

COMPARISON OF DIFFERENT TYPES OF AFFINITY LIGANDS FOR THE PURIFICATION OF COAGULATION FACTOR VIIa

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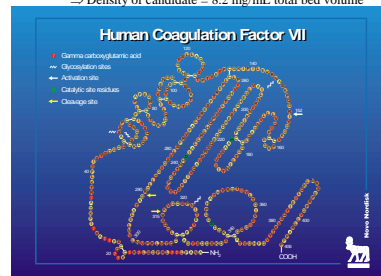


Abstract

In the purification process of human recombinant Factor VIIa (rFVIIa), a monoclonal antibody column is being used as an efficient chromatographic step for removal of media and host cell contaminants. However the immuno affinity matrix have some inherent drawbacks such as high production costs, low capacity and low stability. In order to evaluate alternatives to the immuno affinity matrix, libraries of triazine compounds and synthetic peptides, immobilized to chromatographic adsorbents, were constructed and tested for rFVIIa binding and elution properties. The performance of selected candidates of triazine compound ligands and peptide ligands will be compared to the performance of the immuno affinity ligand in terms of the capability of purifying rFVIIa. Further differences between the matrices will also be discussed.

Description of ligands

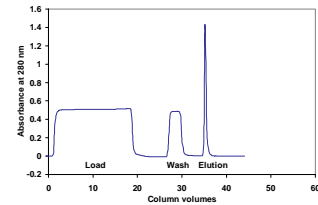
- Antibody** ⇒ Monoclonal, produced at Novo Nordisk
 ⇒ Immobilised to CNBr Sepharose CL-4B
 ⇒ Ligand density = 10 mg anti-FVII/mL gel
 ⇒ Ca²⁺ dependent (load with Ca²⁺, elute with chelator) (ref.1)
- Triazine** ⇒ Trichloro-s-triazine substituted with aromatic amines (ref.2)
 ⇒ Immobilised to activated Sepharose CL-6B
 ⇒ Designed to be Ca²⁺ dependent
- Peptide** ⇒ Linear peptides, 8-13 Amino Acids
 ⇒ Immobilised to pre-activated gels
 ⇒ Density of candidate = 8.2 mg/mL total bed volume



References

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- Boyer PM, Hsu JT. Protein purification by dye-ligand chromatography. *Adv Biochem Eng Biotechnol*. 1993; 49:1-44.

Antibody ligand



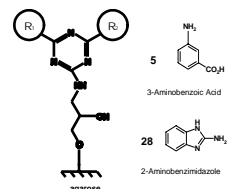
Purification of FVII with antibody. Eluate from capture step (84 % pure) was applied. Binding with Ca²⁺ and elution with citrate. Detergent present in load and wash



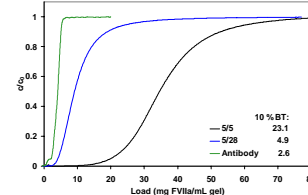
Peptide ligand

- Peptide library designed from the FVIIa binding domains of tissue factor (ref.3) using short overlapping segments
- Identification of binders by spot-synthesis and real-time interaction analysis, as described for the development of a ligand for FVIII (ref.4)
- Candidate (H₂N-NNFTLSRLRYVFGK-COOH) was immobilised on an epoxy-activated CIM-disc (BIA Separations)
- Ca²⁺ dependent binding and elution of FVIIa could not be obtained, elution was carried out with chaotropic agent
- Capacity was measured as 100-200 µg/mL total bed volume

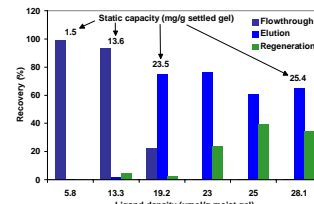
Triazine-based candidate ligands



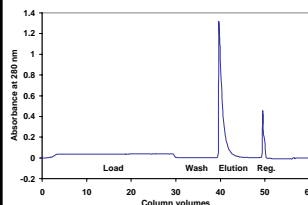
Structure of the immobilised ligand with candidate amines



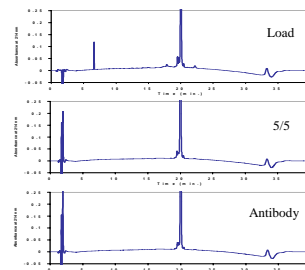
Comparison of capacity with pure FVIIa. Ligand density of 5/5 and 5/28 is 15 µmol/g. BT = Breakthrough



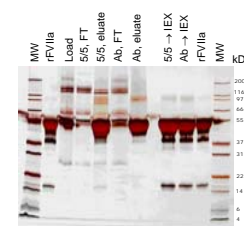
Recovery as a function of ligand density (5/5). Binding with Ca²⁺, elution with citrate and reg. with NaOH/2-propanol



Purification of FVII with 5/5. Eluate from capture step (84 % pure) was applied. Binding with Ca²⁺, elution with citrate and reg. with NaOH/2-propanol



RP-HPLC purity profiles from top: Capture eluate, eluate from 5/5 and eluate from antibody column



Silver stained non-reduced SDS-PAGE. FT = Flowthrough. IEX = Ion-exchange step

Conclusions

Peptide ligand

- Designed from FVIIa - tissue factor interaction
- Capacity needs to be optimised
- In an early phase the project was terminated since the triazine based ligands showed promising results and this project had progressed over a longer time

Antibody ligand

- Monoclonal Ca²⁺ dependent antibody
- Capacity 2 - 2.5 mg/mL gel
- Very efficient removal of non-product related impurities
- Difficult to sanitise

Triazine-based ligands

- Designed to bind FVIIa in the Gla-domain and to be Ca²⁺ dependent
- Dynamic capacity of final candidate (5/5) at process conditions: approx. 5 mg/mL gel (data not shown)
- Performance is strongly dependent on the ligand density as could be expected (ref.5)
- Performance of 5/5 similar to antibody
- Cleaning and sanitisation at low and high pH and in alcohol

Acknowledgements

The technical assistance of Liselotte Lindgreen and Nina Johansen is greatly appreciated.