Preparation and high resolution analysis of a Fab

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Preparation and high resolution analysis of a Fab

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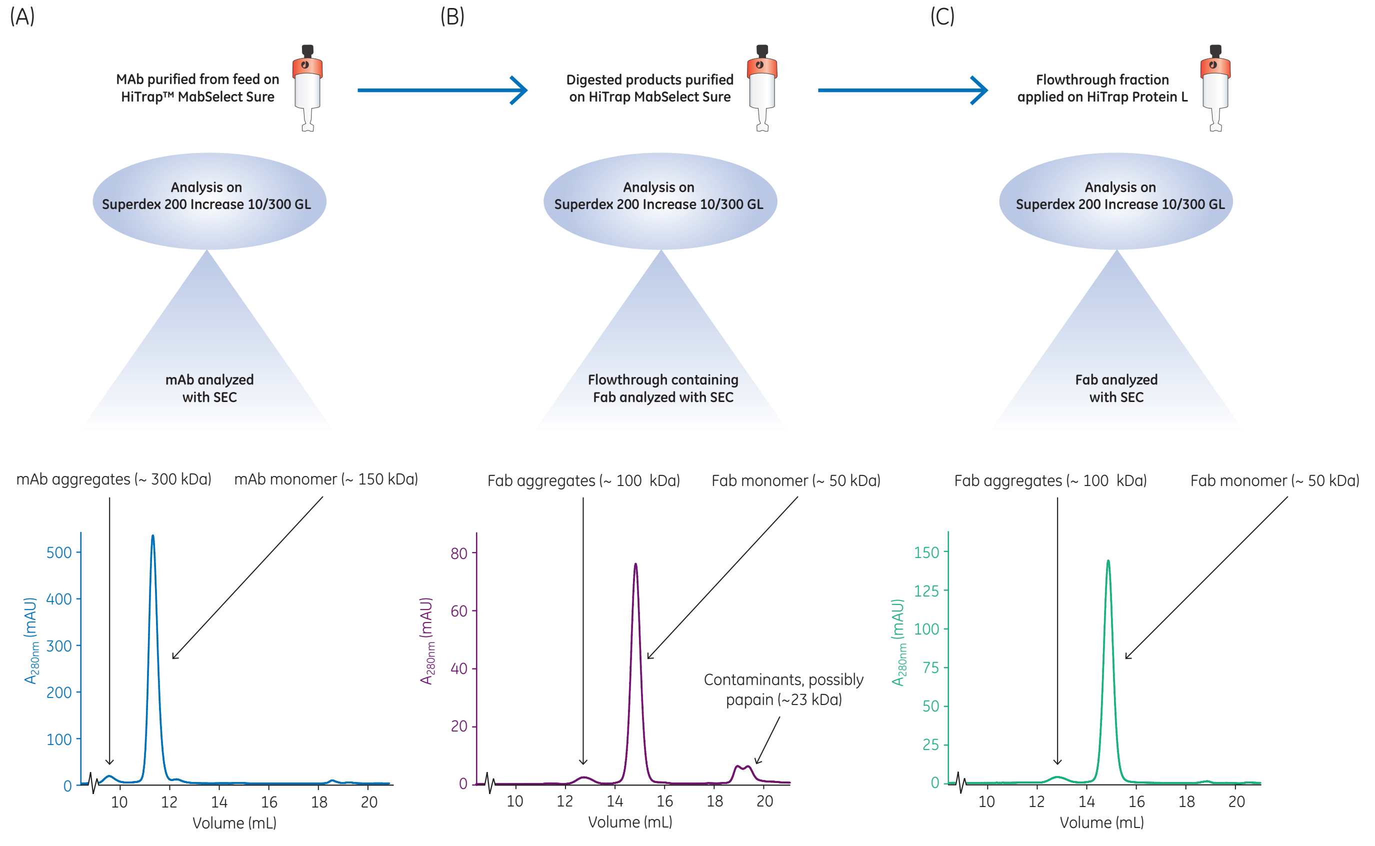
Introduction

Antibody fragments are getting increased attention in research use due to their small size and lower immunogenicity (relative to intact antibodies).

