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Hydrophobic Interaction Chromatography of Recombinant Nipah Virus-Like Particle from Escherichia coli Homogenate: Type of Ligand

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Abstract. The performance of hydrophobic interaction chromatography (HIC) on the recovery of recombinant Nipah virus-like particle (VLP) was evaluated with Sepharose™ 6 Fast Flow (FF) adsorbents coupling separately with four different types of ligand; phenyl low substitution, phenyl high substitution, butyl and octyl. Evaluation of the HIC recovery method was performed in a preparative bench scale. The binding condition of the VLP of NiV was using a salt buffer of 15% ammonium sulfate concentration. The recovery of the VLP of NiV was achieved by a linear decreasing salt gradient. The purification of recombinant VLP of NiV using the phenyl low substitution ligand of HIC medium has recovered 75.5% of VLP of NiV from unclarified E. coli homogenate with a purification factor of 8.8. The product yield achieved in preparative HIC recovery method showed a promising result and the phenyl low substitution ligand was selected for subsequent optimization process.

Keywords: hydrophobic interaction chromatography, Nipah virus, phenyl sepharose, *Escherichia coli*

1. Introduction

The capsid protein of Nipah virus (NiV) have been successfully expressed as intracellular virus-like particle (VLP) in *E. coli* [1]. The VLP of NiV is highly immunogenic and has a potential to be used as diagnostic agents [2]. Due to its wide application, it is important to develop a cost effective production and purification process to manufacture this proteins.

In hydrophobic interaction chromatography (HIC), protein adsorption is promoted by high salt concentrations and elution of the bound protein is achieved by a linear decreasing gradient of salt concentration in the binding buffer [3-5]. The performance of HIC is influenced by the characteristics of protein and the operating conditions of the chromatographic system [6]. Thus, the selection of the right technique and operating condition is important to ensure a successful protein purification process. In this work, the development of a packed bed adsorption protocol using a HIC matrix for the recovery of the VLP of NiV from clarified *E. coli* homogenate was investigated. In order to study the effects of type of ligand on the recovery of the VLP of NiV, a set of experimental work using a commercially available Sepharose™ product with the same matrix base, but different types and densities of ligand is presented here.

2. Materials and Methods

2.1. Hydrophobic Ligand

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All the HIC media: Sepharose™ 6 Fast Flow (FF) phenyl low substitution (low sub), phenyl high substitution (high sub), butyl and octyl were purchased as prepacked 1 ml HiTrap™ from GE Healthcare (Sweden). Separation of protein by the HIC column was performed using the Äkta FPLC chromatography system (GE Healthcare, Sweden).

2.2. Purification of Recombinant VLP of Nipah virus: Selection of Hydrophobic Ligand

Recombinant VLP of Nipah virus was produced by *E. coli* strain BL21 (DE3) harbouring plasmid pTrcHis$_2$ expressing the capsid protein of NiV [1], as that reported by Chong *et al*. [7].

Selection of ligand was carried out in the HiTrap™ 1 ml column (0.7 cm diameter, 2.5 cm length) (GE Healthcare, Sweden) prepacked with the same Sepharose™ FF matrix, but with four different types of ligands: phenyl high sub, phenyl low sub, butyl and octyl.

All experiments were performed at room temperature, 22°C. Columns were equilibrated with 10 column volumes (CV) of Buffer A (20 mM sodium phosphate buffer, 15% ammonium sulfate, pH 7.5). The clarified feedstock was fed to the column at a maintained linear flow rate of 150 cm/h. Unbound protein was washed out with 10 CV of Buffer A. The proteins were eluted by a linear decreasing salt gradient by mixing Buffer A and Buffer B (20 mM sodium phosphate buffer, pH 7.5) over 10 CV, followed by a regeneration step with 5 CV of distilled water and Buffer A.

2.3. Protein Analysis and Quantitation

The recovery of VLP of NiV from the chromatographic process was investigated in this study. The SDS-PAGE of protein sample was performed under denaturing condition with 0.1% (w/v) SDS [1]. The quantity of the VLP of NiV was determined by comparing the intensity of the protein band on the Western blot [7]. The Bradford assay was performed to estimate the total protein content. The distribution of of the VLP of NiV antigenicity was determined with an ELISA-based assay.

3. Results and Discussion

The type and density of the HIC ligands affects the overall performance of HIC with respect to its adsorption capacity, strength of binding, recovery of bound proteins and selectivity [3,8]. In the current study, the suitable type and concentration of ligand have to be determined prior to the scale-up purification process to ensure a high recovery yield and purity. In the experiments, adsorbents coupling separately with four types of ligand (phenyl high sub, phenyl low sub, butyl and octyl) were used to bind the VLP of NiV from the clarified *E. coli* homogenate. The HIC medium bound the VLP of NiV at a reasonable low concentration of salt buffer, which was a 15% saturation of ammonium sulfate. The salt concentration of 15% was below the concentration that caused precipitation of VLP of NiV in the feedstock. Then the bound protein was eluted with gradient salt concentration buffer. The chromatogram in Figure 1 shows the elution pattern obtained after the initial binding step of the VLP of NiV of each type of ligand.

The recovery performance of VLP of NiV from the chromatographic process is shown in Table 1. The data show that the octyl and phenyl low sub achieved a higher elution yield of the VLP of NiV than the butyl and phenyl high sub. One should aware that the higher the n-alkyl ligand, the stronger interaction of solute and ligand.

