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Purification of recombinant trichodysplasia spinulosa–associated polyomavirus VP1-derived virus-like particles using chromatographic techniques

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ABSTRACT

Trichodysplasia spinulosa-associated polyomavirus (TSPyV) has been linked to a rare and recently characterized skin disease occurring in immunocompromised patients. In analogy with other polyomaviruses, the major capsid protein VP1 of TSPyV can self-assemble into virus-like particles (VLPs). VLPs are increasingly applied for the vaccination and diagnostics. Mostly, non-scalable and labor intensive ultracentrifugation-based techniques are used for the purification of VLPs. In this work, we developed a purification procedure for TSPyV VP1 VLPs based on two chromatographic steps, ion-exchange monolith and core bead chromatography. Prior to chromatography, ammonium sulfate precipitation was used for the initial purification of TSPyV VP1 VLPs from yeast lysate. The VLPs were further purified using CIMmultus QA ion-exchange monolith in bind-elute mode. Most of TSPyV VP1 VLPs bound to the monolith and were subsequently eluted by a linear NaCl gradient. After ion-exchange monolith chromatography, the purity of TSPyV VP1 protein was about 75%. The final purification step of TSPyV VP1 VLPs was core bead chromatography using Capto Core 700 resin in flow-through mode. After core bead chromatography, 42% of TSPyV VP1 protein was recovered with a purity of 93%. The assembly of purified TSPyV VP1 protein into VLPs approximately 45–50 nm in diameter was confirmed by electron microscopy analysis. The purification procedure for TSPyV VP1 VLPs described here could be a scalable alternative to the conventional ultracentrifugation-based purification methods.

1. Introduction

Polyomaviruses (PyVs) are non-enveloped, double-stranded DNA viruses of ~45 nm diameter with a small genome of ~5.2 kb [1,2]. Fourteen human polyomaviruses (HPyVs) have been identified to date [3,4]. HPyV infections are prevalent in the general population with rates ranging from 35% to 90% [2]. HPyV generally persist without clinical symptoms, but some HPyV types can cause serious disease in patients with impaired immune functions. One such disease is trichodysplasia spinulosa, characterized by development of papules, spines and alopecia in the face [5].

The trichodysplasia spinulosa-associated polyomavirus (TSPyV) was identified in 2010 [6]. In analogy with other PyVs, the late region of the TSPyV genome encodes three structural proteins VP1, VP2 and VP3 that form the viral capsid [1,2]. The capsid is composed of 72 pentamers of the major structural protein VP1 [1,7]. The pentamer incorporates a single molecule of VP2 or VP3 protein, which occupies the interior of the capsid. The major capsid protein VP1 alone can self-assemble to form virus-like particles (VLPs), which mimic the morphology of the virus but are devoid of genetic material [8,9].

Virus-like particles are increasingly used for biomedical applications such as vaccination, gene delivery and diagnostics to detect antibodies against the virus in serum [10,11]. Several VLP vaccines are available on the market, such as Gardasil® [12] and Cervarix® [13] against human papillomavirus. VLP-based vaccines are safer in comparison to the inactivated or live attenuated virus vaccines since they are devoid of infectious genetic material [10,11].

Yeast and baculovirus expression systems are commonly used for the production of PyV VP1 VLPs [10]. In these systems, the major capsid protein VP1 self-assemble to form VLPs [14–17]. The yeast *Saccharomyces cerevisiae* expression system was successfully used for the generation of VP1-derived VLPs from human, monkey SV40, murine and avian PyVs, including TSPyV [16,17].

The purification and characterization of VLPs is largely based on experience that has been obtained in experiments with various viruses [18]. The precipitation with ammonium sulfate or polyethylene glycol is often used as a first purification step [18–20]. In laboratory-scale, further purification by ultracentrifugation in cesium chloride or sucrose

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gradients is generally sufficient to achieve the required purity of VLP preparations. However, ultracentrifugation-based procedures are non-scalable, labor intensive and might result in very low yields due to the irreversible aggregation [21–23]. In density gradient ultracentrifugation, other particulate contaminants like host cell DNA with a buoyancy density or a rate of sedimentation similar to that of VLPs are likely to sediment in the same fraction [21]. In addition, cesium chloride is a toxic compound that is incompatible with clinical applications and should be removed from VLP preparation using a further purification step. For the large-scale VLP manufacturing, gradient ultracentrifugation-based methods are not practical mainly due to the difficulties in method scale-up [21,22].

Within the purification procedures of viruses and VLPs, a trend is observed from classical purification methods like sucrose or cesium chloride gradient ultracentrifugation towards scalable techniques like tangential flow filtration and liquid chromatography [21,22]. Adsorption chromatography methods offer several important advantages in large-scale purification of viruses and VLPs: high flow rates can be used, thus limiting the processing time; scale-up is relatively easy; large volumes of cell lysates can be processed. It was shown that chromatographic adsorbents developed for protein purification might be employed for the preparation of highly purified and fully assembled VLPs [19,20,24,25]. However, the conventional particle-based chromatography adsorbents are not optimal for the purification of VLPs when operating in bind-elute mode. VLPs are self-assembled from tens to hundreds protein subunits and have size ranging from 20 to 200 nm [22,26], while the pore size of conventional media for protein chromatography ranges from 10 to 100 nm [27,28]. Therefore, VLPs are generally hindered from binding onto the internal surface area of the resin, resulting in low binding capacity and sub-optimal usage of such matrices. In addition, chromatography process of large biomolecules is limited by pore diffusion and becomes slower with increasing molecule size [29].

