Single Step Virus Purification with a New - Calcium Affinity, Cation Exchange – Mixed-Mode Media

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Abstract

CHT™ Ceramic Hydroxyapatite Media/Resins are known as the industry gold standard for aggregate removal during monoclonal antibody purification. The new CHT Media from Bio-Rad — CHT XT — can also be used for single-step purification of viruses. We performed single-step purifications of influenza and dengue viruses on CHT XT Columns. The recovery of pure viruses was quantified by hemagglutinin (HA) assay. We recovered a mean of 88% influenza and 83% dengue virus in the single-step purification. A good resolution between the pure viral peaks and the contaminant peaks was seen. This positions CHT XT as an additional media for simple, fast, and reproducible viral purification and overcomes the shortcomings of conventional purification methods.

Introduction

We have previously demonstrated mammalian virus purification with the mixed-mode CHT Ceramic Hydroxyapatite Media/Resin (Kurosawa et al. 2014). The CHT Media/Resins are a group of calcium affinity, cation exchange media. The high negative surface charge on many viruses allows them to bind tightly to CHT calcium sites, allowing for significant purification with high yields (>80%). Such tight binding has also been utilized to provide robust viral clearance, which can be used to ensure viral safety during biomanufacturing (ICH Expert Working Group 1999, Moritz 2005).

CHT XT is the newest addition to the CHT family of media. It has been designed for superior physical robustness to ensure repeated use over a large number of cycles. It maintains the unique separation properties of CHT for aggregate removal and monoclonal antibody purification. In order to test its virus purification capabilities, we performed single-step purification of influenza and dengue virus using CHT XT Media. The initial results of this ongoing study are shown below.

Materials and Methods

The viral particles used in this study are shown in Table 1.

Virus	Genome	Envelope	Size, nm
Influenza	ssRNA	+	80-120
Dengue	ssRNA	+	50

Table 1. Viral type and size

Viral production

Influenza virus: Strain A/California/7/2009 was cultured on MDCK cells in minimal essential medium (MEM; Gibco) containing 10% fetal bovine serum (FBS) and L-glutamine in 225 cm^2 flasks. When the cells were almost confluent, influenza virus was inoculated onto the cell monolayer at a 1:3,000 dilution in 75 ml of D-MEM/F-12 with trypsin, penicillin, and streptomycin. The supernatant was harvested between days 3–14 and clarified through 0.45 µm filters.

Dengue virus: Type 2 strain ThNH7/93 was cultured on C6/36 cells. Cells were grown in 225 cm^2 flasks that were precoated with poly-L-lysine at 100 µg/ml in phosphate buffered saline (PBS). Cells were cultured in Minimum Essential Medium Eagle (modified) (modified EMEM, MP Biomedicals, Irvine, CA, USA) containing 10% FBS in poly-L-lysine coated flasks at 28˚C for 1 week. After the cells reached confluence, dengue virus type 2 was inoculated at a 1:1,000 dilution onto the cell monolayer in 75 ml of modified EMEM containing 0.5% FBS and MEM Vitamin Solution (Invitrogen Corporation) and cultured at 28˚C. The medium was changed at day 3 and culture fluid (about 75 ml) was collected at day 7. The culture fluid was filtered through a 0.45 µm filter to remove cells and large cell debris.

Chromatography

CHT XT Media was packed into 4.6 x 35 mm columns (0.58 ml bed volume) and viral purification was performed as shown in Table 2. All experiments were carried out in duplicate.

Step	Mobile Phase	pH	Volume, ml
Pre-wash	1M NaOH		5
Wash	600 mM sodium phosphate	7.2	10
Equilibration	10 mM sodium phosphate	7.2	20
Sample loading and washing	10 mM sodium phosphate	7.2	20
Elution	Gradient elution from 10-600 mM sodium phosphate	7.2	15
Column wash	600 mM sodium phosphate	7.2	5

Table 2. Purification protocol.

Viral activity assay

Influenza viral activity in the recovered fractions was determined via hemagglutination analysis (HA test) with chicken red blood cells. Activity of the dengue virus in the recovered fractions was determined via HA test with goose red blood cells as described elsewhere (Kurosawa et al. 2012).

