

TESTING DIFFERENT ACTIVATION CHEMISTRIES FOR IgG IMMOBILIZATION ONTO A MONOLITHIC SUPPORT



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INTRODUCTION

Interactions between antibodies and their antigens are highly selective and therefore immensely popular for affinity chromatography. Consequently, numerous antibody immobilizations were performed on monolithic supports via different activation chemistries in the last decade. Despite the work already done there was no systematic study, where as many as possible activation

chemistries were tested for the immobilization of a model monoclonal antibody with subsequent chromatographic characterization of the affinity support. In this work, various preactivated CIM monolithic columns were used for the immobilization of a model monoclonal IgG.

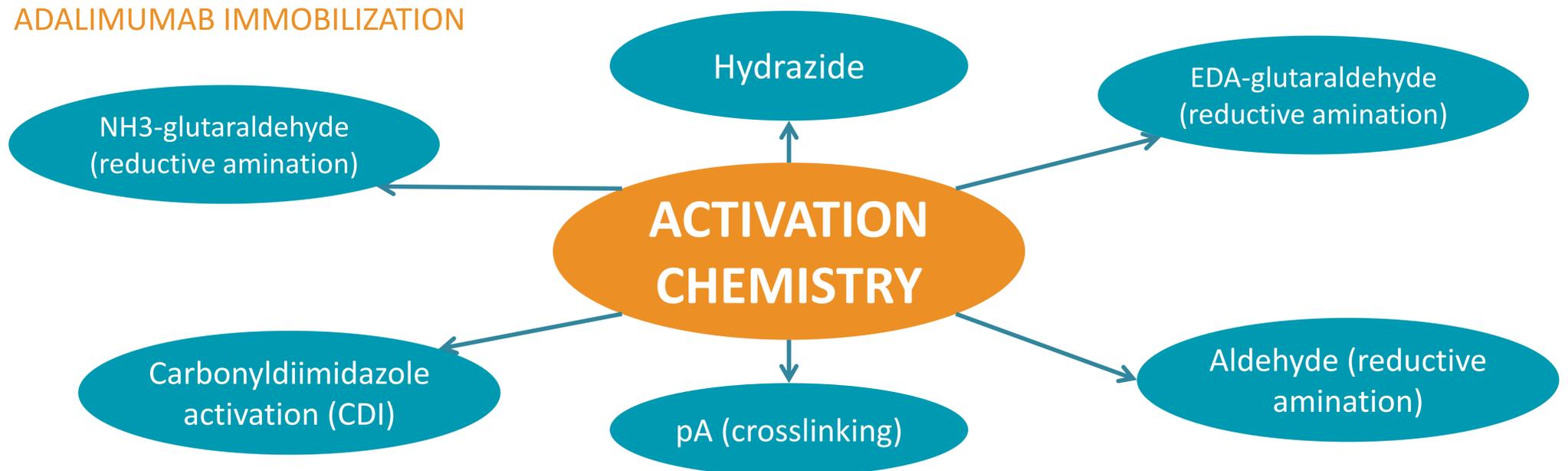
MATERIALS

Main materials:

- Differently activated CIMac columns;
- Adalimumab, a model monoclonal antibody (Abbott Laboratories Ltd.);
- Tumor necrosis factor alpha or TNF α , a specific antigen, recognised by adalimumab (Lek Pharmaceuticals d.d.)

