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Purification of a Murine IgG_1 Antibody Expressed in the Leaves of *Arabidopsis Thaliana*

Annika Bergenstråhle[1], Maria Storgärds[1], Anna Heijbel[1], Chris De Wilde[2], and Ann Depicker[2]

[1] GE Healthcare, SE-751 84 Uppsala, Sweden

[2] Dept. of Genetics, University of Gent, VIB, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium

Introduction

Monoclonal antibodies are mostly produced in animal cell cultures. Transgenic plants are an interesting alternative for antibody production because of the reduced risk of mammalian viral contamination and the ability to produce proteins on a large scale at a low cost. Here we present a purification procedure for the murine MAK 33 antibody expressed in the leaves of *Arabidopsis thaliana* (1). This IgG_1 antibody is targeted to the endoplasmic reticulum and secreted into the intercellular spaces and xylem vessels.

Conclusions

- Affinity chromatography using rProtein A Sepharose™ FF is suitable for purification of recombinant antibodies expressed in plants.
- Minor amounts of the plant pigments adsorb to the rProtein A Sepharose medium and elute with the IgG. The pigments can be removed by a gel filtration step.

HiTrap

Sample preparation

The plants were grown in a greenhouse for five weeks, and the leaves were frozen directly in liquid nitrogen at harvest and stored at -80°C. The protein extraction was performed by mixing the frozen leaves with 50 mM Tris-HCl, pH 8.5, 100 mM KCl, 2 mM EDTA, 0.5 mM PMSF (1 g leaf material to 2 ml buffer) using an ice-chilled food blender. The extract was filtered through Miracloth™, followed by centrifugation twice at 15 000 x g at 4°C for 10 minutes. The expression level of IgG was about 1% of the total soluble protein in the supernatant (protein extract) as detected by Western blotting.

Before application to a HiTrap™ rProtein A FF column, NaCl was added to the protein extract to a concentration of 2.9 M, and the sample was then filtered through a 0.45-µm filter.

Purification and analysis

Efficient adsorption of murine IgG_1 to rProtein A media demands high concentrations of salt, and the sample contained 2.9 M NaCl and 0.1 M KCl. Elution was performed by lowering the pH to 3.0 and excluding the NaCl in the buffer. A chromatogram from the purification of 50 ml of the extract on a 1-ml HiTrap rProtein A FF column is shown in Figure 1.

The fractions were analyzed by SDS-PAGE and Western blotting (Figs 2a and 2b). The primary antibody was rabbit anti-mouse IgG, 1:4000, and the secondary antibody was anti-rabbit-HRP, 1:4000. ECL Plus™ was used for detection. Only one protein band, the IgG, could be detected after silver staining, but minor amounts of the plant pigments were also found in the eluate. The eluate from the HiTrap rProtein A FF column was further purified on Superdex™ 200, 10 × 300 mm (Fig 3), where the IgG was separated from the pigments. The purified antibody was also analyzed using MALDI-ToF MS (Figs 4a and 4b).

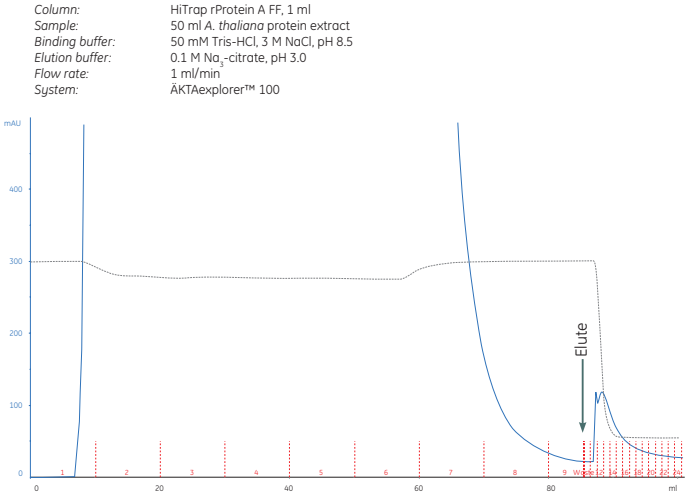


Fig 1. Purification of IgG from *A. thaliana* protein extract on HiTrap rProtein A FF, 1 ml.

