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Affinity purification of viral protein having heterogeneous quaternary structure: Modeling the impact of soluble aggregates on chromatographic performance

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ABSTRACT

Prokaryote-expressed polyomavirus structural protein VP1 with an N-terminal glutathione-S-transferase tag (GST-VP1) self-assembles into pentamer structures that further organize into soluble aggregates of variable size (3.4×10^2–1.8×10^4 kDa) [D.I. Lipin, L.H.L. Lua, A.P.J. Middelberg, J. Chromatogr. A 1190 (2008) 204]. The adsorption mechanism for the full range of GST-VP1 soluble aggregates was described assuming a dual-component model [T.Y. Gu, G.J. Tsai, G.T. Tsao, AICHE J. 37 (1991) 1333], with components differentiated by size, and hence pore accessibility, rather than by protein identity. GST-VP1 protein was separated into two component groups: aggregates small enough to access resin pores (LMW: 3.4×10^2–1.4×10^3 kDa) and aggregates excluded from the resin pores (HMW: 9.0×10^2–1.8×10^4 kDa). LMW aggregates bound to resin at a higher saturation concentration (29.7 g L^{-1}) than HMW aggregates (13.3 g L^{-1}), while the rate of adsorption of HMW aggregates was an order of magnitude higher than for LMW aggregates. The model was used to predict both batch and packed bed adsorption of GST-VP1 protein in solutions with known concentrations of HMW and LMW aggregates to Glutathione Sepharose HP resin. Asymmetrical flow field flow fractionation with UV absorbance was utilized in conjunction with adsorption experimentation to show that binding of HMW aggregates to the resin was strong enough to withstand model-predicted displacement by LMW aggregates. High pore concentrations of LMW aggregates were also found to significantly inhibit the diffusion rate of further protein in the resin pores. Additional downstream processing experimentation showed that enzymatic cleavage of LMW aggregates to remove GST tags yields more un-aggregated VP1 pentamers than enzymatic cleavage of HMW aggregates. This model can be used to enhance the chromatographic capture of GST-VP1, and suggests an approach for modeling chromatographic purification of proteins that have a range of quaternary structures, including soluble aggregates.

1. Introduction

Virus-like particles (VLPs) have current and prospective uses in vaccination [1], drug delivery [2] and gene therapy [3]. Optimizing the VLP manufacturing process is critical to realizing several of these uses as commercially viable products. Conventional paradigms for the production of individual proteins may not be best suited for the production of complex VLPs, which assemble from one or more viral structural proteins in a process highly dependent on environmental conditions [4]. The optimal manufacturing process will ideally: (i) enable precise control of VLP assembly; (ii) ensure that particles are homogeneous; (iii) prevent encapsulation of cellular contaminants, and (iv) enable efficient packaging of specific molecules. This study describes further research into a VLP manufacturing process that may meet these objectives [5], with murine polyomavirus used as a model VLP.

Murine polyomavirus VLPs can be produced by prokaryotic expression of the viral structural protein VP1 fused to glutathione-S-transferase (GST-VP1). Following removal of cellular contaminants by a single affinity chromatography step, GST tags are detached by enzymatic cleavage yielding VP1 protein organized into stable pentamers. Controlled VLP assembly can be subsequently executed in a separate bioreactor, with or without the encapsulation of chosen target molecules [6]. This process approach benefits from the high level expression characteristic of bacterial systems (0.18 g L^{-1} at laboratory shake-flask scale [7]), and yields high-quality building blocks for subsequent VLP assembly. Moreover, because structural protein is purified before VLP assembly, the process approach eliminates encapsulation of cellular contaminants as an issue. The process can also be used to produce purified

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GST-tagged viral protein, which has prospective uses in vaccination even when not assembled into a VLP [8].

A previous study has shown that prokaryote-expressed GST-VP1 exists as soluble aggregates (associated protein aggregates not retained by a 0.22 μm filter [9]) ranging in size from 1 to at least 52 pentamers [5]. During affinity chromatography of GST-VP1 protein, only low molecular weight aggregates (LMW: ≤4 pentamers in size, 3.4×10^2–1.4×10^3 kDa) are able to access the resin pores. High molecular weight aggregates (HMW: 9.0×10^2–1.8×10^4 kDa) can only bind to the limited outer surface area of the resin beads. This study further characterizes the physical nature of LMW and HMW aggregates, and shows that the size range of GST-VP1 aggregates affects the yield of VP1 pentamers when tags are removed by enzymatic cleavage. Attempts were subsequently made to describe the adsorption of protein from a solution containing both LMW and HMW aggregates using an established multiple-component model [10,11] with modifications to accommodate components having variable pore accessibility [12].