RESULTS

ADALIMUMAB IMMOBILIZATION



CHROMATOGRAPHIC TESTING OF AFFINITY COLUMNS

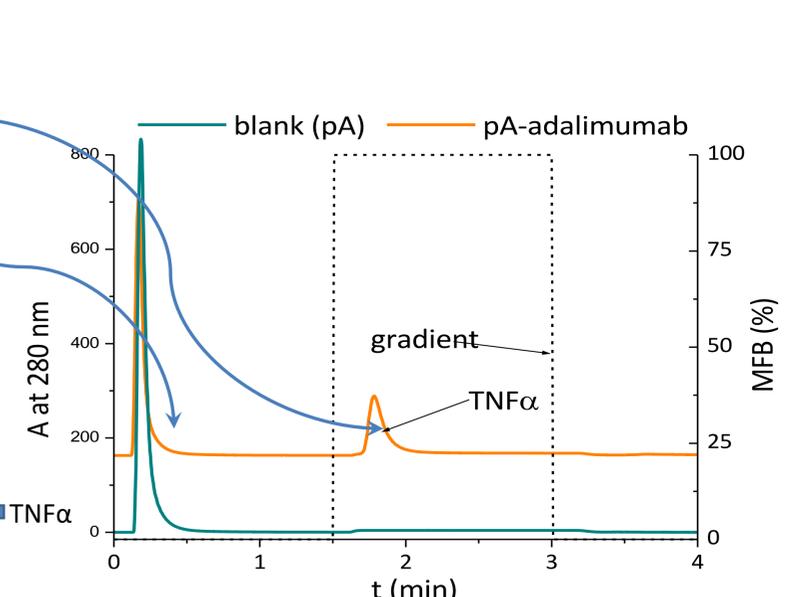
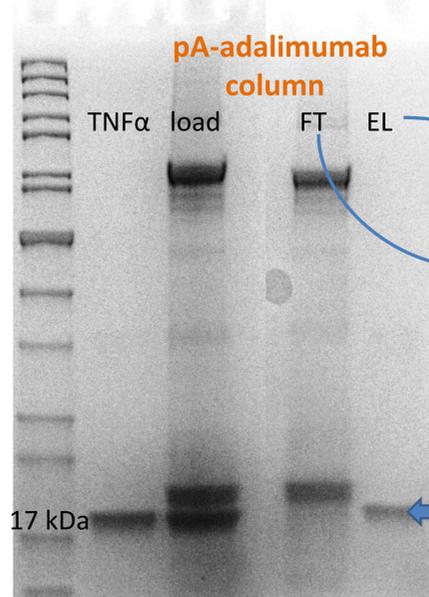
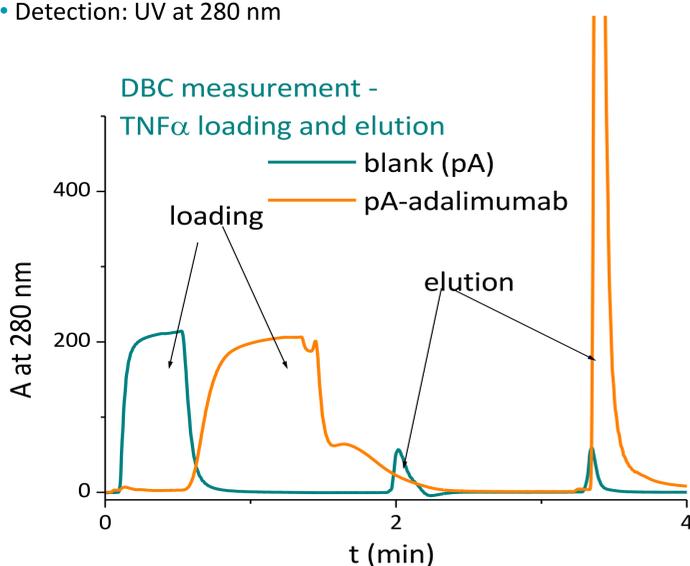
Chromatographic conditions for DBC measurements:

- CIMac columns with immobilized adalimumab
- TNF α (0.16 mg/ml) in 1x PBS buffer, loading till the end of the breakthrough
- Elution buffer: 340mM NaCl in 0.1M acetic acid pH 2,74
- Flow: 1.0 ml/min
- Detection: UV at 280 nm

Adalimumab column - activation	DBC for TNF α (mg/ml)
aldehyde	0.30
NH ₃ -GA	0.30
EDA-GA	0.20
CDI	0.05
Crosslinked pA	0.74
hydrazide	0.80

Chromatographic conditions for selectivity measurements:

- CIMac columns with immobilized adalimumab
- Sample: mixture of TNF α (0.3 mg/ml), Myoglobin (0.2 mg/ml), Conalbumin (0.3 mg/ml), Soybean Trypsin Inhibitor (0.4 mg/ml) in 1x PBS + 0.20 M NaCl buffer, Vinj. = 50 μ l
- Elution buffer: 340mM NaCl in 0.1M acetic acid pH 2,74
- Flow: 1.0 ml/min
- Detection: UV at 280 nm



CONCLUSIONS

- We were able to covalently bind the model monoclonal antibody to a monolithic support using different linkage strategies.
- The immobilization of antibody was proven by measuring the selectivity of the final affinity chromatographic support for the specific antigen.
- The most efficient immobilization of model monoclonal antibody according to the determination of DBC was achieved with oriented immobilization (via hydrazone linkage or via pA crosslinking). CDI preactivated monoliths resulted in almost no final affinity despite high density of the loaded antibody.

Acknowledgments

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