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Purification of a Murine IgG₁ Antibody Expressed in the Leaves of *Arabidopsis Thaliana*

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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 IgG₁ 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.



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 × 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.

Column: HiTrap rProtein A FF, 1 ml
Sample: 50 ml *A. thaliana* protein extract
Binding buffer: 50 mM Tris-HCl, 3 M NaCl, pH 8.5
Elution buffer: 0.1 M NaO_4 -citrate, pH 3.0
Flow rate: 1 ml/min
System: ÄKTAexplorer™ 100

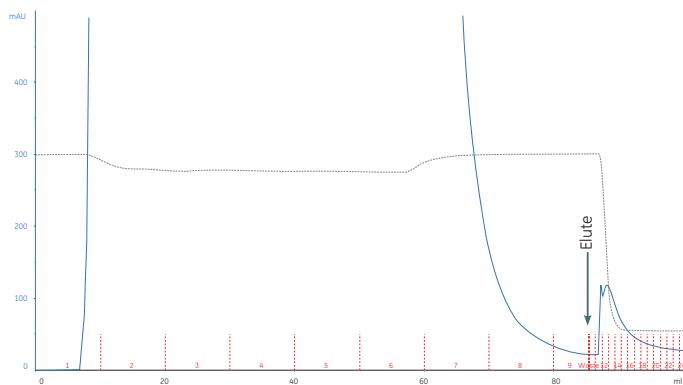


Fig 1. Purification of IgG from *A. thaliana* protein extract on HiTrap rProtein A FF, 1 ml.

Purification and analysis

Efficient adsorption of murine IgG₁ 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).

Column: Superdex 200, 10 × 300 mm
Sample: 250 µl IgG eluate from HiTrap rProtein A FF
Buffer: 50 mM Tris-HCl, 100 mM KCl, 2 mM EDTA, pH 8.5
Flow rate: 0.4 ml/min
System: ÄKTAexplorer 100

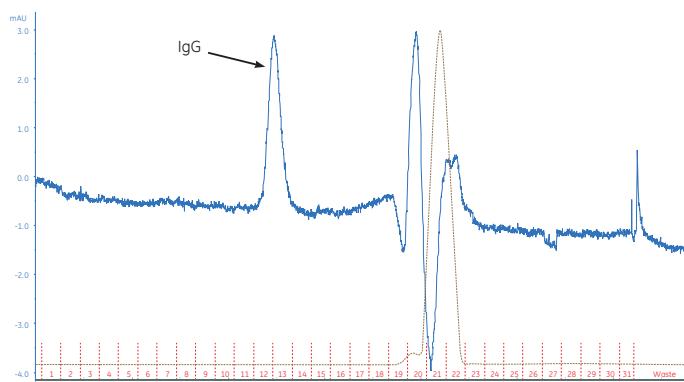


Fig 3. Further purification of IgG from *A. thaliana* on Superdex 200.

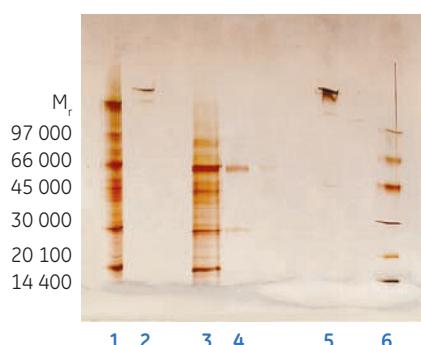


Fig 2a. SDS-PAGE analysis of MAK 33 after purification on HiTrap rProtein A FF using ExcelGel™ SDS Gradient 8-18 and silver staining.

Lane 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)

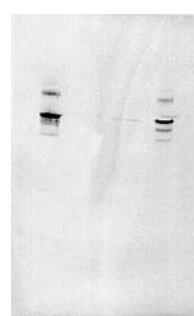


Fig 2b. Western blot analysis of MAK 33 after purification on HiTrap rProtein A FF.

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

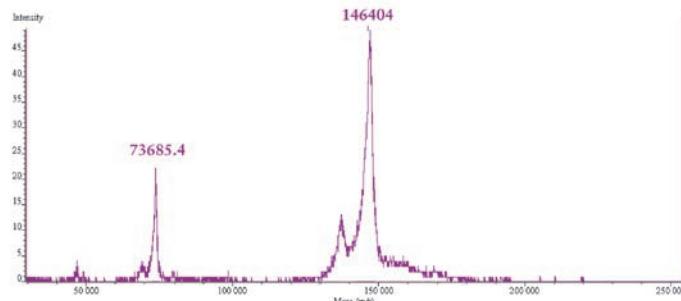


Fig 4a. Full-length analysis of MAK 33 using MALDI-ToF MS.

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.

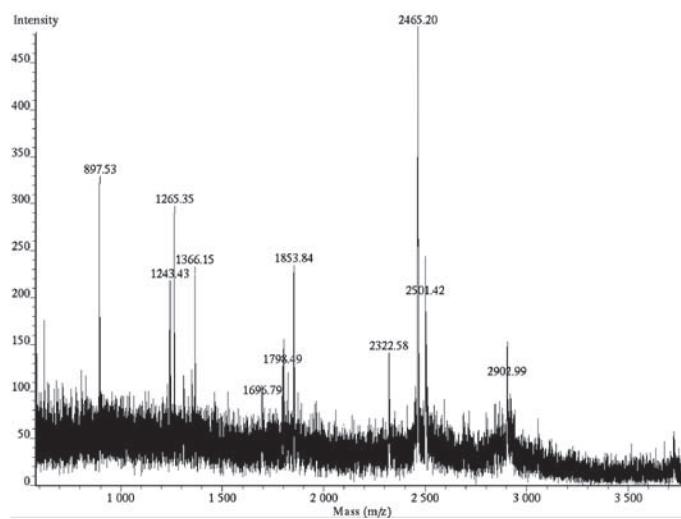


Fig 4b. Peptide map of MAK 33 using MALDI-ToF MS.

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 (trifluoroacetic 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-hydroxycinnamic acid (saturated solution in 50% acetonitrile and 0.9% trifluoroacetic 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).

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