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Improving stability of virus-like particles by ion-exchange chromatographic supports with large pore size: Advantages of gigaporous media beyond enhanced binding capacity

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ABSTRACT

Limited binding capacity and low recovery of large size multi-subunits virus-like particles (VLPs) in conventional agarose-gel based chromatographic supports with small pores have long been a bottleneck limiting the large scale purification and application of VLPs. In this study, four anion exchange media including DEAE-Sepharose FF (DEAE-FF), DEAE-Capto, gigaporous DEAE-AP-120 nm and DEAE-AP-280 nm with average pore diameters of 32 nm, 20 nm, 120 nm and 280 nm, respectively, were applied for purification of hepatitis B virus surface antigen (HBsAg) VLPs. Pore size effects of media on the VLPs adsorption equilibrium, adsorption kinetics, dynamic binding capacity (DBC), and recovery were investigated in detail. According to the confocal laser scanning microscopy observation, adsorption of the VLPs in DEAE-FF and DEAE-Capto was mostly confined to a thin shell on the outer surface of the beads, leaving the underlying pore space and the binding sites inaccessibly, while the large pores in gigaporous media enabled the VLPs to access to the interior pore spaces by diffusion transport efficiently. Compared to the most widely used DEAE-FF, gigaporous media DEAE-AP-280 nm gained about 12.9 times increase in static adsorption capacity, 8.0 times increase in DBC, and 11.4 times increase in effective pore diffusivity. Beyond increasing the binding capacity and enhancing the mass transfer, the gigaporous structure also significantly improved the stability of the VLPs during intensive adsorption-desorption process by lowing the multi-point interaction between the VLPs and binding sites in the pores. At 2.0 mg/mL-media loading quantity, about 85.5% VLPs were correctly self-assembled after the chromatography with DEAE-AP-280 nm media; oppositely about 85.2% VLPs lost their normal assembly with DEAE-FF due to irreversible disassembly. Comparative investigation was made to study the purifying performance of these four chromatographic media for actual VLPs purification from recombinant Hansenula polymorpha. DEAE-AP-280 nm media were demonstrated the best results showing the highest recovery of 68.33% and purification fold of 3.47, at 2.98 mg protein/mL-media loading quantity and a flow rate of 240 cm/h.

1. Introduction

Chromatography is one of the most powerful techniques for separation and purification of biomacromolecules. Among such biomacromolecules, virus and virus-like particles (VLPs) separation by chromatography is becoming more and more important as various types of VLPs application are now available as effective vaccines against diseases. Compared to small and medium size proteins, whose molecular weight are in the 10–100 kDa range and dynamic radius are in the 1–10 nm range, VLPs are self-assembled from unusually more than 100 subunits and have size ranging from tens up to hundreds of nanometers. Hepatitis B virus surface antigen (HBsAg) and human papilloma virus (HPV) vaccine are currently the most successful two types of VLP vaccines that are available on the market against hepatitis B virus and HPV, respectively. The HBsAg VLPs (HB-VLPs) from recombinant Hansenula polymorpha are composed of about 100 subunits, and known to have diameter about 22 nm and molecular weight of more than 3000 kDa [1,2]; and the size of later one was reported to be larger than 50 nm in diameter [3].

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The large size and multi-subunit structure of VLPs make their chromatographic process a challenging task. Agarose-based gel is the most popular commercial chromatographic media [4–6], having pore size generally less than 30 nm [7,8]. Although this pore size is large enough for most proteins, it is apparently too small to accommodate large VLPs. It is well known that protein adsorption capacity on most ion-exchange media decreases drastically when the molecular mass is over 100 kDa [9]. Adsorption capacity of agarose gel-based ion exchange media for most proteins can easily reach more than 100 mg/mL [10–13], while that for VLPs are usually less than 1 mg/mL [14–16]. Obviously, the pore size of the media would play a dominant role in the accessibility of the VLPs to the binding sites on the interior surfaces of the beads. VLPs would be physically excluded from small pores and the binding would be restricted to the outer surface of the beads, thus leading to the low binding capacity. Besides the low binding capacity, slow mass transfer rate of VLPs in the pore of agarose bead would also limit the fast purification by chromatography. The pore diffusivity of HB-VLPs in Sepharose FF gel was estimated to be about 5×10^{-13} m^2/s, which is much lower than the estimated pore diffusivity value (1×10^{-11} m^2/s) for medium size protein such as bovine serum albumin (BSA, M_r 68,000 Da) [14].

