Protein Expression and Purification 113 (2015) 23–29

Contents lists available at ScienceDirect

Protein Expression and Purification

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

A hydrophobic interaction chromatography strategy for purification of inactivated foot-and-mouth disease virus

Hao Li [a,b,1], Yanli Yang [a,b,1], Yan Zhang [a], Songping Zhang [a], Qizu Zhao [c], Yuanyuan Zhu [c], Xingqi Zou [c], Mengran Yu [a,b], Guanghui Ma [a], Zhiguo Su [a,*]

[a] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] China Institute of Veterinary Drug Control, Beijing 100081, PR China

ARTICLE INFO

Article history:
Received 8 April 2015
and in revised form 28 April 2015
Available online 6 May 2015

Keywords:
Foot-and-mouth disease virus
Hydrophobic interaction chromatography
Purification
Downstream processing

ABSTRACT

A purification scheme based on hydrophobic interaction chromatography was developed to separate inactivated foot-and-mouth disease virus (FMDV) from crude supernatant. About 92% recovery and 8.8-fold purification were achieved on Butyl Sepharose 4FF. Further purification on Superdex 200 resulted in another 29-fold purification, with 92% recovery. The columns were coupled through an intermediate ultrafiltration unit to concentrate the virus. The entire process was completed in about 3.5 h, with 75% final FMDV recovery, and 247-fold purification. The final product had purity above 98%, with over 99.5% of host cell DNA removed. High-performance size exclusion chromatography (HPSEC), Western blot, dynamic light scattering (DLS), and transmission electron microscopy (TEM) indicated that the purified virus contained the required antigen, and was structurally intact with a spherical shape and a particle size of 28 nm.

Introduction

Foot-and-mouth disease is an acute and contagious disease of cloven-hoofed livestock such as pig, sheep, and cattle [1]. Recently, widespread breakouts were reported in the United Kingdom, Argentina, and Uruguay, with serious consequences on agriculture and international trade [2–4]. New vaccines, for example, the self-assembling capsid protein of the foot-and-mouth disease virus (FMDV)[2], have been developed [5–7]. Nevertheless, vaccines prepared from inactivated FMDV remains the most effective [2].

FMDV is a spherical particle consisting of one molecule of single-stranded positive-sense RNA and a protein capsid [8]. The capsid contains four structural proteins: VP1, VP2, and VP3 with molecular weights between 25 and 34 kDa, and VP4 of 8–10 kDa [8]. Typically, FMDV is produced in baby hamster kidney cells, separated from cell debris, and then inactivated [9,10]. It remains quite common to formulate the vaccine from this relatively crude inactivated antigen [9]. However, as the public has become increasingly concerned with the safety and quality of livestock products, regulatory agencies are considering raising the quality standard for FMDV vaccines. Therefore, more stringent purification of inactivated FMDV may become necessary to remove host cell proteins and DNA, which may cause unwanted side effects [11].

FMDV has been purified by PEG precipitation [10,12], sodium sulfate precipitation [13], ultrafiltration [13,14], and aqueous two-phase partition [13]. These methods are useful, but not adequate to achieve high purity [15]. High purity has been achieved in the laboratory by ultracentrifugation in a sucrose density gradient [2,12]. However, ultracentrifugation is unsuitable for industrial-scale production, because it suffers from low capacity as well as high capital and operational cost.

Chromatographic techniques may provide an alternative strategy, on the basis of high selectivity and scalability [16]. Indeed, many vaccines, including influenza virus and recombinant hepatitis B surface antigen (HBsAg), have been successfully produced through chromatography [17,18]. In addition, size exclusion chromatography (SEC) was recently used as an analytical tool to quantify inactivated FMDV [2]. Nevertheless, we are not aware of any report that describes a complete, effective program to purify inactivated FMDV by chromatography.

* Corresponding author. Tel.: +86 10 62561817.
E-mail address: zgsu@ipe.ac.cn (Z. Su).

[1] Both authors contributed equally to this work.

[2] *Abbreviations used:* FMDV, foot-and-mouth disease virus; IEC, ion-exchange chromatography; HIC, hydrophobic interaction chromatography; SEC, size exclusion chromatography; HPSEC, high-performance size exclusion chromatography; TEM, Transmission electron microscopy; DLS, dynamic light scattering; HBsAg, hepatitis B surface antigen; DRT, dimensionless residence time.

http://dx.doi.org/10.1016/j.pep.2015.04.011
1046-5928/

A classic chromatographic technique is ion-exchange chromatography (IEC), which is often used to purify antigens such viruses [19,20] and virus-like particles [21,22]. However, interaction with IEC media may sometimes alter the structure of purification targets, with disastrous results. For example, HBsAg disassembles during IEC, resulting in substantial loss [23,24]. For some viruses like FMDV, structural integrity is critical. The virus, with sedimentation coefficient 146S when live, is highly unstable and tends to dissociate into smaller particles with sedimentation coefficient 12S [25]. The immunogenicity of 12S particles is extremely low and unprotective [26]. What is worse, the virus was found more unstable after being inactivated, even though the inactivating agents like binary ethyleneimine were thought to only alter the viral RNA without effects on the capsid proteins [27]. Therefore, any chromatographic separation should preserve the intact FMDV structure.

In this paper, we describe our efforts to purify inactivated FMDV through chromatography. We initially attempted to use IEC to purify FMDV from cell culture supernatant. However, it was difficult to achieve both satisfactory recovery and purification fold even after screening of IEC media and separation conditions. In addition, desalting by dialysis or dilution was typically required as an extra preliminary step to reduce the salt concentration in the initial crude.

