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Purification of porcine reproductive and respiratory syndrome virus using ultrafiltration and liquid chromatography

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) virus causes severe and persistent disease in pigs worldwide. Its heterogeneity poses a major challenge for the effective prevention and control of PRRS. Purified viruses are essential for serological studies. Traditional methods for purifying PRRS virus are time- and labor-intensive and difficult to scale-up and requires long processing time. Here, we describe a rapid, simple, scalable process for PRRS virus purification. Highly pure viral particles were obtained after ultrafiltration and liquid chromatography, as confirmed by SDS-PAGE and electron microscopy. The overall process achieved a recovery of 50% of raw virus, with a purity close to that obtained by CsCl coupled with sucrose density gradients. The purification process described here should be useful in large-scale production of highly pure PRRS virus.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease of swine throughout the world, characterized by severe reproductive problems with late-term abortions in sows and severe respiratory ailments leading to increased mortality in young pigs [22,21]. The etiological agent, PRRS virus, is an enveloped, non-segmented, single positive-stranded virus, which belongs to the genus *Arterivirus*, family *Arteriviridae*, order *Nidovirales* [26]. PRRS viruses display high biological diversity. Nucleotide sequence comparisons show that PRRS virus possesses remarkable genetic variation with two distinct genetic and antigenic groups: Type 1 (European) and Type 2 (North American). The extent of antigenic diversity among PRRS virus strains has hampered vaccine development [18].

Purified viruses are essential for producing antiserum for serological studies and prophylactic vaccines [1,24]. The production of large quantities of highly purified virus requires simple and efficient methods for the purification and concentration of viral particles. Specifically, purification of PRRS virus has traditionally been carried out by cesium chloride or sucrose density gradient ultracentrifugation [17,19,32]. However, these methods are time- and labor-intensive and difficult to scale-up [25]. Compared with traditional density gradient ultracentrifugation, liquid chromatography provides a high level of purity and increased productivity, and has become the method of choice for efficient virus purification. Hu et al. described a novel and scalable process for the purification of PRRS virus using ultrafiltration and heparin affinity chromatography [10]. Although the combination of ultrafiltration and heparin affinity chromatography removed more than 96% of cellular and media proteins, the purification efficiency was still not satisfactory. To improve the currently available methods, we describe here a simple and efficient process for PRRS virus purification based on ultrafiltration and liquid chromatography.

2. Materials and methods

2.1. Preparation of PRRS virus stocks in cell culture

PRRS virus vaccine strain JXA1-R was propagated in the highly permissive MARC-145 cell line. When 90% of the cells showed signs of a visible cytopathic effect, viruses were harvested by freeze thawing infected cells three times. The resulting virus stock solution was frozen at −80 °C until use.

2.2. Clarification and ultrafiltration

The virus-containing cell lysate was clarified by low speed centrifugation at 5000*g* for 20 min at 4 °C. The clarified samples were

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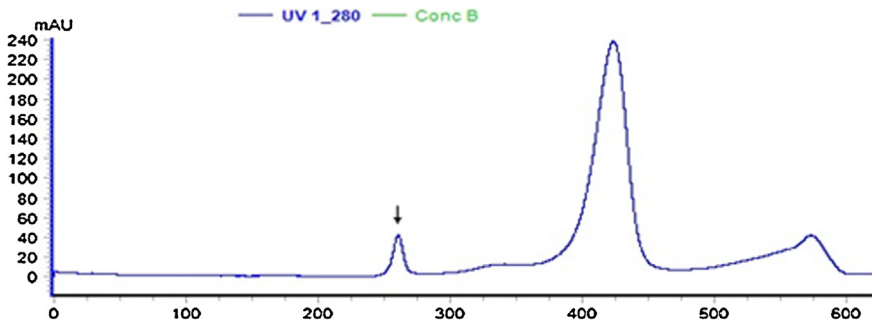

Fig. 1. Purification of 1 ml PRRSV on a C 26/100 column packed with Seoharose 4 Fast Flow. The PRRSV containing peak indicated by arrow.

