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Microcalorimetric study of adsorption and disassembling of virus-like particles on anion exchange chromatography media

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ABSTRACT

Chromatographic purification of virus-like particles (VLPs) is important to the development of modern vaccines. However, disassembly of the VLPs on the solid–liquid interface during chromatography process could be a serious problem. In this study, isothermal titration calorimetric (ITC) measurements, together with chromatography experiments, were performed on the adsorption and disassembling of multi-subunits hepatitis B virus surface antigen virus-like particles (HB-VLPs). Two gigaporous ion-exchange chromatography (IEC) media, DEAE-AP-280 nm and DEAE-POROS, were used. The application of gigaporous media with high ligand density led to significantly increased irreversible disassembling of HB-VLPs and consequently low antigen activity recovery during IEC process. To elucidate the thermodynamic mechanism of the effect of ligand density on the adsorption and conformational change of VLPs, a thermodynamic model was proposed. With this model, one can obtain the intrinsic molar enthalpy changes related to the binding of VLPs and the accompanying conformational change on the liquid–solid interface during its adsorption. This model assumes that, when intact HB-VLPs interact with the IEC media, the total adsorbed proteins contain two states, the intact formation and the disassembled formation; accordingly, the apparent adsorption enthalpy, $\Delta_{app}H$, which can be directly measured from ITC experiments, presents the sum of three terms: (1) the intrinsic molar enthalpy change associated to the binding of intact HB-VLPs ($\Delta_{bind}H_{intact}$), (2) the intrinsic molar enthalpy change associated to the binding of HB-VLPs disassembled formation ($\Delta_{bind}H_{dis}$), and (3) the enthalpy change accompanying the disassembling of HB-VLPs ($\Delta_{conf}H_{dis}$). The intrinsic binding of intact HB-VLPs and the disassembled HB-VLPs to both kinds of gigaporous media (each of which has three different ligand densities), were all observed to be entropically driven as indicated by positive values of $\Delta_{bind}H_{intact}$ and $\Delta_{bind}H_{dis}$; while the nagative $\Delta_{conf}H_{dis}$ values suggested a spontenous enthalpy-driven process for the forming of HB-VLPs disassembled formation at all conditions studied. As ligand density increases, $\Delta_{conf}H_{dis}$ became more negative, which was in agreement with the findings from chromatography experiments, that higher ligand density leads to more serious disassembling of HB-VLPs. Results from thermodynamic studies provided us insight understanding on the mechanism of adsorption and conformational change of VLPs, as well as the effect of ligand densities on the structural stability of VLPs during IEC process.

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1. Introduction

Due to high separation resolution and mild operation, application of purification/separation using chromatographic technology has been extended from proteins and antibodies to a large variety of complex biomacromolecules such as virus, and virus like particles (VLPs). VLPs are self-assembled from unusually more than 100 subunits and have size ranging from tens up to hundreds of nanometers. Using as an important class of vaccines, structural integrity of the VLPs is one of the most important factors

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affecting the potency to induce effective vaccination, although the direct link between antigen structure and the potency or efficacy is still not very clear [1]. For instance, the intact hepatitis B surface antigen particles (HB-VLPs) were reported about 1000-folds more immunogenic than the disassembled HB-VLPs subunits [2], and the HB-VLPs aggregates also have lower antigen activity than the normal assembly [3]. While during chromatographic process, strong interactions between chromatographic media and multi-subunits VLPs, or changes in solution environment might induce significant aggregation or disassembly of VLPs. For example, in one of our previous study, it has been found that intensive adsorption–desorption during ion-exchange chromatography (IEC) process caused severe disassembling of HB-VLPs, leading to low antigen activity recovery [4–6]. Therefore, how to ensure the integrity of the VLPs during chromatographic process is a challenging task due to its large size and multi-subunits structure.

Lowering the ligand density of DEAE-adsorbent [4] or using gigaporous media with pore size over 100 nm [7] have been experimentally found to efficiently increase the antigen recovery during IEC purification process of HB-VLPs. Such improvement was speculated to be benefited from suppressed irreversible disassembly of HB-VLPs on the surface of the adsorbent during the intensive adsorption–desorption process [4]. However, the thermodynamic mechanism accounting for the conformational change of VLPs on the liquid–solid interface during adsorption process remains unclear; furthermore, in an attempt to elucidate the effect of ligand density and other surface properties of chromatographic media on VLPs adsorption and conformational change, there are no any directly measured data, i.e., adsorption enthalpies, available for discussion in view point of thermodynamics.

