Rapid Adenovirus Purification Using Q Sepharose XL

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Introduction

Because of their ability to efficiently transduce many tissues and cells, recombinant adenoviral vectors are useful in both basic research and clinical applications. Adeno and retrovirus are the two vectors most frequently used as delivery vehicles because of a high transduction yield.

Traditionally, purification of virus/viral vectors to high purities is done by cesium chloride (CsCl) density gradient centrifugation. The work presented in this poster illustrates that purification by one-step anion exchange chromatography on Q Sepharose™ XL is a very attractive alternative to gradient centrifugation for the production of adenoviral vectors. Purification of adenoviral vectors with anion exchange chromatography is also more efficient than preparative CsCl density gradient centrifugation, with respect to time and costs.

Experimental and Results

In this study we used the first generation recombinant adenovirus Ad5CMV-GFP, which contains a deletion in region E1 and a concomitant insertion in that region of an expression cassette for green fluorescent protein. A Nunc cell factory containing approximately 1×10^9 cells was infected with 1×10^{12} particles of Ad5CMV-GFP. After 60 hours of infection, maximum cytopathic effect was observed and the cells were collected by centrifugation for 20 minutes at 1 000 × g and resuspended in 10 ml of 50 mM Tris-HCl (pH 8.0) in 5% glycerol. This cell suspension was frozen and thawed three times and debris was removed by centrifugation for 30 minutes at 5 000 × g. One-half (5 ml) of the resulting supernatant was subjected to purification using CsCl equilibrium density gradient centrifugation. The other 5 ml of supernatant was purified using a 20 ml column containing Q Sepharose XL.

An ÄKTA™*explorer* 100 automated chromatography system was used for these chromatography experiments. Elution was performed with a linear gradient from 0 to 1.0 M NaCl in 50 mM Tris/HCl, pH 8.0, in 5% glycerol, for 20 column volumes (CV). The virus, identified by its characteristic A260/280 ratio and confirmed by infection followed by fluorescence microscopy, was eluted in three 5 ml fractions that were subsequently pooled. The column was cleaned with four CV of 0.1 M NaOH between each run.

The chromatogram obtained is shown in Figure 1, and Figure 2 shows part of this chromatogram magnified.

The total yield of the virus purified by CsCl was 4.5×10^{12} viral particles while the total yield of the virus purified using Q Sepharose XL was 6.7×10^{12} viral particles. Each purified virus was titered by plaque assay on 293 cells and the total plaque forming units (PFU) determined. The CsCl banded virus had a particle/PFU ratio of 73 while the virus purified using Q Sepharose XL had a particle/PFU ratio of 40. Results are summarized in Table I.

Table I. Comparison of results, total yield, and particle/PFU ratio, obtained with preparative gradient centrifugation (CsCl) and chromatography on Q Sepharose XL (QXL).

	Yield	Particle/PFU
CsCl	4.5×10^{12}	73
QXL	6.7×10^{12}	40

Fractions were collected from the Q Sepharose XL column (Fig 1 and Fig 2) and analyzed by SDS-PAGE (Fig 3).

The influence of surface modification of the adenoviral vector was investigated by comparing a wild type adenoviral vector with a modified vector. The modification was done in protein (IV), e.g. the fiber protein. Samples of these two vectors (which had been purified by CsCl centrifugation) were chromatographed on columns packed with Q Sepharose XL. Columns used: HR 5/10, volume 2 ml (0.5 × 10 cm) and system used: ÄKTA*explorer* 10. Conditions as described in legends for Figures 3 and 4.

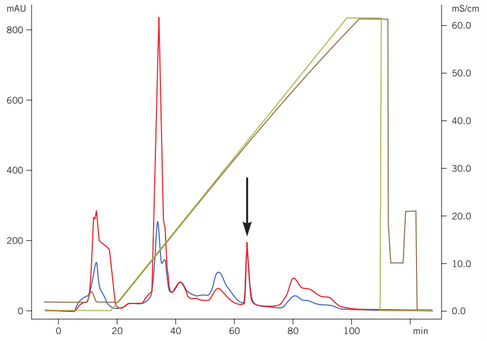

Fig 1. Purification of an adenoviral vector on Q Sepharose XL. Adenoviral vector containing peak indicated by arrow.

Sample volume and type: 5 ml of virus preparation
Column: HiPrep™ 16/10 Q XL
Eluent A: 50 mM Tris/HCl, pH 8.0 in 5% glycerol
Eluent B: A + 1.0 M NaCl in 5% glycerol
Gradient: 0–100% B in 20 CV
Flow: 150 cm/h (except for sample application, when flow was 30 cm/h)

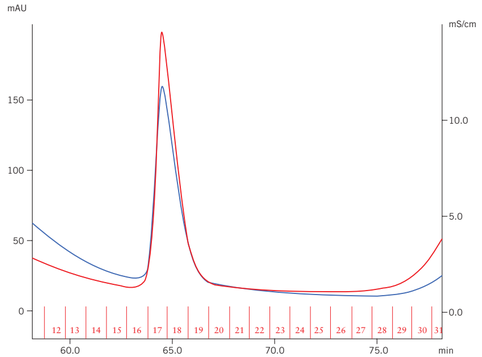

Fig 2. Magnification of part of chromatogram shown in Figure 1.

