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Purification of foamy viral particles

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ABSTRACT

Foamy viruses are non-pathogenic retroviruses and represent a tool for vector development. For gene therapy applications and for analyses of viral protein composition infectious particles need to be purified, which has been difficult for foamy viruses in the past. Here, we describe a novel, simple, and fast purification method for prototype foamy viruses with high purity using size exclusion and affinity chromatography. More than 99,9% of the contaminating proteins were removed. The purified viruses were used to determine the amount of the incorporated Pol protein relative to Gag. The determined Gag to Pol PR-RT ratio of 30:1 confirmed previous studies suggesting FV virions encapsidate fewer number of Pol molecules than orthoretroviruses.

1. Introduction

Foamy virus (FV) replication differs in several aspects from the orthoretroviral life-cycles (Bodem, 2011; Hamann and Lindemann, 2016; Hütter et al., 2013b; Linial, 2007; Löchelt, 2003; Rethwilm and Lindemann, 2013). For instance, the foamy viral Pol protein is translated from a spliced transcript and not as Gag-Pol precursor protein (Bodem et al., 1996; Löchelt and Flügel, 1996; Yu et al., 1996). Thus, Pol is not packaged as Gag-Pol precursor but encapsidated via an interaction with the genomic RNA (for review see Lee et al. (2013), Rethwilm (2013), and Rethwilm and Lindemann (2013)). On the other hand, evidence has been presented that interactions of Gag and Pol are required for genome and/or Pol incorporation (Lee and Linial, 2008).

Gene therapeutic vector systems derived from FV are considered to have low risks of adverse side effects, since FV related diseases are unknown in humans or other mammals (for review see Lindemann and Rethwilm (2011), Rethwilm (2007), Rethwilm and Lindemann (2013)). Foamy viral vector systems provide a promising alternative to MoMLV or lentiviral vector systems, since the parental viruses seem to be non-pathogenic (reviewed in Lindemann and Rethwilm (2011)). In addition, foamy viral vectors were shown to efficiently transduce hematopoietic stem cells (Cai et al., 2008; Morianos et al., 2012; Trobridge et al., 2006; Zhang et al., 2010), mesenchymal stromal cells (Sweeney at al., 2016) and neural progenitor cells (Rothenaigner et al., 2009). Cell toxicity was neither observed in cell cultures nor in animal models (Armbruster et al., 2014; Bauer et al., 2008, 2013; Burtner et al., 2014; Lindemann and Rethwilm, 2011; Olszko and Trobridge, 2013). Furthermore, the generation of sufficient amounts of viral particles at high titers (~1×10^7 to 1×10^8 infectious particles per millilitre) is due to a recently developed expression-optimized packaging constructs relatively easy to achieve (Lindemann and Rethwilm, 2011). However, purification of foamy viral particles, compared to the Human Immunodeficiency Virus type 1 (HIV-1), has been extremely difficult due to the cytopathic effect and subsequent release of cellular proteins and vesicles, which led to impure viral fractions.

The adaptation of HIV-1 gradient centrifugation protocols led to impure viruses and low yield (unpublished observation). Furthermore, concentration of FV particles by ultra-centrifugation resulted in losses of viral infectivity of up to 40% (unpublished observation). In addition, especially gene therapeutic applications need pure recombinant foamy viruses, free of cellular and cell culture contaminants.

Here, we describe a chromatographic purification of recombinant prototype FV (PFV), which is less time consuming, leads to purer particles and was a prerequisite to analyse wild-type PFV particles in greater detail and to determine the ratios of Gag, and Pol.