Fabricating and applying chromatographic media with large pores might provide an ideal remedy to the bottleneck encountered in the large scale chromatographic purification of protein by providing maximum binding capacity and minimum diffusion limitation. Although the conventional chromatographic media like ceramic hydroxyapatite [17–19] and Matrex Cellufine Sulfate affinity chromatography media [20,21] have been successfully used for purification of a variety of VLPs, the rapid developments in the area of VLPs production and application raise continually demand for new materials for a more powerful VLPs purification process. Polymethacrylate monolithic columns, also known as Convective Interaction Media (CIM) monoliths, are one of the most interesting stationary phases for purification of large molecules like pDNA and VLPs [22–24]. They consist of a single block of material that contains highly connected flow through channels as large as 1.5 μm diameter that enable the transport of the solute to the surface solely by convection, thus providing the large biomolecules both high surface accessibility and flow-independent high binding capacities and resolutions. It is notable, however, that the size of these media is currently limited to a maximum of 8 L columns, which will need to be amplified in the future for larger scale applications [25]. Another promising media suitable for large scale VLPs purification are the perfusion chromatography media based on a rigid poly(styrene-divinylbenzene) backbone, commercially known as POROS®, which were developed in 1990s. This microspheres media that contains two sets of pores: through pores (600–800 nm) and diffusive pores (80–150 nm), were designed to overcome slow mass transfer while preserving substantial binding capacity for protein at modest operating pressures. Due to these advantages, POROS® have been applied in purification a variety of VLPs [3,26,27], though one recent study on HPV Type 11 capsid protein VLPs adsorption on POROS® media indicated that the effect of perfusion seemed to vanish and the binding occur exclusively in a thin layer at the particle surface [28].

In the case of VLPs purification, maintaining multi-subunits structure of VLPs during chromatography process is considered as important as perusing high binding capacity. The biological activity of VLPs are known to be strongly dependent on the correct self-assemble of its subunit. Aggregation or disassembly of the subunits during the intensive adsorption-desorption process have been assumed as the main reason for incorrect self-assemble in VLPs and the resulting low activity recovery [15,27,29]. Lowering the ligand density of DEAE-adsorbent was reported to efficiently increase the HB-VLPs recovery during ion-exchange chromatography (IEC) purification process of HB-VLPs by decreasing the irreversible disassembly of HB-VLPs on the surface of the adsorbent during the intensive adsorption-desorption process [15]. Pore size of the media has also been found imposing substantial influence on stability of the adsorbed proteins or enzymes. Nevertheless, most previous studies were carried out with medium size proteins and relative small pores [30–32]. Relationship between the pore size of media on the structure and activity of VLPs, unfortunately, remains unclear.

In principle, increasing pore size of the media will certainly increase the binding capacity for VLPs adsorption and enhance the mass transfer rate of VLPs in the pores. A comparative study have shown that the dynamic binding capacity for the HB-VLPs on a hydrophobic CIM monolith column with OH ligands was approximately three to four times larger than that on Butyl-S 6 Sepharose FF [22]. What is more important, the increased pore size will possibly lower intensive multi-point interaction between proteins and the surface of the media, which has been assumed as the major reason for irreversible disassembly of VLPs [15], therefore recovery will be improved without sacrificing binding capacity. To reveal the advantages of using large pore media in VLPs purification, we choose the IEC process of HB-VLPs purification as a model system, four anion-exchange media with average pore size of 20, 32, 120 and 280 nm, respectively, were applied to make a comparative investigation on the effect of pore size of media on the static and dynamic binding capacity, adsorption kinetics, and recovery as well as structure stability of HB-VLPs.

HB-VLPs are one of the most successful and effective vaccine for preventing strong infectious Hepatitis B. The downstream process for a large-scale purification of HB-VLPs from CHO cell line had been established including hydrophobic chromatography (HIC), IEC, ultrafiltration and gel filtration chromatography (GFC) [33–35]. Among them, IEC with the lowest step recovery (<50%) is still a bottleneck of the whole purification process [34]. Results of the present study will show that compared to the widely used agarose-based Sepharose Fast Flow (DEAE-FF) media, whose pore size is 32 nm, gigaporous media with pore size of 280 nm exhibit the best performance in many aspects such as about 8 times higher dynamic binding capacity, 11.3 times larger HB-VLPs effective pore diffusivity, and 2.8 times higher recovery after IEC operated at 6 times higher flow rate.

2. Materials and methods

2.1. Materials

Two commercially available agarose media, DEAE-FF, and DEAE-Capto, were purchased from GE Healthcare Life Science (Uppsala, Sweden). Both media are weak anion-exchange media composed of rigid, high-flow 6% cross-linked agarose matrix with diethylaminoethyl (DEAE) as ligand, while the DEAE-Capto was grafted with dextran surface extenders prior to coupling of DEAE ligand. Gigaporous polystyrene microspheres-based anion exchange media, DEAE-AP-120 nm and DEAE-AP-280 nm, were obtained from National Engineering Research Center for Biotechnology (Beijing, China). These two media were prepared by coating the gigaporous polystyrene microspheres with average pore size of 120 nm or 280 nm with agarose, followed by functionalizing the agarose layer with diethylaminoethyl chloride [36].

Purified HB-VLPs from recombinant Hansenula polymorpha line was kindly gifted from Hualan Biological Engineering Corporation (Xinxiang, China). Dextran samples with molecular weight ranging from 1 to 2000 kDa were purchased from RI-101 Showa Denko K. K. (Tokyo, Japan). All other chemicals were of analytical grade and all solutions were prepared using Mill-Q grade water (Millipore, USA).