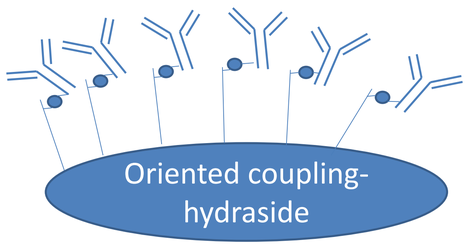
Isolation of RAE-1 from murine cells based on immunoaffinity monolithic chromatography

Urh Černigoj[1], Tihana Lenac Rovis[2], Mateja Simonič[1], Suzana Malić[2], Jernej Gašperšič[1], Karmela Miklić[2], Olga Schevchuk[2], Rene Hennig[4], Alexander Pralow[4], Erdmann Rapp[4], Djuro Josić[3], Stipan Jonjić[2], Aleš Štrancar[1,5]

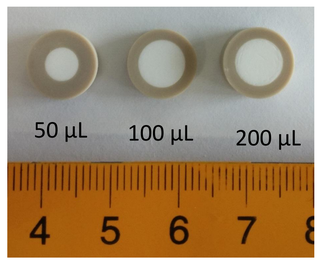
1 BIA Separations d.o.o., Mirce 21, 5270 Ajdovščina, Slovenia | 2 Center for Proteomics, Faculty of Medicine University of Rijeka, 51000 Rijeka, Croatia| 3 University of Rijeka, Trg braće Mažuranića 10, 51000, Rijeka, Croatia | 4 Max Planck Institute for dynamics of complex technical, Sandtorstraße 1, 39106 Magdeburg, Germany | 5 COBIK, Tovarniška 26, 5270 Ajdovščina, Slovenia

INTRODUCTION

Immunoaffinity columns using antibodies as ligands against mammalian proteins could be used for different applications in protein expression control and, if a standard available, for direct protein quantification in complex sample solutions. Additionally, these columns are ideal for polishing step of recombinant proteins, such as mammalian receptor Fc fusion proteins. Most importantly, such columns could extract a significant amount of a single membrane protein from native source, suitable for downstream analyses, such as mass spec analysis of their glycans. Immunoaffinity chromatographic monoliths against RAE-1 GPI anchored glycoprotein were developed (CIMmic αRAE-1 column) as a part of Glycomet project with the main goal to analyze the antigen glycoprofile.



CIMmic chromatographic support (white circles):



CIMmic housing:

Figure 1: Antibodies immobilized on monoliths in oriented direction. On hydraside preactivated support (HIDA), monoclonal antibodies (mAbs) are immobilized through oxidized glycans.
CIMmic® monolithic disks with column volume of 50 µL were used as a chromatographic support for immunoaffinity chromatography

Figure 2: RAE-1 is a GPI anchored protein. To solubilize hydrophobic proteins, detergent is needed before and during separation.

RESULTS

Antibodies

Figure 3: αRAE-1 mAb precipitates RAE-1 from cell lysates of corresponding transfectants. RAE-1 is heavily glycosylated and thus forms smear in WB.

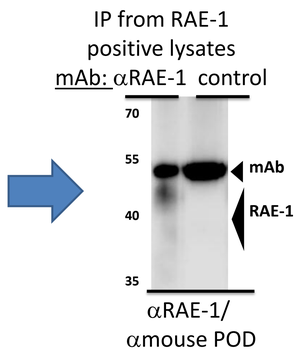
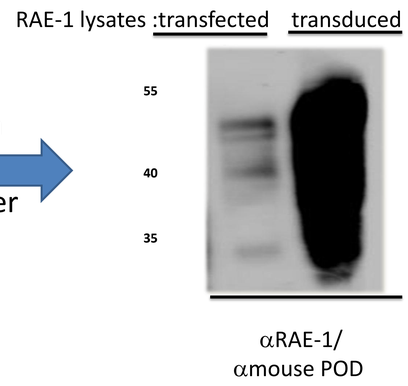


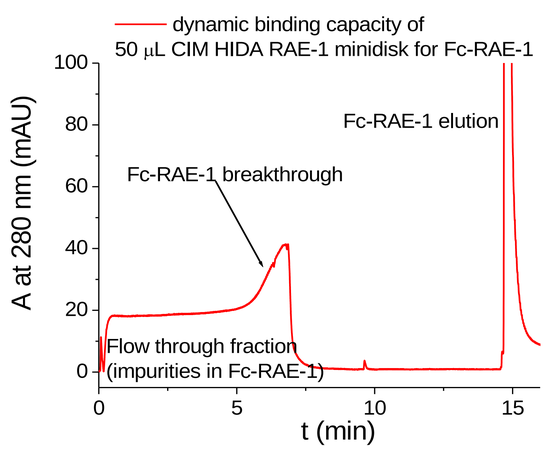
Figure 4: The amount of proteins upon transduction (using a cytomegaloviral vector) is significantly higher than in transfected cells (RAE-1)

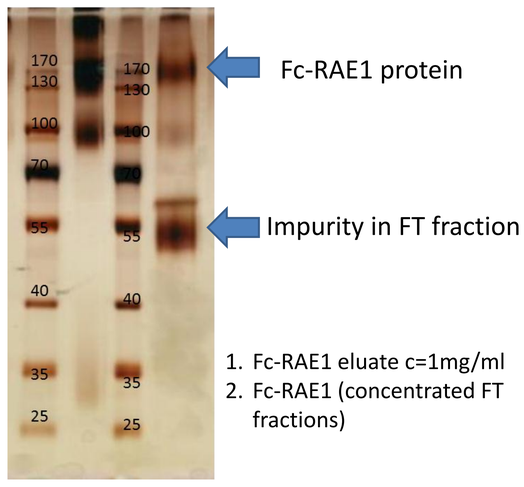


CIMmic αRAE-1 column

αRAE-1 mAb immobilisation: Immobilisation procedure was a platform one, developed before for other IgGs. Approximatelly 500 µg of IgG is needed for a 50 µL minidisk.