2. Results

2.1. Foamy virus purification by size exclusion chromatography

Purification of FVs has been difficult due to the pronounced cytotoxic effect, which leads to impure fractions even after iodixanol gradient centrifugation. To obtain enriched and partially pure recombinant PFV particles, a fast protein liquid chromatography based purification scheme was developed (Fig. 1). Recombinant FV particles were produced by transfecting HEK 293T cells with the expression-

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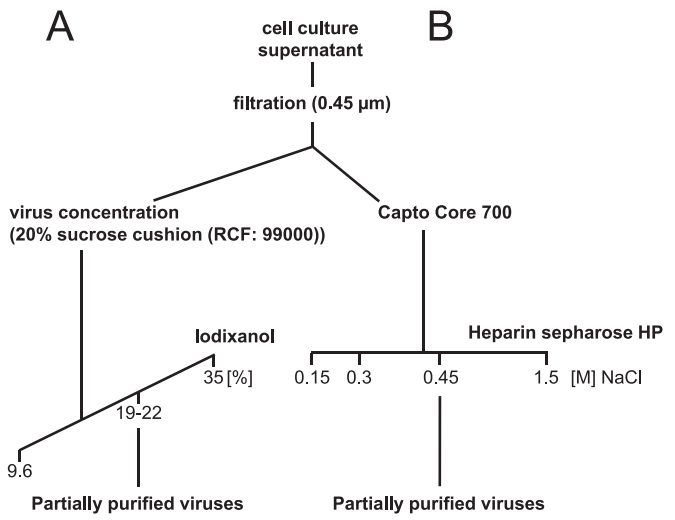

Fig. 1. Scheme of foamy virus purification. (A) Conventional purification scheme using a two-step centrifugation method. **(B)** Novel virus purification scheme using chromatography purification: HiScreen Capto Core 700 column and HiTrap Heparin sepharose HP column. NaCl concentrations are indicated.

optimized packaging system (VS) consisting of *gag*, *pol* and *env* expression plasmids and the viral vector pMD9. The latter contains an internal promoter facilitating eGFP expression. Viral supernatants were collected 3 days post-transfection and filtered (0.45 µm) to remove detached cells. After filtration an initial total protein concentration of 2900 µg/ml was measured by Bradford assay (Table 1). Part of the supernatant was stored on ice and analysed for viral infectivity on BHK21 cells. To visualize viral particles by Western blotting 20% of the initial volume was concentrated by centrifugation and analysed as input control (Fig. 2). Due to the high protein concentration, the sample had to be diluted 1:100 before the purity of the fraction could be determined by silver-stained SDS PAGE (Fig. 2B). PFV Gag, Pol and Env proteins were detected in the undiluted fraction (1×10^6 virus particles) by Western blotting (Fig. 2A). Analysis of a cellular lysate of cells transfected with the VS served as positive control and of non-transfected cells as negative control. GAPDH were determined as loading control.

Next, particles and proteins smaller than 700 kDa were removed from the supernatant by size exclusion chromatography with a HiScreen Capto Core 700 column (Fig. 1). Again viral titers and purity were determined (Fig. 2C and Table 1). The flow-through fractions showed only a non-significant reduction of viral titer (Fig. 2C and Table 1). In contrast, their total protein concentration was reduced to 26 µg/ml. Thus, approximately 99% of the contaminating proteins were removed by this purification step, whereas viral titers were reduced by less than factor 2 (Table 1 and Fig. 2C). The amount of infectious viral particles compared to the total protein content of the fractions was increased by 53fold from the input fraction with 1.2×10^3 [1/µg] to 6.4×10^4 [1/µg] underlining the significant removal of