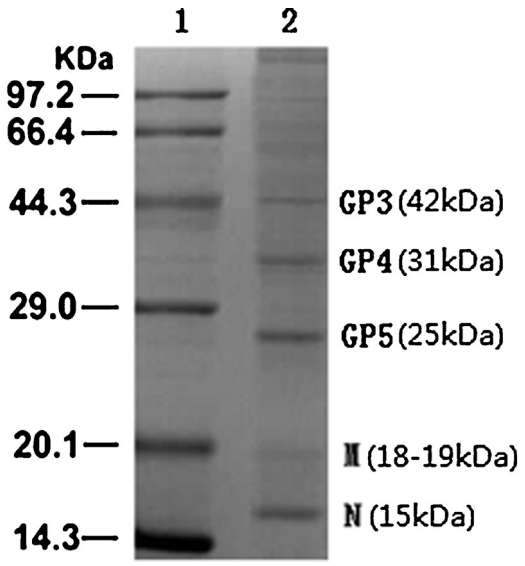

Fig 2. SDS–PAGE analyses for fractions from size-exclusion chromatography elution peak 1. Lane 1, Takara low molecular weight marker. Lane 2, fraction from elutin peak 1.

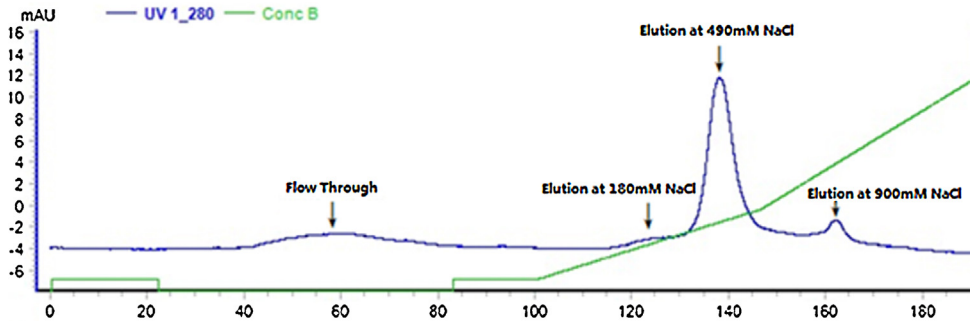

Fig. 3. Linear gradient elution profile of PRRSV in Q Sepharose High Performance column. Loosely bound proteins were eluted in the first peak using 180 mM NaCl. Pure viral particles were eluted in second peak using 490 nM Nacl. The last peak eluted using 900 mM NaCl completely removed. The PRRSV containing peak indicated by arrow.

collected into sterile containers, and further concentrated using an Amicon® ultra 100 k device at 8000*g* for about 1 h at 4 °C.

2.3. Size-exclusion chromatography

All chromatographic procedures were carried out on an ÄKTA Explorer 150 (GE Healthcare, Uppsala, Sweden) automated chromatography system controlled by the Unicorn software (version 3.10).

Then, 1 ml of concentrated PRRS virus stocks was applied to a C26/100 sepharose 4 fast flow column (GE Healthcare) equilibrated with 0.02 M Tris-HCl (pH 7.5). The column was eluted with the same buffer at a flow rate of 0.8 ml/min. The OD_{280} absorbance of eluents was monitored continuously. The fractions from the first elution peak were collected and further purified by ion exchange chromatography (IEC).

2.4. Anion exchange chromatography (AEX)

Q sepharose highperformance was packed into an XK 16/20 glass column (GE Healthcare) to a 5 ml bed volume for AEX. The column was equilibrated with 20 mM Tris-HCl (pH 7.5) that had been previously filtered and degassed. The flow rate used for all steps was 5 ml/min. The fractions from the first elution peak of size-exclusion chromatography containing 0.1 M NaCl were applied to the column, the flow-through was collected, and the column was