A key challenge of this study was to quantitatively determine the adsorption properties of a mixture of soluble aggregates of variable size and degree of pore accessibility. Existing studies of multi-component systems with pore accessibility differences have utilized proteins that can be easily distinguished by size exclusion chromatography (SEC) [13,14] or ultraviolet (UV) absorbance at different wavelengths [15]. This study focused on a complex mixture of components that are difficult to distinguish from each other in terms of protein composition or by using SEC. Asymmetrical flow field flow fractionation (AFFFF) [16] was successfully used to measure the relative quantities of different sized GST-VP1 aggregates during adsorption, allowing for a fundamental investigation of the adsorption behavior of proteins organized into different quaternary structures (such as soluble aggregates).

2. Model

Protein in all GST-VP1 solutions was separated into two groups: aggregates that could access resin pores (LMW) and aggregates that were excluded from resin pores (HMW). For the purposes of simplifying modeling calculations, it was assumed that all aggregates within a group (LMW or HMW) were uniform in size, shape, pore accessibility and adsorption properties. In this dual-component system, aggregates were assumed not to interact with each other and adsorption to the resin surface was limited to a single monolayer. Modeling equations derived by Arve and Liapis [10,11] to describe rate-controlled mass transfer in the bulk, in the resin pores and at the resin surface were used to describe the adsorption of protein to chromatography resin. Modifications to these equations to account for proteins with variable pore accessibility were adapted from Gu et al. [12].

The initial molar fraction of LMW and HMW GST-VP1 in a solution was defined using the parameter α.

$$c_{0,\mathrm{L}} = \alpha c_{0,\mathrm{T}} \tag{1}$$

$$c_{0,\mathrm{H}} = (1-\alpha) c_{0,\mathrm{T}} \tag{2}$$

Mass transfer between the bulk and the outer surface of the resin beads in batch and packed bed systems was described using Eqs. (3) and (4), respectively [10,11].

$$\frac{dc_i}{dt} = -\frac{3V_{\mathrm{M}}(1-\varepsilon)k_{\mathrm{f},i}}{(V+V_{\mathrm{M}}\varepsilon)r_{\mathrm{p}}}(c_i - c_{\mathrm{p},i}|_{r=r_{\mathrm{p}}}) \tag{3}$$

$$\frac{\partial c_i}{\partial t} - D_{\mathrm{L}}\frac{\partial^2 c_i}{dz^2} + \frac{\partial c_i}{\partial z}u = -\frac{3(1-\varepsilon)}{r_{\mathrm{p}}\varepsilon}k_{\mathrm{f},i}(c_i - c_{\mathrm{p},i}|_{r=r_{\mathrm{p}}}) \tag{4}$$

Boundary conditions for the packed bed system [11]:

$$t>0,\begin{cases} z=0,\ \varepsilon u c_{0,i} = \varepsilon u c_i|_{z=0} - \varepsilon D_{\mathrm{L}}\left.\dfrac{\partial c_i}{\partial z}\right|_{z=0} \\ z=L,\ \left.\dfrac{\partial c_i}{\partial z}\right|_{z=L} = 0 \end{cases} \tag{5}$$

Mass transfer within the resin pores was described using Eq. (6). $\varepsilon_{\mathrm{p,i}}$ has been incorporated into this equation as a component-specific particle porosity value with a range of $0 \le \varepsilon_{\mathrm{p,i}} \le \varepsilon_{\mathrm{p}}$ [12].

$$\varepsilon_{\mathrm{p},i}\frac{\partial c_{\mathrm{p},i}}{\partial t} = \varepsilon_{\mathrm{p},i}D_{\mathrm{E},i}\left(\frac{\partial^2 c_{\mathrm{p},i}}{\partial r^2} + \frac{2}{r}\frac{\partial c_{\mathrm{p},i}}{\partial r}\right) - (1-\varepsilon_{\mathrm{p}})\frac{\partial q_i}{\partial t} \tag{6}$$

