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Purification of histidine-tagged nucleocapsid protein of Nipah virus using immobilized metal affinity chromatography

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ABSTRACT

Nucleocapsid (N) protein of Nipah virus (NiV) is a potential serological marker used in the diagnosis of NiV infections. In this study, a rapid and efficient purification system, HisTrap™ 6 Fast Flow packed bed column was applied to purify recombinant histidine-tagged N protein of NiV from clarified feedstock. The optimizations of binding and elution conditions of N protein of NiV onto and from Nickel Sepharose™ 6 Fast Flow were investigated. The optimal binding was achieved at pH 7.5, superficial velocity of 1.25 cm/min. The bound N protein was successfully recovered by a stepwise elution with different concentration of imidazole (50, 150, 300 and 500 mM). The N protein of NiV was captured and eluted from an inlet N protein concentration of 0.4 mg/ml in a scale-up immobilized metal affinity chromatography (IMAC) packed bed column of Nickel Sepharose™ 6 Fast Flow with the optimized condition obtained from the method scouting. The purification of histidine-tagged N protein using IMAC packed bed column has resulted a 68.3% yield and a purification factor of 7.94.

1. Introduction

Nipah virus (NiV) is a deadly zoonotic virus of bat origin and has been classified as biosafety level 4 (BSL4) pathogens [1]. Since its presence in the 1990s, lethal infections of NiV in human and/or livestock animals are reported almost every year in Southeast Asia [2–4]. In 1998 and 1999, the outbreak of NiV in Malaysia has claimed 105 human lives and resulted in the culling of about 1.1 million pigs [5], bringing tremendous economic and social impact to the nation. Hence, vigorous serological monitoring of the NiV infection is necessary to prevent the occurrence of the major outbreak.

The N protein of NiV has been successfully expressed in *Escherichia coli* (*E. coli*), and it is highly antigenic and immunogenic [6]. The recombinant N protein assembles automatically into herringbone-like structure [6,7], which resembles the native NiV nucleocapsid. Yu et al. [8] developed a NiV-N protein-based ELISA system as a diagnosis tool for NiV infection. Ndifuna et al. [9] recommended that the N protein can be used in large-scale epidemiological investigations and to be applied in developing countries.

The major problem during the production and purification of the N protein of NiV is the low recovery yield due to the proteolytic degradation [6]. The protein degradation can be reduced by shortening the purification time [10]. The lab scale purification of the N protein using sucrose gradient ultracentrifugation is time consuming, and thus provides sufficient time for the protease to attack the N protein. Therefore, the development of a rapid and simplified purification of N protein is desired. The placement of a histidine hexamer tag at the C-terminus of the N protein has enabled the purification of the N protein by Ni^{2+} based immobilized metal affinity chromatography (IMAC) [6]. IMAC can be applied in the early stage of protein purification before the protein precipitation and dialysis steps, hence significantly shorten the purification time.

The protein adsorption on IMAC is governed by various factors such as the type of chelating ligand, metal ion, the surrounding chemical environment and experimental parameters on the dynamic binding capacity [11–14]. Furthermore, Sharma et al. [12] demonstrated that the role of ionic strength and pH of the chromatographic medium is significant in governing the binding interaction between the protein and the adsorbent. The performance of a chromatography process such as dynamic binding capacity and recovery throughput can be assessed by frontal breakthrough analysis. Finette et al. [14] examined frontal breakthrough

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measurements in packed bed IMAC with different flow rates and different inlet concentrations of hen egg white lysozyme and human serum albumin.

The benefits of the uses of IMAC as a ligand are its stability, high protein loading, mild elution conditions, simple regeneration and low in cost. These factors are important to be considered when large-scale purification procedures are involved. In this respect, we have selected the Nickel Sepharose™ 6 Fast Flow with iminodiacetic acid (IDA) chelating group, since it has low Ni^{2+} leakage, high protein binding capacity, high flow properties and reproducible chromatographic performance. The detailed investigations on the adsorption and elution behaviors of proteins in IMAC were performed. The yield and efficiency of purification procedure of various operating parameter for Nickel Sepharose™ 6 Fast Flow in packed bed column were evaluated.

2. Experimental

2.1. Materials and equipment

Precharged Nickel Sepharose™ 6 Fast Flow affinity adsorbent and prepacked HisTrap™ FF 1 ml column containing the same adsorbents were purchased from GE Healthcare (Uppsala, Sweden). XK 16/20 column (GE Healthcare) with a 16 mm diameter and 20 cm length and a cross-sectional column area of 201 mm^2 was used to perform a lab scale IMAC purification of N protein of NiV. The packed bed column was connected to the Äkta FPLC chromatography system (GE Healthcare) throughout the protein purification process.

2.2. Feedstock preparation

E. coli strain BL21 (DE3) harbouring plasmid $pTrcHis_2$ expressing the N protein of NiV [6] was cultured in Luria–Bertani (LB) medium containing ampicillin at 25 °C with vigorous shaking at 200 rpm. The expression of N protein was induced by adding isopropylthio-β-D-galactoside (IPTG) to a final concentration of 1 mM when the biomass concentration of the culture reached absorbance at 600 nm (A_{600}) about 0.6–0.8. The induced culture was further incubated for another 5 h at 25 °C. The cells were harvested by centrifugation at 8,000 × *g* (JLA 16.25 rotor, Beckman, USA) for 20 min at 4 °C.