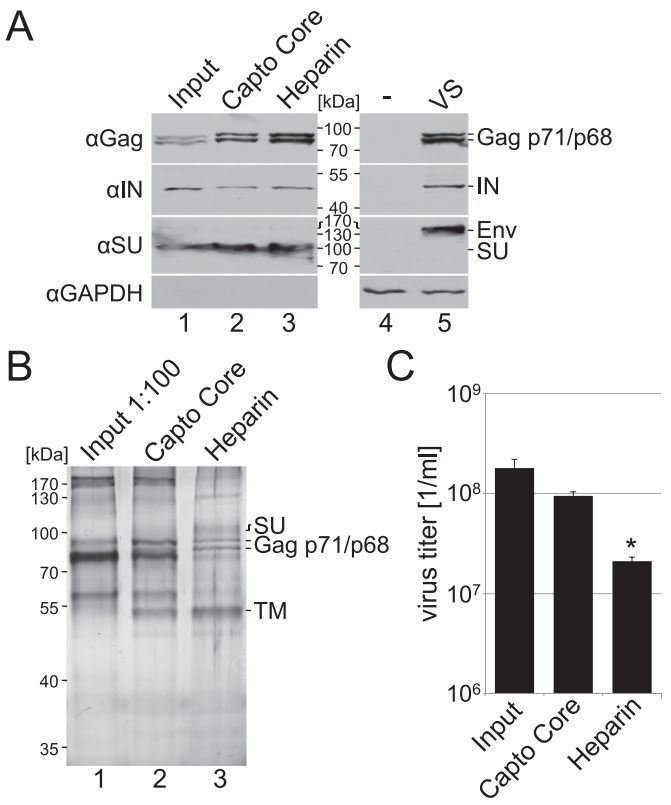

Fig. 2. Foamy virus purification by chromatography. Viruses were purified with a HiScreen Capto Core 700 column followed by a HiTrap Heparin sepharose HP column. **(A)** Western blotting analyses of the purification steps and the lysates of the transfected cells with anti-PFV Gag and anti-PFV SU antibodies and anti-PFV IN antiserum. Amounts of the virus fractions were normalized on viral infectivity (1×10^6). Positions of the size marker proteins are indicated. **(B)** Purity of the fractions were analysed by silver staining. Protein amounts were normalized on viral infectivity (1×10^6) except lane 1 (1:100 diluted input sample due to high total protein content). Positions of the Gag p71/p68, TM and SU proteins as well as the size marker proteins are indicated. **(C)** Viral infectivity was determined by titrating the supernatant and column fractions on BHK21 indicator cells. GFP positive cells were counted. Mean viral titers (n=3) were calculated and normalized on the input volume. Error bars represent standard deviation. The statistical significant reduction of infectivity compared to the input fractions, as determined by the statistical *t*-test, are indicated by asterisk (p-value < 0.05).

contaminating proteins. To analyse the purity and viral protein amounts equivalent to input fractions of the HiScreen Capto Core 700 flow-through fraction were concentrated by centrifugation as described above (25% of Capto Core flow-through corresponds to 20% of the initial supernatant). Viral proteins were quantified by Western blotting analyses and silver-stained PAGE was used to display purity of the fractions (equivalent to 1×10^6 viral particles) (Fig. 2A and B). This purification by size exclusion chromatography represents a fast and easy to use system, which could surely be adapted for other virus purification protocols as well.

Table 1
Purification of foamy viruses.

Columns	Volume [ml]	Protein conc. [µg/ml]	Total protein content of the faction [mg]	Purification[a] [%]	Infectivity [1/ml]	Total infectivity*[b]	Infectivity/protein [1/µg]
Input	50	2900	145	–	$3.6\times10^6 \pm 8\times10^5$	$1.8\times10^8 \pm 4\times10^7$	1.2×10^3
Capto Core 700	60	26	1.5	99	$1.2\times10^6 \pm 1\times10^5$	$9.5\times10^7 \pm 9\times10^6$	6.3×10^4
Heparin Seph. HP	5	0.48	0.0024	99.8	$2.5\times10^6 \pm 2\times10^5$	$2.1\times10^7 \pm 2\times10^6$	8.7×10^5
Total	–	–		99.9[c]	–	–	

[a] The purity of the fractions was defined as the reduction of protein content compared to the previous purification step.
[b] Total infectivity of the complete fraction compensated for the volume taken for centrifugations.
[c] Total purification was defined as the reduction of the protein content compared to the input fraction.