Estimating double-stranded DNA (dsDNA)

The concentrations of dsDNA in fractions were determined using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen Corporation) according to the manufacturer's instructions.

Results

The chromatographic results from the virus purification are shown in Figure 1. Influenza virus elutes at approximately 250 mM phosphate, with good separation from the bulk of UV-absorbing material and partial resolution from dsDNA (Figure 1A and 1B). Dengue virus eluted from CHT XT at approximately 400 mM phosphate, well separated from virtually all UV-absorbing material and dsDNA (Figure 1C and 1D). The viral activity recovery was over 80% for both viruses as measured using the hemagglutinin assay (HA test) (Table 3). The decrease in DNA contamination by this one step purification in dengue virus is shown in Table 4.

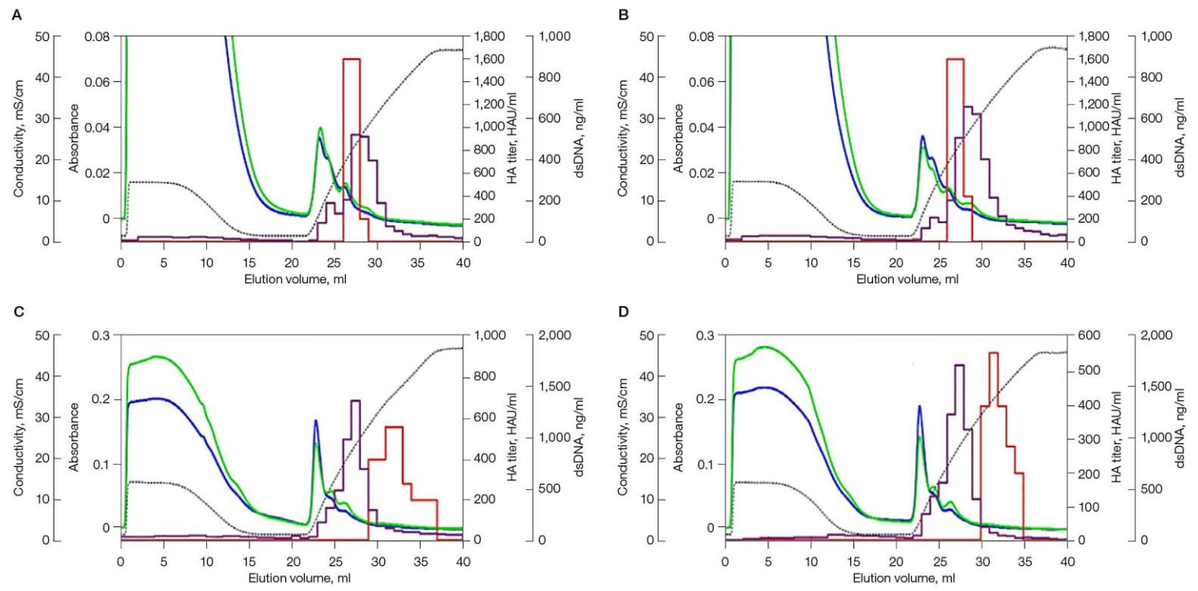

Figure 1. Elution of influenza virus (A and B) and dengue virus (C and D) from CHT XT. HA titer (red), DNA (purple), A280 (blue), A260 (green) and conductivity (black).

Virus	Exp. 1	Exp. 2	Mean
Influenza	85	90	88
Dengue	100	66	83

Table 3: Hemagglutination activity of the recovered viruses on CHT XT

Dengue Virus	Exp. 1	Exp. 2	Mean
Culture Fluid DNA Load (ng)	4934	6263	5598
Purified fraction DNA load (ng)	732	321	526

Table 4: Comparison of dsDNA contaminants in culture fluid and purified fractions of dengue virus

Conclusion

Chromatography media are continuously evolving to meet the increasing demands of the bioprocessing industry. We developed the new CHT XT Media to meet the demand for a robust media with an excellent life time for repeated use to improve process economics. With this set of virus purification studies, we show that the functionality of the new media still remains. Yields from a single-step purification of both influenza and dengue virus were above 80% as shown by the activity assay. In addition, the chromatograms demonstrated significant purification of host cell impurities from active virus. This positions CHT XT as an additional media for simple, fast, scalable, and reproducible viral purification.

References

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