As in Table 1 also, where an increase in ligand concentration of a phenyl ligand in medium results in an increase in both selectivity and yield of VLP of NiV. The lower elution yield of the phenyl high sub adsorbent compared to that of phenyl low sub adsorbent. Such a problem may be due to the high interaction of VLP of NiV and phenyl high sub ligand as a result of its higher ligand density. This leads to the denaturation of the VLP of NiV upon elution from the column. Similarly according to To and Lenhoff [9], high ligand density had resulted higher binding of proteins but lower elution.

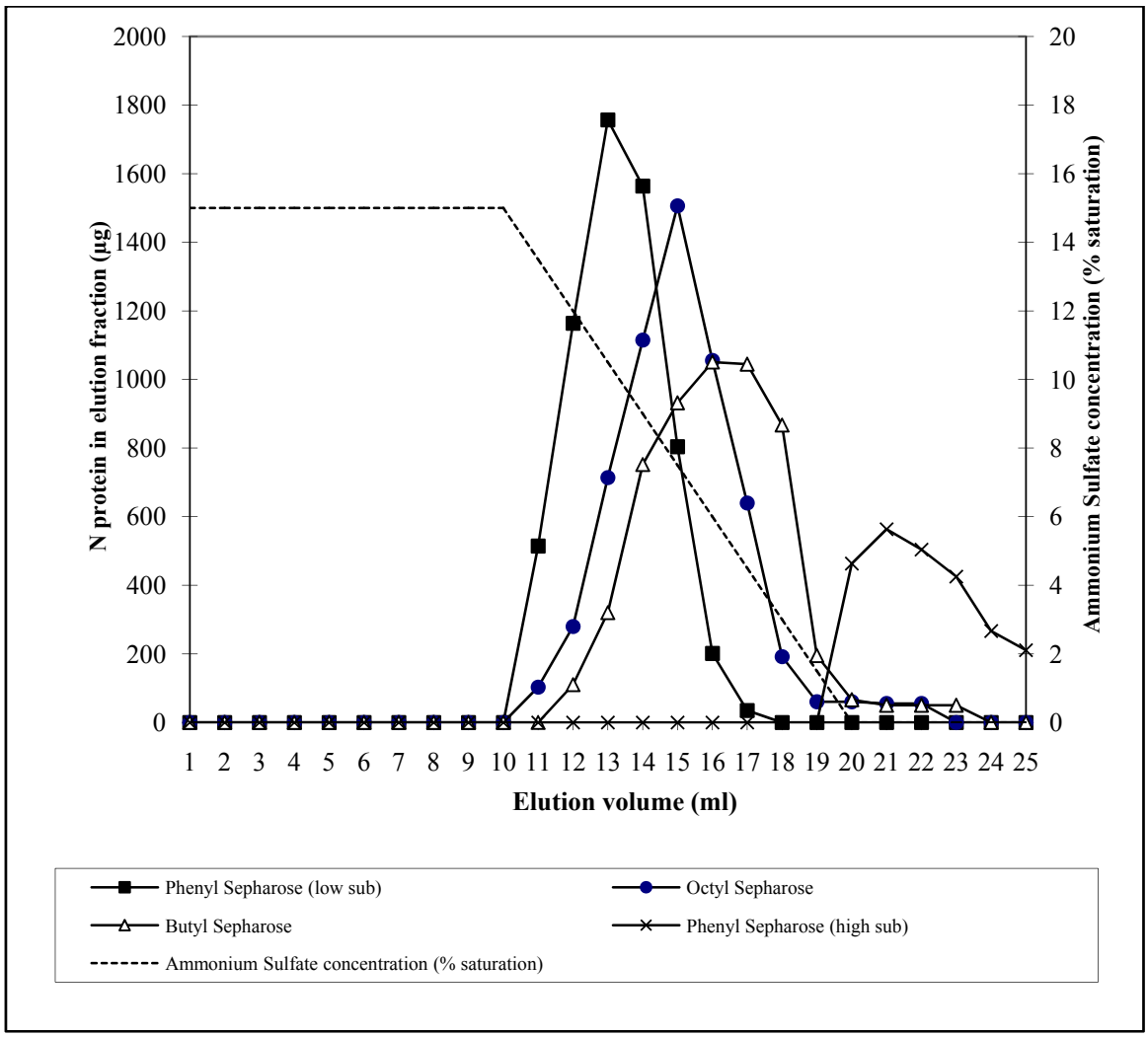

Figure 1 Chromatography of a VLP of NiV on phenyl high sub, phenyl low sub, butyl and octyl Sepharose

Table 1: The performance profile of VLP of NiV in four different types of HIC ligands: phenyl high sub, phenyl low sub, butyl and octyl.

Type of ligand	Percentage of bound N protein (%)	Purity of N protein eluted	Yield of N protein eluted (%)	Purity factor
Phenyl low sub	75.6 ± 4.3	0.86 ± 0.02	75.5 ± 4.0	8.8
Phenyl high sub	82.2 ± 3.8	0.41 ± 0.04	30.4 ± 3.8	4.2
Butyl	70.5 ± 7.0	0.75 ± 0.05	56.1 ± 4.3	7.7
Octyl	72.3 ± 2.9	0.93 ± 0.07	73.5 ± 5.4	9.6

4. Conclusion

The highest recovery of VLP of NiV was achieved by the HIC medium coupled with the phenyl low sub ligand, with 75.5% yield and purity factor of 8.8. The type of ligands for synthesizing HIC media has profound effects on the adsorption and elution characteristics of the HIC media. Thus, the correct choice of HIC medium can lead to a successful purification process with a high recovery.

5. Acknowledgements

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6. References

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