In this work Fab was produced by papain cleavage of a purified monoclonal antibody (mAb) followed by purification on MabSelect Sure™ and Capto™ L resins (Protein A and Protein L immobilized to high flow agarose, respectively). Fractions from the different steps were analyzed with a new generation size exclusion chromatography (SEC) resin, Superdex™ 200 Increase.



(A)

MAb purified from feed on HiTrap™ MabSelect Sure

Analysis on Superdex 200 Increase 10/300 GL

mAb analyzed with SEC

(B)

Digested products purified on HiTrap MabSelect Sure

Analysis on Superdex 200 Increase 10/300 GL

Flowthrough containing Fab analyzed with SEC

(C)

Flowthrough fraction applied on HiTrap Protein L

Analysis on Superdex 200 Increase 10/300 GL

Fab analyzed with SEC

Fig 1. SEC analyses of (A) Purified mAb (B) Flowthrough containing Fab, and (C) Purified Fab. Affinity chromatography run on HiTrap 5 mL MabSelect Sure and Protein L. Buffers and flow rates according to resins instructions. All SEC analyses were run on Superdex 200 Increase 10/300 GL with PBS, 0.5 mL/min and 50 µL sample on ÄKTA™ pure chromatography systems.

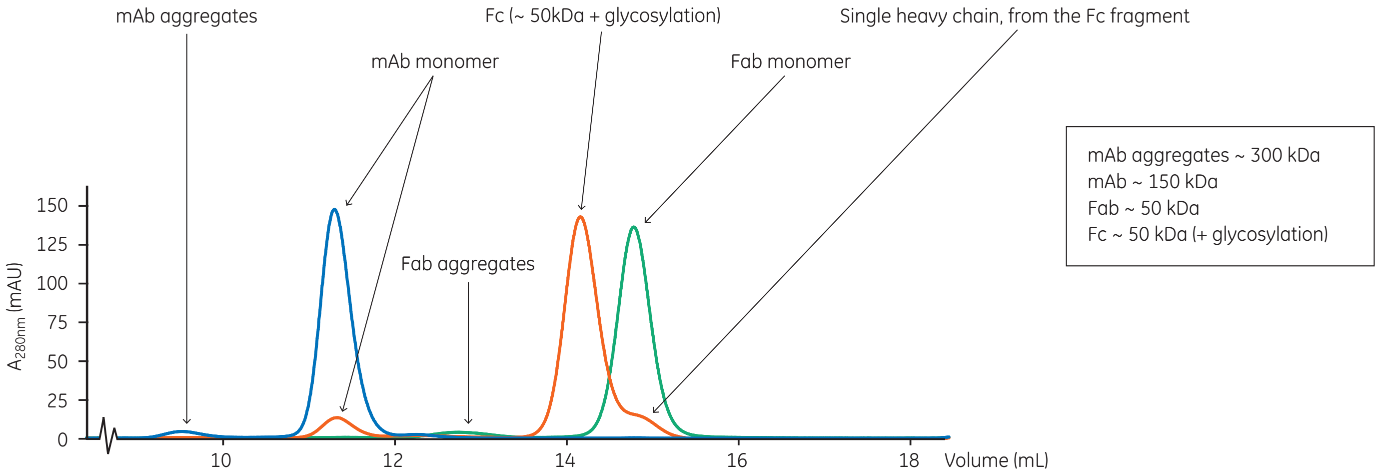


Fig 2. Overlay of individual SEC analyses of mAb, Fab, and Fc fragments. For comparative purposes, the curves have been normalized against the peak height of each monomer species.

Conclusions

- HiTrap MabSelect Sure and HiTrap Protein L enable a simplified workflow to produce Fab by papain digestion of mAb
- High resolution power of Superdex 200 Increase allows baseline separation between monomer and aggregates from both mAb and Fab
- Broad separation range (M_r 10 000 to M_r 600 000) with optimized resolution for antibodies (M_r 100 000 to M_r 300 000) allows separation of a large number of different biomolecules

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