Boundary conditions for pore mass transfer of LMW aggregates ($\varepsilon_{\mathrm{p,L}} > 0$) [12]:

$$t>0,\begin{cases} r=0,\ \dfrac{\partial c_{\mathrm{p,L}}}{\partial r} = 0 \\ r=r_{\mathrm{p}},\ \varepsilon_{\mathrm{p,L}}D_{\mathrm{E,L}}\dfrac{\partial c_{\mathrm{p,L}}}{\partial r} = k_{\mathrm{f,L}}(c_{\mathrm{L}} - c_{\mathrm{p,L}}|_{r=r_{\mathrm{p}}}) \end{cases} \tag{7}$$

For HMW components, which are excluded from the resin pores ($\varepsilon_{\mathrm{p,H}} = 0$), the lack of a relationship between r and $c_{\mathrm{p,H}}$ yields different boundary conditions (Eq (8)). This causes the pore mass transfer equation (Eq. (6)) to degenerate into Eq. (9) for $0 \le r < r_{\mathrm{p}}$, and Eq. (10) when $r = r_{\mathrm{p}}$ [12].

$$t>0,\ r=0,\ \frac{\partial c_{\mathrm{p,H}}}{\partial r} = 0$$

$$t>0,\ r=r_{\mathrm{p}},\begin{cases} \varepsilon_{\mathrm{p,H}}D_{\mathrm{E,H}}\dfrac{\partial c_{\mathrm{p,H}}}{\partial r} = k_{\mathrm{f,H}}(c_{\mathrm{H}} - c_{\mathrm{p,H}}|_{r=r_{\mathrm{p}}}) \\ \varepsilon_{\mathrm{p,H}}D_{\mathrm{E,H}}\dfrac{\partial^2 c_{\mathrm{p,H}}}{\partial r^2} = \dfrac{k_{\mathrm{f,H}}}{r_{\mathrm{p}}}(c_{\mathrm{H}} - c_{\mathrm{p,H}}|_{r=r_{\mathrm{p}}}) \end{cases} \tag{8}$$

$$\left.\frac{dq_{\mathrm{H}}}{dt}\right|_{0\le r<r_{\mathrm{p}}} = 0 \tag{9}$$

$$\left.\frac{dq_{\mathrm{H}}}{dt}\right|_{r=r_{\mathrm{p}}} = \frac{3k_{\mathrm{f,H}}}{(1-\varepsilon_{\mathrm{p}})r_{\mathrm{p}}}(c_{\mathrm{H}} - c_{\mathrm{p,H}}|_{r=r_{\mathrm{p}}}) \tag{10}$$

When one of the components in a dual-component system is excluded from the resin pores, competitive adsorption between the two components will occur only at the outer surface of the resin bead. Mass transfer at the resin surface must therefore be described using Eqs. (11)–(12) [12].

$$\frac{\partial q_{\mathrm{L}}}{\partial t} = k_{\mathrm{a,L}}c_{\mathrm{p,L}}(q_{\mathrm{max,L}} - \theta_{\mathrm{LH}}q_{\mathrm{H}} - q_{\mathrm{L}}) - k_{\mathrm{d,L}}q_{\mathrm{L}} \tag{11}$$

$$\frac{\partial q_{\mathrm{H}}}{\partial t} = k_{\mathrm{a,H}}c_{\mathrm{p,H}}(q_{\mathrm{max,H}} - q_{\mathrm{H}} - q_{\mathrm{L}}\theta_{\mathrm{HL}}) - k_{\mathrm{d,H}}q_{\mathrm{H}} \tag{12}$$

$$\theta_{ij} = \frac{q_{\mathrm{max},i}}{q_{\mathrm{max},j}} \tag{13}$$

The factor θ_{ij} accounts for the difference in saturation capacity between the two components at the *outer* surface of the resin bead. In a LMW/HMW dual-component system where $q_{\mathrm{max,L}} \gg q_{\mathrm{max,H}}$, θ_{HL} reduces q_{L} in Eq. (12) to reflect the fact that adsorption of LMW components occupies fewer binding sites on the outer surface of the resin bead than HMW components. θ_{LH} increases q_{H} in Eq. (11) to reflect the fact that adsorption of one HMW component to the outer surface of the resin bead blocks adsorption of several LMW components.

At equilibrium, adsorption of both components at the *outer* surface of the resin bead can be described by the Langmuir equation for multiple-component adsorption (Eq. (14)) [17]. Within the resin pores, there will be no adsorption of HMW components at equilibrium due to $\varepsilon_{\mathrm{p,H}} = 0$ (Eq. (15)). Overall adsorption capacity of LMW components will therefore not be significantly affected by