Isothermal titration calorimetry (ITC) is an useful technique for directly measuring the thermodynamic parameters of interactions in solution, for example, adsorption of proteins on media [8], binding of small molecules (such as medicinal compounds) to larger macromolecules (proteins, DNA, etc.) [9,10]. ITC has found a variety of important applications in protein–ligand interaction studies and drug discovery. By directly measuring the heat released or taken up upon binding, ITC provides a sensitive and rapid method for determining the enthalpy of adsorption without having to resort to the indirect path of van't Hoff analysis [11]. The binding mechanism of proteins on adsorbents may involve several of sequential sub-processes, therefore the directly measured endothermic or exothermic enthalpy presents an integrated parameter which can be affected by numerous factors including types of adsorbents, ligand chain length and density, pH values, salt concentrations, temperature, etc. [12–17]. By measuring adsorption enthalpies of partially denatured protein and resolving the enthalpy change accompanying the conformational change in presence of denaturants, the thermodynamic mechanism of protein unfolding on a liquid–solid interface were also studied by ITC [18,19]. Although investigations on these effects have been widely taken for protein adsorption process, information relating to thermodynamics of VLPs adsorption is still lacking.

In the past, the adsorption mechanism for ion exchange chromatography was thought to be mainly driven by ionic interactions between oppositely charged protein and adsorbents, whose adsorption mechanism is therefore often described as an exothermal enthalpy-driven process [20]. However, microcalorimetric studies showed that in some of the technically interesting operating ranges, an endothermic process was often observed for proteins adsorption on ion-exchange adsorbents [16,21]. The presence of endothermic enthalpies indicates that the binding process of an ionized protein onto the charged media surface has other sub-processes in addition to the expected electrostatic interactions. For instance, entropic effects associated to water release might make major contributions over electrostatic interactions to the adsorption process. As regards to the IEC process of VLPs, where possible conformational changes in VLPs's structure may occur on the liquid–solid interface, the process would be more complicated. The measured apparent adsorption heat by ITC may involve contributions from several of terms, i.e., retention adsorption heat of both intact VLPs and possible dissembled and/or aggregated VLPs, as well as the heat related to the conformational change of VLPs. Considering the first two terms are associated with electrostatic interaction between VLPs and ion-exchange media, the ligand density of the media would have important influence. While the information of molar enthalpy changes induced by conformational change of VLPs during its adsorption to IEC media with different ligand densities will be more useful, we might be able to elucidate the thermodynamic mechanism of the conformational change. Such insight theoretical analysis will further provide us one more useful criterion for optimization of the surface properties of the media, so that the unexpected conformational change of VLPs during adsorption can be eliminated.

To this end, a series of ITC measurements were performed in this study for adsorption of HB-VLPs to two types of ion-exchange media, DEAE-POROS and DEAE-AP-280 nm, both having gigaporous structure allowing VLPs to penetrate. Based on thorough analysis of the results from chromatography experiments, a thermodynamic model was proposed and the molar enthalpy changes corresponding to both VLPs' binding and their conformational change on the liquid–solid interface were determined with the aid of ITC measurement. Finally, the relationships between ligand density of IEC media and the thermodynamic parameters were analyzed and discussed.

2. Materials and methods

2.1. Materials

POROS OH was purchased from Applied Biosystems Corporation (Foster, USA). According to the supplier, POROS OH is one of perfusion chromatography media with pore size of 50–1000 nm based on a rigid poly(styrene-divinylbenzene) backbone functionalized with OH groups. Gigaporous polystyrene microspheres-based anion exchange media, Agap-co-PS, were obtained from National Engineering Research Center for Biotechnology (Beijing, China). The media were prepared by coating the gigaporous polystyrene microspheres having average pore size of 280 nm with agarose [22].

Purified intact HB-VLPs standard from recombinant *Hansenula polymorpha* line was kindly gifted from Hualan Biological Engineering Corporation (Xinxiang, China). All other chemicals were of analytical grade and all solutions were prepared using Mill-Q grade water (Millipore, USA).

2.2. Preparation of anion exchange media with different ligand densities

DEAE groups were introduced onto Agap-co-PS microspheres and POROS OH according to the method reported by Wang et al. [23]. Typically, the gigaporous Agap-co-PS microspheres and POROS OH were soaked and swollen in deionized water for 24 h aforehand, then filtered through sintered glass funnel to remove the external water. The drained microspheres (2 g) were suspend in 10 mL of DEAE·HCl (3.5 mol/L) and shaken at 130 rpm for 10 min in an incubator at 70 °C. For making various ligand densities, 10 mL NaOH solution with concentration of 3.0, 5.0, and 7.0 mol/L was pre-heated to 70 °C and added to the reactant, respectively. After reaction for 1 h, the microspheres were rinsed with deionized water thoroughly to remove the residual DEAE·HCl on the microspheres. The microspheres were collected with a sintered glass funnel and stored in 20% ethanol–water solution. The prepared anion exchange

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