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Analysis of adsorption of a baculovirus bioreaction bulk on an ion-exchange surface by surface plasmon resonance

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ABSTRACT

The binding and elution of the key components of a bioreaction bulk for the production of recombinant baculoviruses—a promising viral vector for gene therapy and vaccination—on a model ion-exchange surface have been successfully measured and interpreted by surface plasmon resonance (SPR) spectroscopy. The micro-scaled, ion-exchange surface was produced by immobilizing a typical ion-exchange ligand, diethylaminoethyl, onto commercially available planar gold sensor chip surfaces, which were pre-derivatized with a self-assembled monolayer of 11-mercaptoundecanoic acid. Each isolated analyte was injected into the SPR cell at defined operating conditions of salt and solute concentrations to determine the adsorption equilibrium plateau, and then eluted at the same salt concentration, upon which a well-defined, residual desorption equilibrium plateau was observed. From the analysis of the binding and elution curves and equilibrium plateaus for seven key biomolecules, it is possible to determine the adsorption isotherms over a broad range of equilibrium conditions for the three main cuts of the baculovirus bioreaction bulk: the product (the infective baculovirus), the main product-related impurities, and the main process-related impurities. A model that quantitatively explains the SPR-derived adsorption/desorption data was successfully developed for this complex biological system.

1. Introduction

Ion-exchange processes are ubiquitously used in downstream processing of complex biologics for clinical use. The optimization of charge-dependent unit operations relies on the evaluation of pivotal operating conditions, such as ionic strength and pH of the suspension buffer. These procedures tend to become exceptionally costly when a high added-value product is recovered from a complex preparation, such as an animal cell derived bioreaction bulk. Hence, the optimization of such unit processes becomes mandatory in the interest of cost and time savings. Scaled-down models, often used in high-throughput technologies, are being considered as options of choice for early-stage process development (Coffman et al., 2008; Rege et al., 2006; Wensel et al., 2008).

Recombinant baculoviruses (rBVs) constitute a challenging model system within the collection of promising complex biopharmaceuticals for clinical therapies. Besides being used as a robust expression system with insect cell lines (BEVS), the potential of these viral vectors has evolved to human gene therapy or vaccination due to important (and alternative) advantages over other more studied vectors such as adeno- or retroviruses (Kost et al., 2005; Airenne et al., 2009). Since the mid-nineties (Hofmann et al., 1995), it has been shown that these insect-specific viruses can be genetically modified to transduce vertebrate cells, by means of a proper mammalian promoter, without replicating themselves or causing cytotoxic effects. Due to this non-pathogenicity to mammalian cells, the ability to accommodate very large foreign DNA inserts and the easiness of production at high viral titers in insect cell cultures, this system presents significant potential and important advantages over other vectors used in clinical trials (Hu, 2008; Airenne et al., 2009).

The typical insect cell clarified bioreaction bulk comprises a complex mixture of components: the desired product—the recombinant baculoviruses, infective/bioactive particles—the product-related impurities, which decrease the quality of the product, and the process-related impurities—DNA, host-cell protein, and/or eventual endotoxin. The understanding of how these impurities behave in an ion-exchange process provides useful information for

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Nomenclature

Symbols

c_B	solute concentration in bulk solution
c_0	salt concentration in bulk solution
c_B^o	solute concentration in outer compartment
R	Biacore sensor's signal
z	distance from the sensor surface
$c_{0,ref}$	reference salt concentration
d_p	penetration depth of the evanescent field
d_m	thickness of adsorbate monolayer
d_s	thickness of sensor surface film
c_m	adsorbate concentration
m	correlation coefficient between RI and Biacore's ΔR signal
m_0	slope of linear fitting of ΔR vs $(c_0 - c_{0,ref})$
m_B	slope of linear fitting of ΔR vs c_B
D	diffusion coefficient
k_m	mass-transfer coefficient from bulk to sensor chip surface
h_i	height of the inner compartment
$\bar{u}$	average velocity of the fluid in the flow cell, $Q/(hw)$
h	height of the flow cell
w	width of the flow cell
l	length of the sensor surface
k_a	reversible adsorption rate constant
k_d	desorption rate constant
k_r	rate constant for irreversible adsorption from the bulk solution
k'_r	rate constant for irreversible adsorption from the reversibly adsorbed phase
q	total adsorbed concentration
q_1	concentration of irreversibly bound solute
q_2	concentration of reversibly bound solute
q_1^∞	capacity of the derivatized surface for irreversible binding
q_2^∞	capacity of the derivatized surface for reversible binding
q^∞	total capacity of the stationary phase
$\tilde{q}_1^\infty$	apparent capacity of the derivatized surface for irreversible binding
$\tilde{q}_2^\infty$	apparent capacity of the derivatized surface for reversible binding
$\bar{q}_1$	equilibrium concentration of irreversibly bound solute
$\bar{q}_2$	equilibrium concentration of reversibly bound solute