Figure 5: Dynamic binding capacity for Fc-RAE-1 on CIMmic αRAE-1 column was determined to be cca 5 mg Fc-RAE-1 per mL of chromatographic support .
A strong signal in FT fraction was proven to be an impurity (see SDS-PAGE on the right) after standard purification protocol for recombinant Fc fusion proteins. This opens up new perspectives to obtain high-quality protein samples needed for certain functional and immunological assays.





RAE-1 isolation from cells

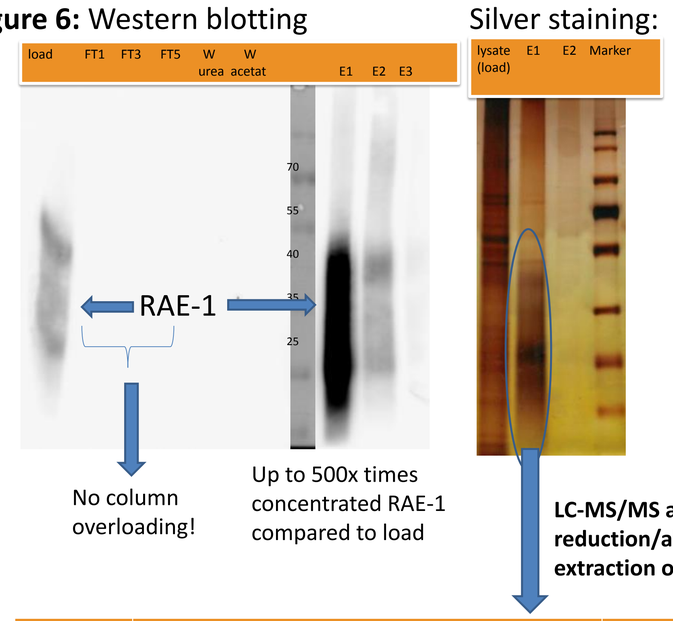
Sample preparation:

- Cell lysis (0.1% NP40), followed by centrifugation, supernatant collected
- Addition of CPI (optionally another centrifugation)
- Acidification to pH 3 using glycine
- Filtration through 0.22 µm filters
- Neutralisation with TRIS to pH 7.5
- 4.5 times dilution with phosphate buffer containig 0.5% Tween 20
- Filtration through 0.22 µm filter
- Chromatography

Chromatography:

- Sample loading (up to 200x10⁶ cells) to CIMmic αRAE-1 column (flow rate: 40 CV/min)
- Wash with 20 CV **1x PBS** + 0.5 % Tween 20 (MPA)
- Wash (W urea) with **2 M urea**/PBS + 0,5% Tween 20
- Wash (W acetate) with **50 mM acetate pH 4.5** + 0.5% Tween 20
- **Elution (E1) with 20 CV 0.1M glycine pH 2.0 + 0.5 % Tween 20 (MPB)**
- Wash with 20 CV **1x PBS** + 0.5 % Tween 20 (MPA)

RAE-1 isolation from transduced NIH cells;
Figure 6: Western blotting

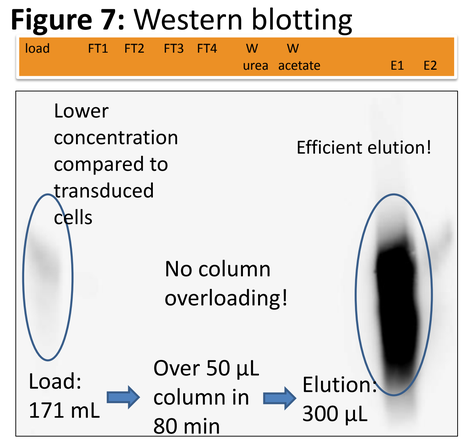


Silver staining:

LC-MS/MS analysis from Coomassie-stained gel after: 1) reduction/alkylation; 2) deglycosylation; 3) tryptic digestion; 4) extraction of digested proteins from band

Accession	Description	Score	Coverage	# Proteins	# Unique peptides	MW (kDa)
O08604	Retinoic acid early-inducible protein 1-gamma	6162	32.02	1	5	28.5

RAE-1 isolation from 160 million transfected NIH cells;
Figure 7: Western blotting



CONCLUSIONS

- Transduced cells are ideal for massive production of mammalian membrane glycoproteins.
- Hydraside coupling chemistry was proven as optimal for preparation of immunoaffinity αRAE-1 monolithic chromatographic support.
- Microgram amounts of low-abundant native RAE-1 protein were purified from cell lysates.
- The protein sample preparation allows N-glycosylation analysis of purified RAE-1.
- The N-linked glycosilation comparison of transfected and transduced proteins might elucidate the mechanism exploited by viruses to evade immune response.