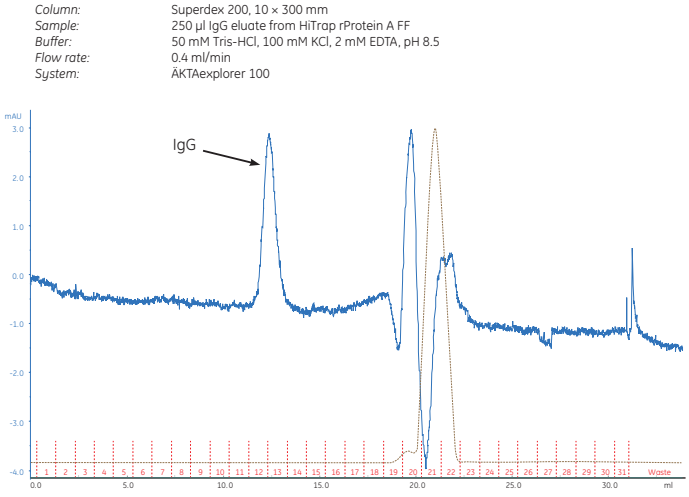
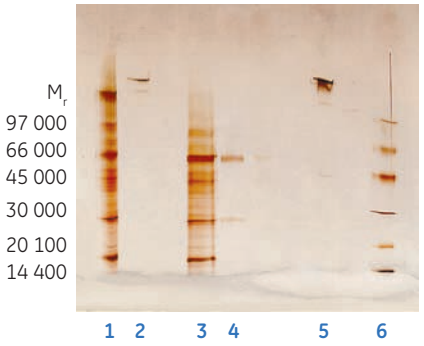
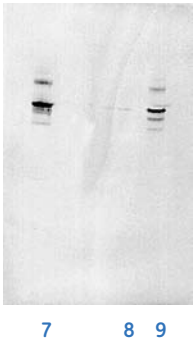


Fig 3. Further purification of IgG from *A. thaliana* on Superdex 200.



Lane 1: *A. thaliana* protein extract
Lane 2: Eluate from HiTrap rProtein A FF
Lane 3: *A. thaliana* protein extract, reduced with 2.5% β-mercaptoethanol
Lane 4: Eluate from HiTrap rProtein A FF, reduced with 2.5% β-mercaptoethanol
Lane 5: MAK 33 antibody standard
Lane 6: Low molecular weight standard (LMW)

Fig 2a. SDS-PAGE analysis of MAK 33 after purification on HiTrap rProtein A FF using ExcelGel™ SDS Gradient 8–18 and silver staining.



Lane 7: MAK 33 antibody standard
Lane 8: *A. thaliana* protein extract
Lane 9: Eluate from HiTrap rProtein A FF

Fig 2b. Western blot analysis of MAK 33 after purification on HiTrap rProtein A FF.

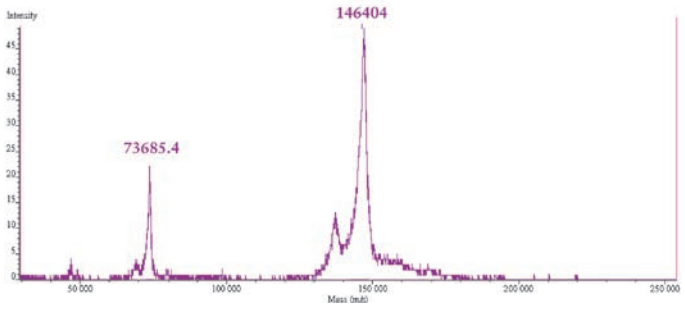


Fig 4a. Full-length analysis of MAK 33 using MALDI-ToF MS.

Figure 4a shows the MALDI-ToF spectrum of the purified antibody MAK 33. The spectrum was obtained with Ettan™ MALDI-ToF Mass Spectrometer operated in positive linear mode at 20 kV accelerating voltage. The purified antibody was desalted on a NAP™ 10 column, using 20 mM ammonium bicarbonate as elution buffer. The antibody (2 pmol/µl) was mixed in a 1:2 ratio with a matrix solution (sinapinic acid, saturated solution: 50% acetonitrile and 0.9% trifluoroacetic acid), and 0.5 µl was applied on the target. Size determination using an external standard resulted in a M_r of 146 400, which is in accordance with the M_r calculated from the known DNA sequence.

