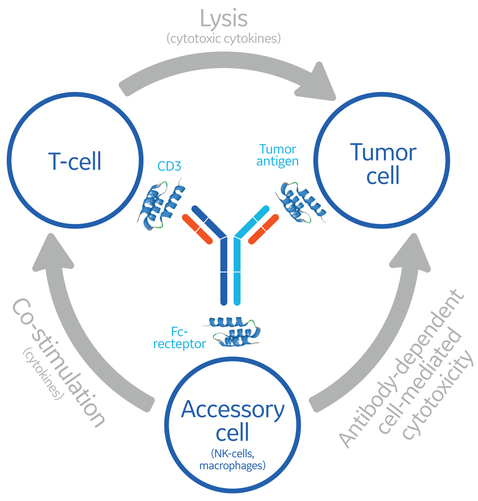

Fig 1. Typical mode of action of a bispecific monoclonal antibody.

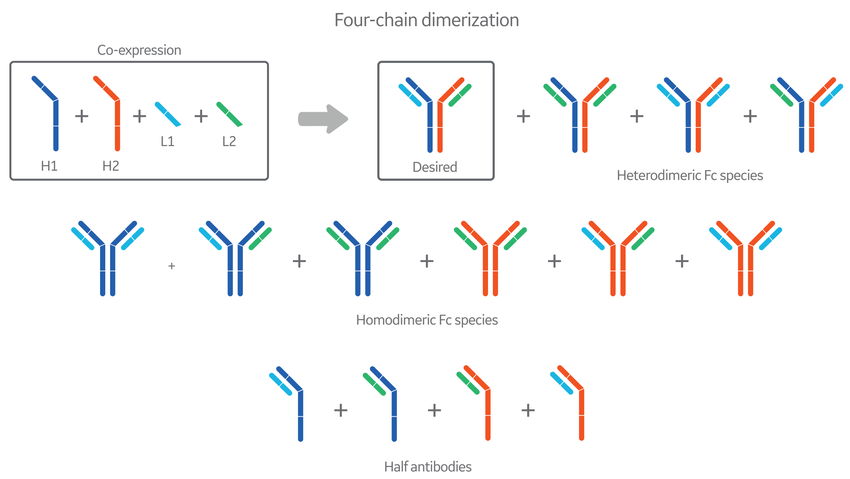

Fig 2. Different genetically engineered variants of bispecific antibodies investigated in this study. Various combinations of the heavy- and light-chain regions as well as half BsAb are shown.

Materials and methods

Resin screening

Three resins from GE were screened for the capture step—MabSelect SuRe™ LX, MabSelect SuRe pcc, and MabSelect PrismA. Table 1 lists the products and their properties in terms of relative dynamic binding capacity at specified residence times. Conditions for the screening study are listed in Table 2.

This protocol was optimized and verified using a different BsAb. The screening was performed using ÄKTA™ avant 25.

Table 1. Resins included for the protein A capture step

Resin	Properties
MabSelect SuRe LX	Protein A resin providing high DBC at short RT: ~ 60 g IgG/L resin at 6 min RT
MabSelect SuRe pcc	Protein A resin providing high DBC at short RT: ~ 60 g IgG/L resin at 2.4 min RT
MabSelect PrismA	Protein A resin providing high dynamic binding capacity (DBC) at short residence times (RT) and high alkaline stability ~ 65 g IgG/L resin at 4 min RT, or ~ 80 mg IgG/mL resin at 6 min RT

Table 2. Running conditions

Column	Tricorn™ 5/5, Bed height = 5 cm, CV = 1 mL
Equilibration	5 CV of phosphate buffered saline (PBS)
Sample application	3 mL (0.35 mg/mL) BsAb-containing cell culture feed
Wash	10 CV of 25 mM sodium citrate, pH 6
Elution	0% to 100% of 25 mM sodium citrate, pH 3, in 20 CV
Flow rate	0.25 mL/min

Analysis

BsAb purity was determined by size exclusion chromatography (SEC) on a Superdex™ 200 Increase 10/300 GL column. Automation of the SEC was achieved using an ÄKTA chromatography system. Peaks were integrated and percentage of whole BsAb, half-antibody fragments, and BsAb aggregates was determined and plotted.

Results

Of the screened protein A resins, MabSelect SuRe pcc has the smallest particle (bead) size (~ 50 µm) followed by MabSelect PrismA (~ 60 µm), and MabSelect SuRe LX with the largest particle size (~ 85 µm). As expected, MabSelect SuRe pcc and MabSelect PrismA offered improved resolution over MabSelect SuRe LX. However, MabSelect PrismA was selected for the capture step due to its higher capacity and alkaline stability compared with MabSelect SuRe pcc. Figure 3 shows chromatograms from the separations using these resins.

The method was optimized with respect to gradient length (more shallow) and a change in buffer salt (from citrate to acetate) and verified using two different BsAb. The result from one of these constructs at two different loads is shown in Figure 4.