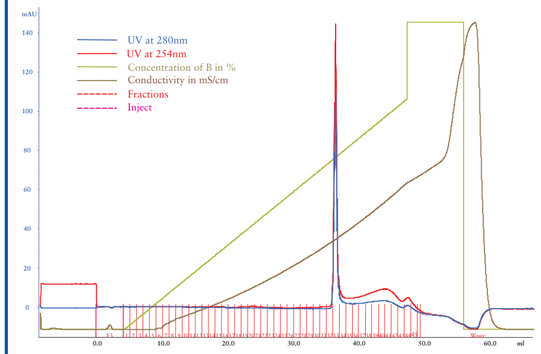

Fig 4. Adenoviral vector on Q Sepharose XL.

Sample volume and type: 0.5 ml of a 1:5 dilution (with buffer A) of a sample of wild type adenoviral vector containing 5×10^{12} virus particles
Column: Q Sepharose XL packed in a 0.5 × 10 cm column
Eluent A: 50 mM Tris/HCl, pH 8.0 in 5% glycerol
Eluent B: A + 1.0 M NaCl in 5% glycerol
Gradient: 0–75% B in 20 CV, followed by step to 100% B
Flow: 75 cm/h

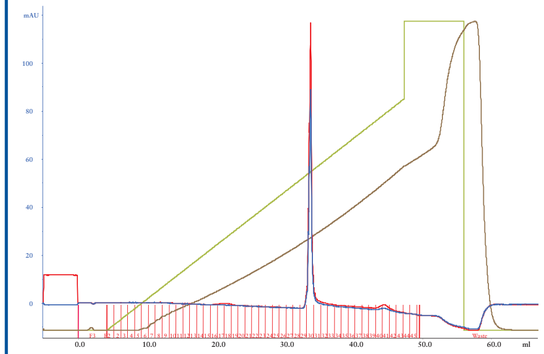

Fig 5. Adenoviral vector on Q Sepharose XL.

Sample volume and type: 0.5 ml of a 1:5 dilution (with buffer A) of a sample of a modified adenoviral vector containing 5×10^{12} virus particles
Column: Q Sepharose XL packed in a 0.5 × 10 cm column
Eluent A: 50 mM Tris/HCl, pH 8.0 in 5% glycerol
Eluent B: A + 1.0 M NaCl in 5% glycerol
Gradient: 0–75% B in 20 CV, followed by step to 100% B
Flow: 75 cm/h

Discussion

Because of the large size of the adenoviral vector (approximate diameter 80 nm), only the surface of the chromatography medium is utilized for binding.

The purity of the adenoviral vector purified by chromatography corresponds to the purity obtained with preparative CsCl density gradient centrifugation. However, the yield of vector is well above that achieved with centrifugation.

From the SDS-PAGE (Fig 3), it can be seen that the results are very similar between the purification made by CsCl density gradient centrifugation (Lane 2) and by Q Sepharose XL chromatography, although not identical. The protein pattern obtained after the chromatographic purification is almost identical to that described in reference 1.

As can be seen from Figures 3 and 4, both the wild type and the modified adenoviral vectors elute in sharp, narrow peaks, and at the same ionic strength (40 mS/cm). This indicates that the purification method described works for both the "wild type" adenoviral vector, as well as, a modified vector.

Chromatographic purification of adenoviral vectors is a very attractive alternative to preparative CsCl density gradient centrifugation. It is easily scaled-up to industrial production scale and at a lower cost compared with centrifugation. The medium used in this study, Q Sepharose XL, is developed specifically for process chromatography of therapeutics. Chromatographic purification, which takes a few hours is also considerably faster than CsCl density centrifugation, which takes up to 24 hours to complete. Chromatographic purification thus allows purification of more vectors per unit time. Furthermore, only relatively inexpensive buffers and salts are used in chromatography, and removal of CsCl from the vector is not an issue as it is for adenoviral vectors purified by centrifugation.

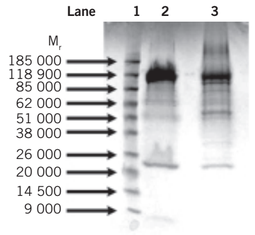

Fig 3. SDS-PAGE (4–20%) of collected material from Figures 1and 2. Gel run in a Tris/Glycine buffer system and stained by Coomassie Blue.
Lane 1, molecular weight standards; **Lane 2**, adenoviral vector purified by CsCl density gradient centrifugation; **Lane 3**, adenoviral vector purified by chromatography on Q Sepharose XL (Fig 1).

References
[1]. F. Blanche, B. *et al.*, *Gene Therapy* 7, 1055–1062 (2000).

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