Monoliths are an alternative to the conventional particle-based chromatography media in the purification of large biomolecules. A monolith can be considered a porous material that is cast in a single, continuous block and inserted into a chromatography column [29]. The pore size of monoliths for separation of large biomolecules ranges from 1000 to 5000 nm. The pores of monolith are interconnected, forming a network of channels [29]. Due to this inherent structure the solutes are transported by convection and the efficiency of mass transfer is high for molecules of different size. Monoliths exhibit high binding capacity for large molecules [29,30]. Another advantageous feature of monoliths is that dynamic binding capacity does not change with velocity.

Methacrylate monoliths, commercially available as CIM (convective interaction media) columns [31], have been used for the purification of VLPs [32–35]. With the use of monoliths the purification time was significantly reduced compared to the ultracentrifugation [32,35]. High dynamic binding capacity of CIMac QA monolith for adenovirus type 3 dodecahedric virus-like particles (Ad3 VLPs) was reported [32]. The dynamic binding capacity of hydroxyl derivatised monolith for VLPs based on the hepatitis B surface antigen (HBsAg) was approximately three times larger compared to that of bead based resin and the recovery of VLPs was equivalent [33]. Using chromatographic purification procedure with CIMmultus SO3 monolith approximately three times higher recovery and higher purity of porcine circovirus type 2 capsid protein (PCV2 Cap) VLPs was achieved compared to the purification procedure with conventional particle-based adsorbent [34].

Several years ago GE Healthcare Life Sciences released core bead chromatography medium, Capto Core 700, which is aimed at intermediate purification and polishing of large biomolecules in flow-through mode [36]. The beads of Capto Core 700 are composed of a core activated with multimodal octylamine ligands and an outer layer without ligands. The bead pores have an approximate exclusion limit of 700 kDa for proteins. Smaller molecules can enter into the core and bind to the ligands, while molecules larger than exclusion limit will pass by the beads in the flow-through. Core bead chromatography using Capto Core 700 resin was fully comparable to ultracentrifugation in removing ovalbumin from influenza virus preparation [37].

There are no published studies on the use of chromatographic techniques for the purification of TSPyV VP1 VLPs. In this work, we developed a purification procedure for TSPyV VP1 VLPs based on chromatographic techniques. This procedure consists of ammonium sulfate precipitation followed by ion-exchange chromatography with CIMmultus QA monolith and core bead chromatography with Capto Core 700 resin.

2. Materials and methods

2.1. Materials

CIMmultus QA Advanced Composite Column (bed volume 1.0 ml) was purchased from BIA Separations (Ajdovščina, Slovenia). Capto Core 700 chromatography medium was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Chemicals were obtained from Merck (Darmstadt, Germany) or Sigma-Aldrich (Munich, Germany).

2.2. TSPyV VP1 VLP production in yeast and preparation of cell lysates

Plasmid pFX7-TSPyV-VP1 with gene encoding TSPyV-derived VP1 protein (GenBank KP293746) was transformed into the yeast *S. cerevisiae* strain AH22-214 (*a*, *leu2-3,112*, *his4-519*) and TSPyV VP1 VLPs were produced in yeast as described previously [17]. Yeast cells harboring recombinant proteins were harvested by centrifugation, washed with distillate water and stored at −20 °C until purification.

An equal volume of glass beads, particle size 425–600 µm (Sigma-Aldrich, Munich, Germany), was added to the plastic tube containing harvested yeast cells (5 g). 10 ml of DB buffer (50 mM Tris-HCl, pH 7.6, 0.15 M NaCl, 1 mM $CaCl_2$), containing 100 µl Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific, Vilnius, Lithuania) was added to the tube and a total of ten cycles of vortexing and incubation on ice (1 min each) were performed. Sample was centrifuged at 805 × *g* for 10 min. The supernatant was collected and then cleared by centrifugation at 12000 × *g* for 25 min.

2.3. Ammonium sulfate precipitation

The trial experiment was carried out in order to determine the optimal concentration of ammonium sulfate for TSPyV VP1 VLPs to be precipitated. Samples of cleared yeast lysate were poured into the beakers. While stirring at 4 °C, solid ammonium sulfate was slowly added in small portions to 30% saturation for the first sample, 35% for the second and so on to 55% for the last sample. After the addition was complete, samples were left to stand overnight at 4 °C and then centrifuged at 12000 x *g* for 25 min. Next, the supernatants were carefully removed and pellets were resuspended into 50 mM Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl and 1 mM $CaCl_2$. The suspensions were centrifuged at 12000 × *g* for 25 min to remove any remaining debris. Supernatants and dissolved pellets were analyzed for the presence of TSPyV VP1 protein.

2.4. Chromatographic purification of TSPyV VP1 VLPs

Chromatographic experiments were carried out using AKTApurifier 100 chromatography system equipped with the sample pump P-960 and the fraction collector Frac-920 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The UNICORN 5.20 software was used for the control and data acquisition.

All chromatographic purification steps were performed at 4 °C temperature. Prior to chromatography, solution of resuspended VLP-containing pellets was filtered through Millex-HV filter with a 0.45 µm hydrophilic PVDF membrane (Merck Millipore, Hertfordshire, UK).