Journal of Chromatography B, 1049–1050 (2017) 16–23

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Purification of foot-and-mouth disease virus by heparin as ligand for certain strains

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ARTICLE INFO

Article history:
Received 10 August 2016
Received in revised form 7 December 2016
Accepted 21 December 2016
Available online 26 December 2016

Keywords:
Foot-and-mouth disease virus
Heparin affinity chromatography
Anion exchange
Virus purification

ABSTRACT

The goal of this project was to develop an easily operable and scalable process for the recovery and purification of foot-and-mouth disease virus (FMDV) from cell culture. Heparin resins HipTrap Heparin HP and AF-Heparin HC-650 were utilized to purify FMDV O/HN/CHA/93. Results showed that the purity of AF-Heparin HC-650 was ideal. Then, the O/HN/CHA/93, O/Tibet/CHA/99, Asia I/HN/06, and A/CHA/HB/2009 strains were purified by AF-Heparin HC-650. Their affinity/virus recoveries were approximately 51.2%/45.8%, 71.5%/70.9%, 96.4%/73.5, and 59.5%/42.1%, respectively. During a stepwise elution strategy, the viral particles were mainly eluted at 300 mM ionic strength peaks. The heparin affinity chromatography process removed more than 94% of cellular and medium proteins. Anion exchange resin Capto Q captured four FMD virus particles; 40% of binding proteins and 80%–90% of viral particles were eluted at 450 mM NaCl. Moreover, ionic strength varied from 30 to 450 mM had no effect on the immunity to FMDV. The results revealed that heparin sulfate may be the main receptor for CHA/99 strain attachment-susceptible cells. Heparin affinity chromatography can reach perfect results, especially when used as a ligand of the virus. Anion exchange is useful only as previous step for further purification.

1. Introduction

Foot-and-mouth disease (FMD) is a political and economic concern. It is caused by the foot-and-mouth disease virus (FMDV) and affects the husbandry development of the world [1]. At present, many developed countries, such as Australia and England, have eradicated FMD. However, FMD is still a serious epidemic in many developing countries, especially in Africa and Asia; the immunization of susceptible animals through a vaccine has been the main method of these countries to control FMD [2]. For the past few years, the quality of FMD vaccines made in China has obviously improved. The technique of cell suspension cultivation was widely applied to scalable inactivated vaccine production; concentrating and purifying the FMDV is next to be resolved in scalable vaccine production. Ultrafiltration and deposition by PEG are the most common methods utilized to concentrate and purify FMDV in scalable vaccine production. However, both have some drawbacks [3]. The superiority of ultrafiltration lies in its very high virus recovery. In general, ultrafiltration can reach 90% virus recovery. However, the efficiency of wipping off nonviral protein was only about 40%. On the contrary, concentrated and purified FMDV with PEG can dislodge 90% of the nonviral proteins. However, the purification process was very complicated and costly in terms of time. At least 2 days were spent finishing the purity procedure. In addition, purification results were not very stable.

FMDV enters cells via the mechanism of receptor-mediated endocytosis [4]. Many studies have reported that heparan sulfate and four integrin proteins, namely, αnβ1, ανβ3, ανβ6, and ανβ8, were the primary receptors of FMDV. Different serotype FMDV strains could utilize different receptors infecting target cells; and the role of integrin in mediating infection is more extensive than heparan sulfate, especially integrin ανβ6 [4–7]. Although heparan sulfate was not necessary for many FMDV strains during the process of infecting animal cells, reports have indicated that O1BFS strains attach and internalize into cells and multiply passages through cultured cell lines only by heparan sulfate [8]. Given that some FMDV strains bind to heparan sulfate in the course of infection, purifying FMDV using a heparin affinity column should be possible. A researcher from Argentina utilized heparin-Ultrogel A_4R concentration to purify type O FMDV; this method resulted in

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http://dx.doi.org/10.1016/j.jchromb.2016.12.033
1570-0232/

90% intact viral particles recovery [3]. In addition, heparin was also utilized to purify other viruses. For instance, Hu et al. purified porcine reproductive and respiratory syndrome virus (PRRSV) [9], and Einarsson et al. utilized Heparin-Sepharose and sulfonated derivates of polysaccharides to purify hepatitis B surface antigen (HBsAg) [10,11].

O/HN/CHA/93, O/Tibet/CHA/99, Asia I/HN/06, and A/CHA/HB/2009 are four virus strains in China. No reports have been made on whether they can utilize heparin sulfate as a cell receptor to infect animal cells. In this paper, we utilized heparin as a ligand to concentrate and purify these four vaccine strains. The study's goal is to develop an easily operable and scalable process for the recovery and purification of FMDV from cell culture; the study analyzed the interaction between strains and ligand and also compared the purification results between affinity chromatography and anion exchange.

2. Materials and methods

2.1. FMDV strains

FMDV strains O/HN/CHA/93, O/Tibet/CHA/99, Asia I/HN/06, and A/CHA/HB/2009 were obtained from the National FMD Reference Laboratory of China in LVRI of CAAS; four strains were separately propagated in the BHK-21 cell line by suspension culture method in fermentation tanks. The culture medium is MEM and fed-batch cultivation substrate, which was developed by the authors. The virus suspension was collected after the cytopathic effect appeared. The virus was subsequently inactivated by BEI at 30 °C for an appropriate number of hours; the efficacy of inactivity was then detected. The virus was stored in suspension at 4 °C until it was used. All experiments were performed in a grade III biology safety laboratory.