Greek letters

η_B	refractive index of a salt solution with a dissolved biological solute
$\tilde{\eta}_B$	refractive index of the (dry) solute
η_0	refractive index buffer solution with salt concentration c_0
ΔR	Biacore signal
$\eta_{0,ref}$	reference salt solution's RI (baseline buffer)
ϕ_s	exponential ratio, $\exp(-d_s/d_p)$
$\tilde{\eta}_0$	refractive index of the salt ions
$\tilde{\eta}_w$	refractive index of pure water
ν_B	specific volume of the biological solute
ϕ_m	exponential ratio, $\exp(-d_m/d_p)$
σ_{12}	steric factor for shielding of irreversible binding by reversibly bound adsorbates
σ_{21}	steric factor for shielding of reversible binding by irreversibly bound adsorbates
ΔR_a	sensorgram signal during adsorption
ΔR_d	sensorgram signal during desorption
$\bar{\Delta} R_a$	equilibrium sensorgram signal upon adsorption
$\bar{\Delta} R_d$	equilibrium sensorgram signal upon desorption

designing and optimizing the chromatographic purification process.

Surface plasmon resonance (SPR) spectroscopy is an extremely sensitive analytical technique with many applications in fundamental surface science (Hoa et al., 2007; Pattnaik, 2005; Rich and Myszka, 2008). SPR biosensors allow label-free, real-time, quantitative and qualitative characterization of the specific binding of a mobile analyte to a ligand immobilized on a metal surface (Schuck, 1997). When working with highly expensive biological materials, such as viral vectors produced in cell culture, SPR can play a supporting role for the optimization of adsorption-based, downstream purification processes, including ion-exchange chromatography, by providing quantitative information on the binding kinetics and equilibria of the various biomolecules of the bioreaction bulk to an immobilized functionalization layer mimicking the adsorbent. Because of the highly reduced sample volume used in a typical SPR experiment, a critical amount of data on the adsorbed film for a broad range of analyte loading and salt concentration can be obtained with a small amount of material.

We have recently applied SPR sensing to the monitoring and quantitative analysis of protein binding and elution over a micro-scaled, ion-exchange surface under cyclic adsorption conditions (Vicente et al., 2010). It is the purpose of the present study to employ SPR and its mathematical formalism for quantitative interpretation of ion-exchange equilibria for a much more complex biological system: a recombinant baculovirus bioreaction bulk.

For convenience, we shall split the biological bulk into three cuts or fractions—the product, the main product-related impurities, and the main process-related impurities—and identify the key biomolecules governing the chromatographic behavior of each fraction. We will then use SPR sensing to measure the adsorption equilibrium of each isolated biomolecule over a broad range of solute and salt concentrations. Overall, we shall report on seven different adsorbates. A kinetic model is proposed to explain the measured SPR responses for the adsorbed films of the various biomolecules. Our results show that SPR can be a handy predictive tool by providing fundamental data for the design of downstream chromatographic purification processes, and thus very useful in early-stage process development of complex biopharmaceuticals.

2. Materials and methods

2.1. Cell culture and baculovirus production

Sf9 insect cells (ECACC #89070101, UK) were routinely grown in Gibco™ Sf-900 II SFM (serum-free) culture medium (Invitrogen, Paisley, UK) using spinner (stirred at 150 rpm in a magnetic stirrer) or Erlenmeyer vessels (shaken at 110 rpm in an orbital shaker). Cell concentration and viability were routinely assessed by haemocytometer (Brand, Wertheim, Germany) with cell viability evaluated by 0.4% trypan blue exclusion dye (Merck, Darmstadt, Germany) in phosphate-buffered saline.

The vector used, rBV-green fluorescent protein (GFP), encodes for the baculovirus major structural capsid protein, vp39, containing on its *N*-terminal a GFP protein reporter (Kukkonen et al., 2003). Stock aliquots of rBV-GFP were produced in Sf9 cells grown in a