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"Salt tolerant" anion exchange chromatography for direct capture of an acidic protein from CHO cell culture

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ABSTRACT

The present study describes the use of the new HyperCel STAR AX "salt tolerant" anion exchange sorbent for the capture from Chinese Hamster Ovary (CHO) cell culture supernatant (CCS) of an acidic model protein (α-amylase). HyperCel STAR AX sorbent and other conventional anion exchangers were evaluated to purify biologically-active enzyme. Salt tolerance of the sorbent allowed reaching 5-fold higher dynamic binding capacity than conventional anion exchange during capture of the enzyme from neat (undiluted) CCS. After optimization of operating conditions, HyperCel STAR AX turned out to be the only sorbent allowing efficient protein capture directly from both neat and diluted CCS with consistent and satisfying purity, yield and productivity. Therefore implementation of the salt tolerant sorbent in industrial purification processes should allow avoiding time and cost consuming steps such as dilution or UF/DF that exclusively aim at establishing suitable conditions for ion exchange step without bringing any added value to the purification process performance. Altogether this study highlights the flexibility and cost-reduction potential brought in process design by the HyperCel STAR AX salt tolerant sorbent.

Introduction

Ion exchange chromatography is extensively used for biomolecule purification at different steps in downstream processing. As an example, anion exchange (AEX) and cation-exchange (CEX) sorbents are routinely used for intermediate or polishing steps for monoclonal antibody (mAb)[1] production to remove host cell proteins (HCP) or DNA contaminants [1]. Alternative mAb purification schemes designed to avoid the use of protein A also rely on ion exchange sorbents for capture steps [2]. In other purification schemes such as plasma fractionation or purification of recombinant proteins, ion-exchangers are often positioned at the first step for the capture of target proteins [3,4].

However, efficient use of conventional ion exchangers requires feed adjustments to low to moderate conductivity to achieve sufficient capacity [5–8]. As a consequence, dilution or diafiltration operations are required [9–11]. Such sample manipulations are resource-, cost- and time-consuming and imply the use of important volumes of water and tankage. To respond to the increasing pressure to reduce manufacturing costs [12], elimination of such operation units would be beneficial [13]. Therefore, the use of salt tolerant ion exchange sorbents, being able to deliver satisfying performance at physiological conductivity may greatly participate in improving downstream process economics.

In this context, the recently launched HyperCel™ STAR AX sorbent, a "salt tolerant" anion exchanger, provides high dynamic binding capacity at moderate to high conductivity over a wide range of pH. The "salt tolerance" of the sorbent is based on a primary amine ligand which offers the possibility to capture proteins at higher conductivity than conventional anion exchangers by exploiting both electrostatic and hydrogen bond interactions [14]. Thus, HyperCel STAR AX sorbent can be applied for the direct capture of proteins (target or contaminants) from feedstocks without conductivity adjustment by dilution or diafiltration.

In order to investigate potential associated process benefits, the optimization of the operating parameters for the capture of α-amylase was conducted. α-amylase from *Aspergillus oryzae* (pI 3.5, 51 kDa), a starch-degrading enzyme, was chosen as a model acidic protein for purification from a CHO cell culture supernatant (CCS). This enzyme is used in a variety of biopharmaceutical applications, and its acidic pI makes it a good target for a capture step using anion exchange sorbents [15]. HyperCel STAR AX sorbent was challenged for the capture of α-amylase and compared to standard anion exchangers. Finally, purification performances of the sorbents were compared in terms of yield and purity of the final α-amylase product as well as in terms of productivity.

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[1] *Abbreviations used:* mAb, monoclonal antibody; CHO, Chinese hamster ovary; CCS, cell culture supernatant; HCP, host cell proteins; DBC, dynamic binding capacity

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Material and methods

Materials

Biological material

CHO CCS was produced at Pall Life Sciences, (Portsmouth, UK). Target protein α-amylase from *Aspergillus oryzae* (E.C. 3.2.1.1) was obtained from Sigma–Aldrich (Saint Quentin Fallavier, France) and was spiked at 0.5–2 mg/mL in the CCS (pH 7.5, conductivity 12 mS/cm).

Materials and equipments

Analytical grade reagents were from Sigma–Aldrich (Saint Quentin Fallavier, France). HyperCel STAR AX sorbent (Pall ,Saint-Germain-en-Laye, France) as well as rigid agarose (Capto™ Q, GE Healthcare, Velizy-Villacoublay, France) and polymer-based (Toyopearl® GigaCap Q, Tosoh Europe N.V., Lyon, France) Q anion exchange sorbents were packed according to manufacturer's instructions into glass columns (Kronlab, YMC Europe, Dienslaken-Hiesfeld, Germany). Akta™ Explorer 100 and Akta Avant 25 systems (GE Healthcare, Velizy-Villacoublay, France) were used for all chromatographic runs. The ionic capacity is 160–220 μeq/mL for rigid Q agarose and 100–200 μeq/mL for polymer-based Q according to manufacturers, and is 115–196 μeq/mL for HyperCel STAR AX.