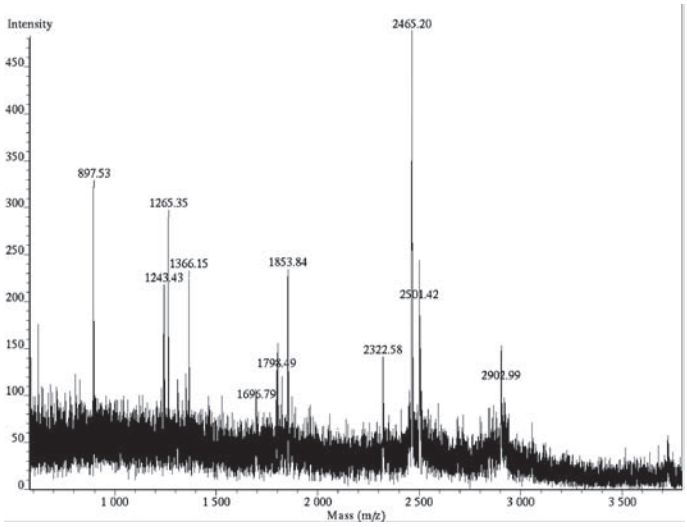


Fig 4b. Peptide map of MAK 33 using MALDI-ToF MS.

Figure 4b shows the MALDI-ToF spectrum of the purified and trypsin digested MAK 33 antibody. No identification was achieved since the sequence information on this antibody could not be found in the public databases. However, the result could be used as a unique peptide map for this antibody.

The sample was reduced and denatured in 8 M urea, 50 mM Tris-HCl, pH 8.0, and 50 mM DTT for 30 minutes, followed by alkylation using 150 mM iodoacetamide for 1 hour in the same buffer as above. The sample was desalted on a NAP-10 column, using 20 mM ammonium bicarbonate as elution buffer. Trypsin digestion was then done overnight at 30°C with 1 µg of trypsin per 100 µg of sample. The digestion reaction was stopped by adding TFA (triflouroacetic acid) to a final concentration of 1%.

The spectrum was obtained with Ettan MALDI-ToF Mass Spectrometer operated in reflectron mode at 20 kV accelerating voltage. The matrix was α-cyano-4-hydroxy-cinnamic acid (saturated solution in 50% acetonitrile and 0.9% triflouroacetic acid). Angiotensin III (M_r 897,52) and ACTH18-39 (M_r 2465,19) were used as internal calibrants for peak size determination. The digested antibody was mixed in 1:1 ratio with the matrix solution, and 0.5 µl was applied on the target.

References

1. De Neve, M. *et al. Transg. Res.* **2**: 227–37 (1993).

www.gehealthcare.com/hitrap

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

Asia Pacific Tel +85 65 62751830 Fax +85 65 62751829 • Australasia Tel +61 2 8820 8299 Fax +61 2 8820 8200 • Austria Tel 01 /57606 1613 Fax 01 /57606 1614 • Belgium Tel 0800 73 890 Fax 02 416 8206 • Canada Tel 1 800 463 5800 Fax 1 800 567 1008 • Central & East Europe Tel +43 1 972 720 Fax +43 1 972 722 750 • Denmark Tel +45 70 25 24 50 Fax +45 45 16 2424 • Eire Tel 1 800 709992 Fax +44 1494 542010 • Finland & Baltics Tel +358 9 512 3940 Fax +358 9 512 39439 • France Tel 01 69 35 67 00 Fax 01 69 41 98 77 • Germany Tel 0800 9080 711 Fax 0800 9080 712 • Greater China Tel +852 2100 6300 Fax +852 2100 6338 • Italy Tel 02 26001 320 Fax 02 26001 399 • Japan Tel 81 3 5331 9336 Fax 81 3 5331 9370 • Korea Tel 82 2 6201 3700 Fax 82 2 6201 3803 • Latin America Tel +55 11 3933 7300 Fax +55 11 3933 7304 • Middle East & Africa Tel +30 210 96 00 687 Fax +30 210 96 00 693 • Netherlands Tel 0800-82 82 82 1 Fax 0800-82 82 82 4 • Norway Tel +47 815 65 777 Fax +47 815 65 666 • Portugal Tel 21 417 7035Fax 21 417 3184 • Russia, CIS & NIS Tel +7 495 956 5177 Fax +7 495 956 5176 • Spain Tel 902 11 72 65 Fax 935 94 49 65 • Sweden Tel 018 612 1900 Fax 018 612 1910 • Switzerland Tel 0848 8028 10 Fax 0848 8028 11 • UK Tel 0800 515 313 Fax 0800 616 927 • USA Tel +1 800 526 3593 Fax +1 877 295 8102

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