Thus, we evaluated hydrophobic interaction chromatography (HIC) as an alternative, which separates molecules based on hydrophobicity instead of charge. The technique has never been used to purify FMDV; indeed, HIC is far less frequently used than IEC in vaccine preparation. Nevertheless, recovery of over 98% was achieved when HBsAg was purified by HIC, more than twice the recovery from IEC [28]. In addition, HIC may be more appropriate than IEC for inactivated viruses, the structure of which may be sensitive to ionic interactions with IEC media.

The final purification scheme combines HIC with ultrafiltration and SEC. The scheme was optimized by screening various chromatographic media and separation conditions. High-performance size exclusion chromatography (HPSEC), Western blotting, dynamic light scattering (DLS), and transmission electron microscopy (TEM) were used to evaluate the purified product.

Materials and methods

Materials

All reagents were analytical grade, and solutions were prepared using Milli-Q water (Millipore, USA). Separations were executed on an ÄKTA Purifier 100 from GE Healthcare (USA). HIC, IEC, and SEC media were obtained from GE Healthcare (Uppsala, Sweden). For HIC, we evaluated Butyl-S Sepharose 6FF, Butyl Sepharose 4FF, Octyl Sepharose 4FF and Phenyl Sepharose 6FF (high sub). DEAE-Sepharose FF and ANX Sepharose 4FF (high sub) were evaluated for ion-exchange. SEC was performed on chromatography media with different separation range, including Sepharose 4FF, Sepharose 6FF and Superdex 200. Chromatography columns (200 × 16 mm I.D., 1000 × 26 mm I.D.) were from GE Healthcare (USA).

Virus

FMDV strain O China 1999 was propagated at industrial scale in BHK-21 cell suspension cultures and inactivated by binary ethyleneimine [2]. Cell debris was removed, and the supernatant containing virus particles was collected. The FMDV supernatant was originally obtained from Lanzhou Veterinary Research Institute (Chinese Academy of Agricultural Sciences, China) after cultivation and pretreatment as described above.

Evaluation of hydrophobic interaction chromatography media

To evaluate the suitability of hydrophobic interaction to separate FMDV, we first analyzed the media by static adsorption with increasing hydrophobicity, including Butyl-S Sepharose 6FF, Butyl Sepharose 4FF, Octyl Sepharose 4FF and Phenyl Sepharose 6FF (high sub). The effect of ionic strength was studied using 0–1 M $(NH_4)_2SO_4$. Generally, about 0.2 g of drained media, pre-equilibrated in an appropriate buffer at pH 8.0 with a defined concentration of $(NH_4)_2SO_4$, was mixed with 0.8 mL FMDV suspension that had been pre-diluted with 5.6 mL buffer. After static adsorption for 24 h at 25 °C on a shaking incubator at 170 rpm, the supernatant was analyzed for remaining FMDV and proteins.

Purification by hydrophobic interaction chromatography and ion-exchange chromatography

For chromatographic purification by HIC, $(NH_4)_2SO_4$ was added to 125 mL FMDV supernatant in amounts required to achieve a target concentration, and the resulting mixture was gently stirred until the salt dissolved. Then the supernatant was loaded onto a HIC column (50 × 16 mm I.D.) individually packed with the HIC media pre-equilibrated with phosphate buffer (pH 8.0) containing a desired concentration of $(NH_4)_2SO_4$. FMDV was eluted stepwise with decreasing $(NH_4)_2SO_4$ concentration. Fractions were collected and analyzed.

To compare purification efficiency with HIC, FMDV was also purified on IEC media, including DEAE-Sepharose FF and ANX Sepharose 4FF (high sub). Thus, 125 mL FMDV was diluted with 20 mM phosphate buffer pH 8.0 until the ionic strength dropped below 4 mS/cm. Suspensions were then passed through IEC media packed in 50 × 16 mm I.D. columns and pre-equilibrated with 20 mM phosphate buffer pH 8.0. Samples were eluted stepwise with increasing NaCl concentration. As before, fractions were collected and analyzed.

All experiments were executed on ÄKTA Purifier 100 at 2.0 mL/min. Residence time is presented as dimensionless residence time (*DRT*), which is defined as $DRT = RT/(CV/Flow\ rate)$, where *RT* is residence time and *CV* is column volume.

Concentration of HIC-purified FMDV

FMDV fractions from HIC were concentrated by pump filtration (Masterflex L/S, USA) through a PES membrane with molecular weight cut-off 100 kDa (VivaFlow50, Sartorius, Germany). Briefly, about 60 mL FMDV fractions were concentrated to about 20 mL at room temperature and 130 mL/min. The membrane was flushed between samples using 0.2 M NaOH at pressure less than 1 bar until flux returned to the initial value.

Size exclusion chromatography

Size exclusion chromatography was used to refine purification products. SEC columns with different separation ranges, namely Sepharose 4FF, Sepharose 6FF, and Superdex 200, were evaluated. Samples of 5–10 mL concentrated, HIC-purified FMDV was loaded on a column packed with one of the three media (900 × 26 mm I.D.), followed by elution with pH 8.0 phosphate buffer containing 0.15 M NaCl. The flow rate was 3.0 mL/min controlled by ÄKTA purifier 100 and the detection wavelength was set to 259 nm. Residence time was converted to dimensionless residence time as described.