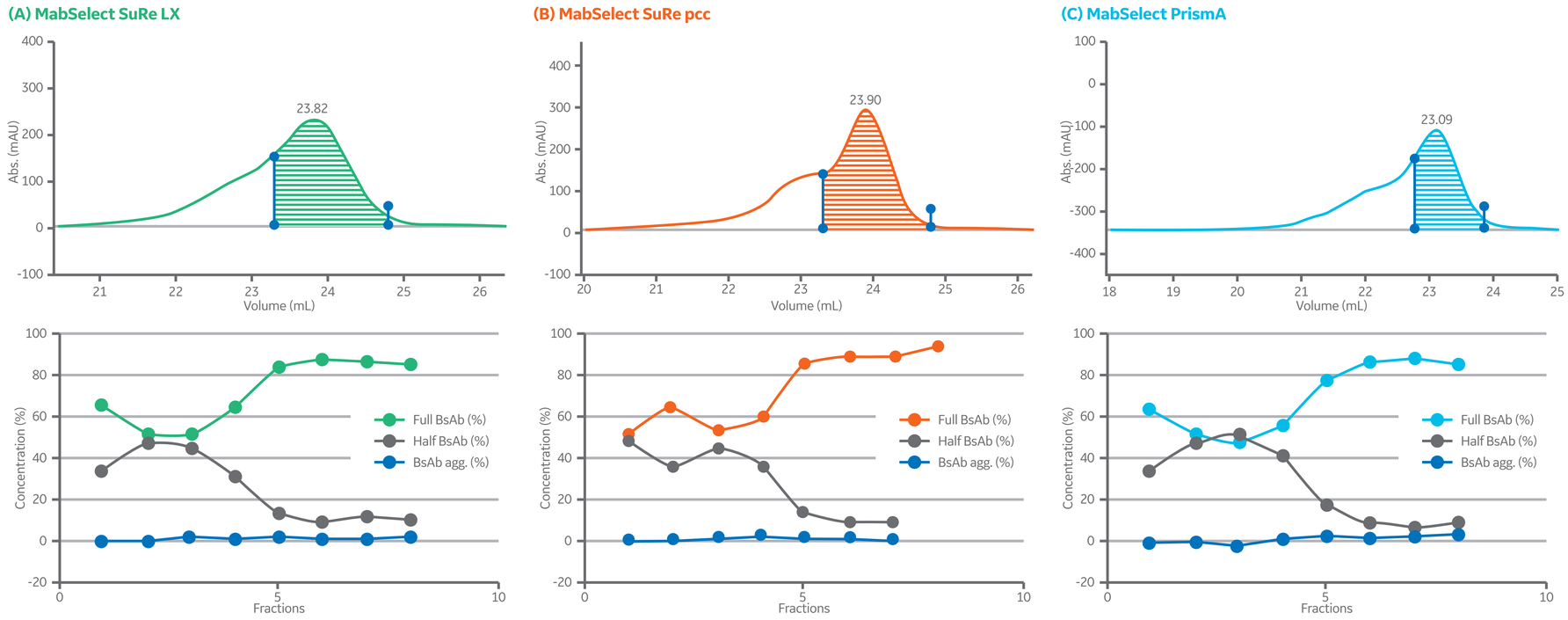

Fig 3. Upper images show chromatograms from separation of whole BsAb , half BsAb, and BsAb aggregates on (A) MabSelect SuRe LX, (B) MabSelect SuRe pcc, and (C) MabSelect PrismA. Lower images show results from analysis by size exclusion chromatography.

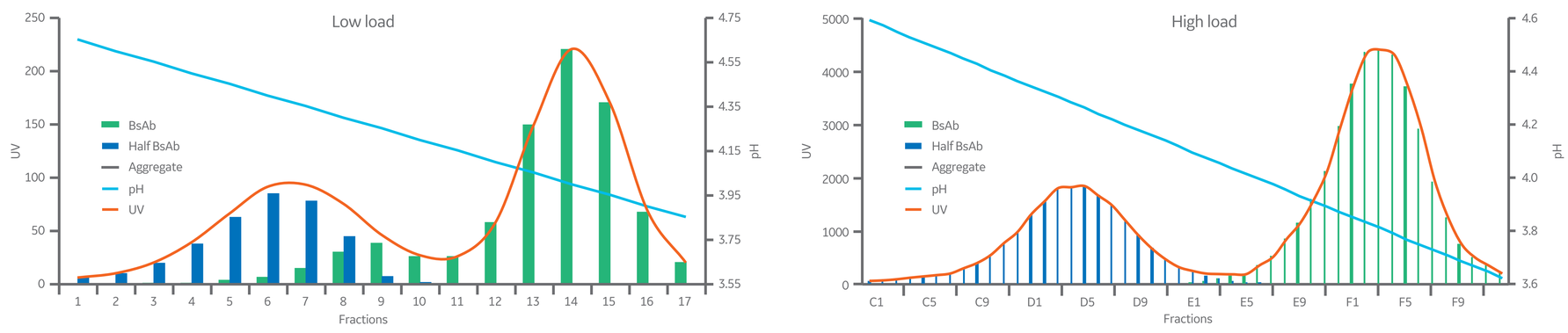

Fig 4. Separation of a 3-chain BsAb heterodimer in a 25 mM acetate gradient shows good separation of half- and whole BsAb.

Conclusions

- MabSelect PrismA provided good separation of BsAb from unassembled fragments in the initial capture step.
- Rapid screening of different protein A resins and elution conditions allowed a one-step purification protocol, which resulted in a time-reduction of 4 to 6 wk for each candidate compared to the original three-step process.
- The suitability of the process for early candidate screening was verified with two different constructs.
- Both purity and yield fulfilled the set requirements of > 90% purity and > 85% yield.

References

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2. Muller, D. and Kontermann, R. E. Bispecific antibodies for cancer immunotherapy: Current perspectives. *BioDrugs* **24(2)**, 89–98 (2010). https://www.ncbi.nlm.nih.gov/pubmed/20199124?dopt=Abstract

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