2.2. Chromatography resins and column

Two heparin affinity chromatography resins, namely, HipTrap Heparin HP (GE Healthcare Science), Toyopearl AF-Heparin HC-650 (Tosoh, Japan), were evaluated to purify FMDV. Anion exchange resin Capto Q (GE Healthcare Science) was also utilized to purify four FMDV vaccine strains (with a column volume of 5 ml). Tricorn 5/20 column (GE Healthcare Science) was packed with various resins to a final of 2 ml bed volume. Virus purification experiments were performed at 18 °C using an AKTATM purifier 100 system (GE Healthcare, Uppsala, Sweden). Fractions (2 ml) were collected using an auto fraction collector frac-900 (GE Health Sciences).

2.3. Clarification and ultrafiltration

The virus suspension containing cell lysate was clarified by continuous flow centrifuge at 8000 rpm/min at 4 °C. Then, clarified virus suspensions were filtered through a 0.22 um nealon membrane filtration sterilization and subpackaged into sterile containers. Next, an ultrafiltration system (Merck Milpore, Germany) was used to concentrate the four FMD antigens five times; the nominal molecular weight limit (NMWL) of the ultrafiltration membrane was 300 KD. During the process of ultrafiltration, the pressure of the inlet and backflow was constantly maintained under 0.9 Mpa and 0.4 Mpa, respectively. Then, five folds of equilibrium buffer (0.01 M NaCl, 20 mM Tris-HCl, pH 7.6) were added to the concentrated solution to exchange buffer and desalt. The desalinated virus supernatant was filtered through a 0.22 um filter and kept at 4 °C until used.

2.4. Heparin affinity chromatography

HipTrap Heparin HP and Toyopearl AF-Heparin HC-650 resins were very adaptive to purified FMDV. To evaluate these resins, a comparative study was performed using O/HN/CHA/93 strains. After screening out the adaptive resins, which were then utilized to concentrate and purify other FMDV vaccine strains. A description of the particular chromatography process follows. A column with 10 column volumes of binding buffer (0.1 M NaCl, 20 mM Tris-HCl, pH 7.6) were pre-equilibrated; five column volumes of desalted samples were loaded onto the chromatography column. The interaction between heparin resins and FMDV lasted for 5 min. Elution was achieved using a stepwise elution strategy at a linear rate of 95 cm/h, followed by a wash step at 0.1 M NaCl, 20 mM Tris-HCl (pH 7.6) and three virus elution steps at 300 mM NaCl, 500 mM NaCl, and 1000 mM NaCl in 20 mM Tris-HCl (pH 7.6) in sequence. Flowthrough fractions and eluted fractions were determined; the concentration of FMDV 146 s was quantified using enzyme-linked immunosorbent assay (ELISA) and sucrose density gradient centrifugation.

2.5. Anion exchange chromatography

Anion exchange resin Capto Q was utilized to concentrate and purify FMDV. The loaded volume was 10 column volumes, the retention time was 5 min, and the equilibrated buffer was 0.01 M NaCl and 20 mM Tris-HCl (pH 8.0). Elution was achieved using a stage elution strategy at a linear rate of 180 cm/h followed by a wash step with 0.01 M NaCl and 20 mM Tris-HCl (pH 8.0). Then, three virus elution steps were performed at 100 mM NaCl, 450 mM NaCl, and 1000 mM NaCl in 20 mM Tris-HCl (pH 8.0) in sequence. The column was regenerated with 0.5 N sodium hydroxide at a linear rate of 100 cm/h. Flowthrough fractions and eluted fractions were collected to purify and identify the concentrations.

2.6. Total protein assays

Total protein concentration was determined by bicinchoninic acid (BCA) protein assay using a Piece BCA kit (Thermoscientific) according to the manufacturer's instructions; bovine serum albumin was utilized as the standard, and the detection range was 5–250 ug/ml. Before the experiment, all samples were diluted 10 times by a buffer solution.

2.7. Purification results assess

Samples from all methods were analyzed by SDS-PAGE electrophoresis to assess the obtained purification; electrophoresis was performed in 15% polyacrylamide gels containing SDS. These gels were then stained by Coomassie brilliant blue. In addition, elution peak1 and the elution of CHA/93 purified by heparin and anion exchange chromatography separately were also analyzed by Western blot.

2.8. Quantitative analysis of intact virus particle

The concentration of intact virus particles was detected for FMDV 146 s by enzyme-linked immunosorbent assay (ELISA) and sucrose density gradient centrifugation. ELISA methods were developed by the Lanzhou Veterinary Research Institute. Sucrose density gradient centrifugation was performed according to previous reports, but with improved methods. Briefly, 1.0 ml FMDV supernatant was loaded on the top of 15%–45% (w/w) sucrose gradients. All samples were centrifuged at 8000 rpm for 5 min to remove debris before ultracentrifugation; a quantitative sample was used