The pelleted cells were resuspended in buffer solution supplemented with 0.2 μg/ml lysozyme and 4 mM $MgCl_2$. Protease inhibitor (1 mM phenylmethylsulphonyl fluoride (PMSF)) was included in the buffer to inhibit the activity of host proteases that released together with the N protein during cell disruption process. The cell suspension was subjected to ultrasonication at 200 W for 30 s with 60 s intervals in an ice bath for duration of 50 min as described previously by Ho et al. [15]. The cell lysate was treated with DNase (5 μg/ml) and incubated for 1 h at 4 °C. The lysate was clarified by centrifugation at 18,000 × *g* (JA 20 rotor, Beckman, USA) for 20 min at 4 °C. The clarified supernatant was used as feedstock for the subsequent purification process.

2.3. Method scouting and optimization

2.3.1. Optimization of binding buffer condition

An experiment to determine the optimal binding buffer condition for purifying the N protein of NiV was performed by using the HisTrap™ FF 1 ml column. The HisTrap™ FF 1 ml column was loaded with the same buffer solution in each equilibration step and binding step. The compounds chosen for preparing the binding buffer solutions were 2-(*N*-morpholino)ethanesulfonic acid (MES) for pH 6, piperazine-*N*,*N*-bis(2-ethanesulfonic acid) (PIPES) for pH 6.5, sodium phosphate for pH 7 and 7.5, 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES) for pH 8 and tris-(hydroxymethyl)-methylamine (Tris) for pH 8.5. The concentration of imidazole and NaCl added into all the buffer solutions was 20 mM and 500 mM, respectively, before the pH was adjusted. The pH range of the binding buffer solutions was prepared from 6.0 to 8.5. The HisTrap™ FF 1 ml column was equilibrated with imidazole prior to chromatographic purification to avoid drastic pH drop due to the effect of imidazole proton™ pump [16].

The HisTrap™ FF 1 ml column was rinsed with 5 column volume (CV) of distilled water and equilibrated with 5 CV of binding buffer. The column was then loaded with clarified lysate at 0.8 mg N protein of NiV/ml adsorbent. Fractions of unbound protein sample at 0.5 ml volume throughout the binding stage were collected and analysed to determine the amount of total protein and N protein of NiV. The amount of bound total protein and N protein was calculated by applying mass balance established from the initial and unbound amount of protein [17].

2.3.2. Optimization of elution conditions

Prior to the elution, the prepacked HisTrap™ FF 1 ml column was loaded with the N protein as described in Section 2.3.1. The column was then washed with 5 CV of binding buffer to remove loosely bound protein. The N protein was eluted from the column in stepwise elution using binding buffer added with different concentrations of imidazole of 50, 150, 300 and 500 mM (5 ml for each concentration). Eluted protein fractions were collected and analysed for the amount of N protein and total protein. The purity and yield of the amount N protein obtained in the purified fractions were calculated as described by Ng et al. [17] and Tan et al. [18].

2.4. Operation of packed bed column

2.4.1. Packing of affinity adsorbent

Nickel Sepharose™ 6 Fast Flow affinity adsorbent was packed in an XK 16/20 column according to the procedure recommended by GE Healthcare [19]. Briefly, slurry of 70% settled medium to 30% distilled water was prepared and poured into the column. The remainder column was filled up with buffer and the column adapter was mounted. By connecting to a pump, a packing flow rate of 2.5 ml/min was run in first step and 8.7 ml/min in second step. The packing flow rate was maintained for 3 column volume after a constant bed height was reached. The pump was stopped and the adapter was locked in position. The column was washed with 5 CV of distilled water and equilibrated with 5 CV binding buffer (20 mM sodium phosphate, 500 mM NaCl and 20 mM imidazole, pH 7.5) [19].

2.4.2. Breakthrough curve and dynamic binding capacity

The breakthrough curve was performed to determine the dynamic capacity and the productivity of the Nickel Sepharose™ 6 Fast Flow affinity adsorbent in the XK 16/20 column. Nickel Sepharose™ 6 Fast Flow affinity adsorbent was packed in an XK 16/20 column at a packed bed height of 10 cm which was installed onto the Äkta FPLC. The breakthrough curve for the packed bed system was obtained by loading clarified feedstock of 0.4 mg/ml N protein concentration at a superficial velocity of 1.25 cm/min. Protein fractions (10 ml) collected throughout the operation were then analysed to determine the concentration of N protein. The dynamic binding capacity, Q_B, is the total amount of N protein adsorbed in the packed bed column per unit adsorbent volume, when the outlet concentration of N protein is 10% of the inlet concentration. Recovery throughput is another parameter in affinity purification. This refers to the amount of adsorbed protein at 10% breakthrough divided by the volume of adsorbent and the processing time; both were calculated according to Chang and Chase [20]. The time for protein adsorption was recorded once the 10% breakthrough was reached. The processing time in one purification cycle was defined