Platform for purification of V_HH-type antibody fragments

Tomas Björkman, Mats Ander, Eva Heldin, Jelena Vasic, Jamil Shanagar, Gustav Rodrigo, Joakim Galli, and Ulrika Knutsson

Cytiva, Uppsala, Sweden

Abstract

Protein A affinity chromatography resins have for a long time been used to purify monoclonal antibodies (mAbs) by the bioprocess industry at all scales. The binding of protein A mainly takes place between CH2 and CH3 in the Fc region of the mAb (constant domains 2 and 3 of the Ab heavy chains). However, it is known that protein A also can bind to V_H3 sequences located on the variable heavy chain of the Fab region. Here we will show examples of purification of mAb derived fragments, including bispecific antibodies (BsAb) and V_HH, with a focus on binding characteristics, e.g., binding capacities, elution conditions, and purification performance. It will be demonstrated that a good yield of V_HH can be purified directly from a high load of an *E. coli* harvest.

Introduction

Traditionally protein A chromatography has been used for purifying mAb using the interaction with the Fc region of IgG. Today, we see a greater diversity of mAb derived molecules (Fig 1), include some that lack the Fc region which require resins with ligands binding to other parts of the molecule (Fig 2).

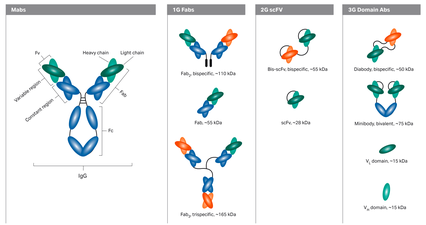

Fig 1. Example of the diversity of mAb derived molecules.

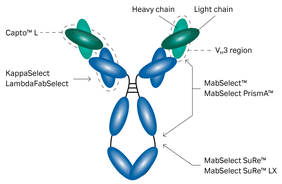

Fig 2. Different interaction points on mAb for alternate purification possibilities.

One possibility is to use affinity resins for polishing product specific impurities, e.g., bispecific antibodies with a κ chain on one arm and λ chain on the other arm, a resin with binding to either κ (Capto™ L) or λ (LambdaFabSelect) chains could be utilized. Another interaction that is possible to use for polishing on product related impurities, as well as for capture, is the V_H3 interaction displayed by protein A resins. However, protein A is not as base stable as more modern resins, MabSelect SuRe™ and MabSelect PrismA™ in particular, but the modern resins have not been fully evaluated for the V_H3 interaction. Table 1 shows the V_H3-determining amino acids and their positions (Kabat nomenclature).

Table 1. V_H3-determining amino acids.

VH	H15	H17	H19	H57	H59	H64	H65	H66	H68	H70	H81	H82a	H82b
VH3	G	S	R, K	K, I, T	Y	K	G	R	T	S	Q	N, G	S
Kabat	HFR1			CDR-H2				HFR3					

Results

In Figure 3, three different columns, MabSelect™, MabSelect SuRe™, and MabSelect PrismA™, were loaded with purified V_HH fragments and eluted with acidic pH. MabSelect™ and MabSelect PrismA™ show the same chromatographic behavior while MabSelect SuRe™ displays a weaker interaction, if any, with the fragment.

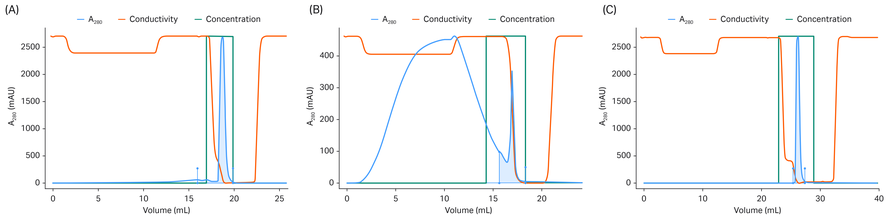

Fig 3. Binding of a V_HH fragment to (A) MabSelect™, (B) MabSelect SuRe™, and (C) MabSelect PrismA™.

V_HH sequence	MabSelect™	MabSelect SuRe™	MabSelect PrismA™
T57	Yes	Yes	Yes
T57K	Yes	No	Yes
T57I	No	No	No
T57E	No	No	No
N82aT, S82bD	No	No	No
T57P	Yes	No	Yes
T57S	Yes	No	Yes
T57R	Yes	No	Yes
K64R	Yes	Yes	Yes
K64T	Yes	Yes	Yes
K64E	Yes	N/A	Yes
G65D	No	No	No
S70T	Yes	No	Yes
S82bD	No	No	No
S82bG	Yes	Yes	Yes

Table 2. Interaction of MabSelect™, MabSelect SuRe™, and MabSelect PrismA™ to V_HH fragments, i.e., mimic of the V_H3 region of IgG, mutated at different positions. The data in the table demonstrates that MabSelect SuRe™ can interact with the V_H3 region in some cases, but MabSelect™ and MabSelect PrismA™ are less sensitive to sequence variation.

Purification of a V_HH fragment on MabSelect PrismA™

The binding capacity for V_HH and Fab was determined by frontal analysis and elution pH using a pH gradient from pH 6 to 3 in a Tricorn™ 5/50 column packed with 1 mL MabSelect PrismA™, shown in Table 3. 150 mL *E.coli* harvest containing 0.2 g/L V_HH (30 g V_HH/L resin) was loaded at 6 minutes residence time to a MabSelect PrismA™ column (CV 1 mL) as displayed in Figure 4. The fragment was eluted in 50 mM Na-acetate pH 3.5.

In Figure 4 the MabSelect PrismA™ capture of the V_HH from an *E. coli* harvest is shown.

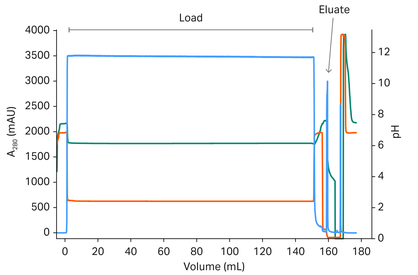

Fig 4. Purification of V_HH using MabSelect PrismA™.

Table 3. MabSelect PrismA™ capacity and the V_HH elution pH

Entity	Q_{b10} (g/L)	Q_{b10} (mM)	Elution pH
V_HH	42	2.7	3.8

Table 4. Analytical data from the purification in Figure 4.

Sample	Pool volume (CV)	eHCP (ppm*)	Leached ligand (ppm**)
Load	NA	4252500	NA
Elution pool	1	200	10

*ng eHCP/mg V_HH. **ng leached ligand/mg V_HH

Materials and methods

The V_HH's were fermented in *E. coli* in shake flasks or in 5 L fermentors. In Table 2, 1 mL HiTrap™ packed with the different resins. In Figure 4, 150 mL harvest of the T57 V_HH material from a 5 L fermentor was loaded to a Tricorn™ 5/50 column packed with 1 mL MabSelect PrismA™ at 6 minutes residence time. In Table 3 and 4 the same column was used to determine the breakthrough capacity and elution pH.

Conclusion

The modern base stabilized protein A resin MabSelect PrismA™ can be used for purification of molecules lacking the Fc region by utilizing the V_H3 interaction, resulting in:

- High capacities
- High purities are achieved for *E. coli* harvest containing V_HH (4.3 logs reduction of HCP)

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