Purification Process Development of Protein Subunit based Vaccine Candidates Produced using Recombinant *E. coli* Expression System-Case Study

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Part I

ABSTRACT

Proteins are key components of prophylactic vaccines against infectious diseases. Protein subunit based vaccine is an attractive alternative to the traditional detoxified bacterial or inactivated viral vaccine approach due to its highly purified and well characterized product nature. Purification of protein antigens to achieve consistent product purity and quality is an integral part of the protein subunit vaccine product development process. Expression levels of the recombinant proteins in bacterial expression system may be extremely high following rapid technology advancement. The challenges and approaches used to develop purification processes for novel protein vaccine candidates expressed at g/L level are being discussed.

Introduction and Objective

- The concept of Platform Technology (capturing, purification and polishing) has been successfully applied to the Mab purification (Figure 1).
- The objective of this study was to assess whether a similar concept can be applied to the purification of *E. Coli* expressed soluble recombinant proteins in order to facilitate vaccine antigen purification process development.
- A case study with a specific challenge will also be discussed (Part II)

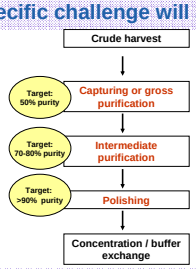


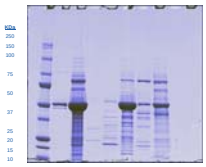
Figure 1: Typical Purification Process

Methods

- Chromatographic methods such as Anion or cation exchange, Hydrophobic interaction chromatography, mixed mode chromatography methods were used.
- AKTA Explorer was used for small-scale method development and processes were scaled-up using AKTA PILOT.
- In-Process samples were tested using inline monitoring by UV, conductivity etc.
- Fractions were analyzed by SDS-PAGE or BCA protein assay.

Results (1)

- Several protein purification processes were developed using different protein antigens expressed in *E. coli* expression systems.
- A trend was observed in most of these purification schemes i.e. strong anion exchange such as Q column worked well as primary capture step and had enough capacity to capture the target protein where the expression in upstream was 1-3 g/L (Figure 1)
- Binding and elution conditions of each protein including the buffers to purify these protein varied and were dependent on the individual characteristics of these proteins (data not shown).
- The purity of in-process fractions after 1st unit operation was typically >50%. (Figure 2)



1= M.W. std
2= Standard
3= Crude
4= Flow through
5=Intermediate wash step
6= Elution Peak
7= Elution Tail
8= Strip

Figure 2: SDS-PAGE Analysis of Fractions from 1st unit operation

Results (2)

- The 2nd unit operation selected was either a cation exchanger (such as SP) or Hydrophobic column chromatography (HIC) step.
- The purity of in-process samples after 2 unit operation was typically >80% and for one protein was even >90% (Figure 3).
- Binding and elution conditions of each protein including the buffers to purify these proteins on cation exchanger or HIC varied and were dependent on the individual characteristics of these proteins (data not shown).

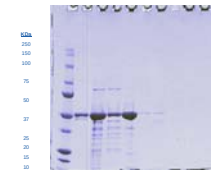


Figure 3: SDS-PAGE Analysis of Fractions from 2nd unit operation

1=M.W. Std
2=Standard
3=First column purified
4=Flow Through
5= Elution
6. Elution Tail
7= Strip

Results (3)

- The 3rd unit operation selected was almost always a mixed mode column chromatography step such as Ceramic Hydroxyapatite or Q membrane. The purity of in-process samples after 3 unit operation was typically >95% and met the criteria (Figure 4).
- Binding and elution conditions of each protein including the buffers to purify these proteins on 3rd column were dependent on the individual characteristics of these proteins (data not shown).
- Three or two column purified material was used for final buffer exchange using Tangential Flow Filtration.

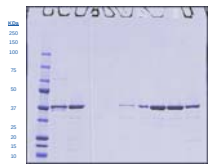


Figure 4: SDS-PAGE Analysis of Fractions from 3rd unit operation

1= M.W. Std.
2=Standard
3= Two column purified
4= Flow Through
5=Intermediate Wash
6-9=Elution
10-Strip

Conclusions

- A common theme or the standardized approach can be used to develop new purification processes for new recombinant *E. Coli* antigens (Figure 5).

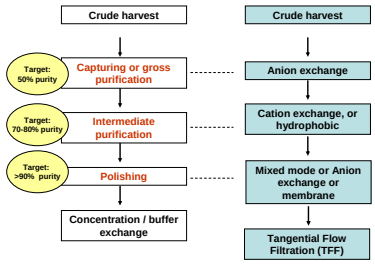


Figure 5: Common theme in the purification of soluble recombinant proteins in *E. Coli*