Methods

Analytical assays

α-Amylase quantification. α-Amylase activity was measured using a Ceralpha enzymatic assay from Megazyme (Wicklow, Ireland) performed in a 96-well plate format following a protocol adapted from manufacturer's instructions. 20 μl of samples or standards were transferred into the wells. Then 20 μl of substrate reagent were dispensed in all wells and the microplate was shaked vigorously for 5 s. The plate was left for incubation at ambient temperature for 10 min. Finally, 300 μl of stopping solution were added in the wells and absorbance was measured at 400 nm. Samples and standards (from 3.6 μg/mL to 0.06 μg/mL) were diluted with extraction buffer supplied in the kit. A calibration curve was plotted with standards absorbance to calculate α-amylase concentration.

Host cell proteins quantification

CHO host cell proteins (HCP) were quantified with an ELISA assay kit (Cygnus Technologies, Southport, USA) following manufacturer's protocol.

Total protein quantification

Total protein concentration was measured using BCA assay kit (Thermo Fisher Scientific, Courtaboeuf, France) according to manufacturer's protocol using BSA as protein standard.

SDS–PAGE electrophoresis

SDS–PAGE analysis in non-reducing conditions was done with NuPAGE® 4–12% Bis-Tris precast gels and stained with Coomassie SimplyBlue Safe stain (Life Technologies Invitrogen, Saint Aubin, France) according to manufacturer's protocol.

Evaluation of α-amylase pH and conductivity stability range

α-Amylase stability was investigated by preparing 1 mg/mL α-amylase solutions at different pH and conductivity using 50 mM sodium acetate buffer and NaCl. The different solutions and a control solution made in extraction buffer provided in the α-amylase assay kit were stored overnight at 4 °C and then assayed for activity.

Chromatographic runs

Runs were performed on HyperCel STAR AX sorbent, conventional rigid Q agarose and polymer-based Q sorbents packed in 1 mL columns (0.5 × 5.0 cm) at 1 min residence time. Neat or diluted (in 50 mM Tris–HCl pH 7.5) CCS were used as loading feed.

Evaluation of dynamic binding capacity (DBC)

DBC at 10% breakthrough was measured at different loading conditions utilizing neat (undiluted) or diluted CCS. The column was first equilibrated for 10 CV with 50 mM Tris–HCl pH 7.5 buffer at the same conductivity as the loading feed. Then CCS, adjusted to the desired conductivity, was loaded and flow through was fractionated. α-Amylase concentration was measured in the resulting fractions to determine the 10% breakthrough volume. The dynamic binding capacity was calculated according to Eq. (1).

$$DBC_{10\%\,B.T.} = C_\alpha \frac{V_{10\%} - V_0}{V_c} \quad (1)$$

where $DBC_{10\%\,B.T.}$ is the dynamic binding capacity (mg/mL), C_α is the concentration of α-amylase in the CCS loaded (mg/mL), $V_{10\%}$ is the volume of CCS at 10% breakthrough (mL), V_0 is the holdup volume of the system (mL), and V_c is the packed bed volume of the sorbent (mL).

Optimization of elution conditions

Optimization of elution was carried out using a salt gradient with 2-fold diluted CCS as loading feed. The column was first equilibrated with 10 CV of 50 mM Tris–HCl pH 7.5 adjusted to the same conductivity as the load. After loading a volume of CCS corresponding to 60% of the DBC, a wash with 15 CV of equilibration buffer was applied to remove unbound material. Then a linear salt gradient was performed during 30 CV from equilibration buffer to 50 mM Tris–HCl containing 1 M NaCl. A final 5 CV strip using 50 mM Tris–HCl pH 7.5 containing 2 M NaCl and a clean-in-place procedure using 1 M NaOH (1 h contact time) were performed to regenerate the sorbent.

Optimization of wash conditions

Different wash conditions were evaluated using either neat or 2-fold diluted CCS as loading feed. The column was first equilibrated with 50 mM Tris–HCl pH 7.5 at the same conductivity than the feed for 10 CV. A volume of CCS corresponding to 60% of the DBC was loaded followed by a first wash with equilibration buffer during 15 CV. Then, a second 10 CV wash using 50 mM sodium acetate pH 4.5 at 2 mS/cm or 5 mS/cm, 50 mM Tris–HCl pH 7.5 at 15 mS/cm, or equilibration buffer was applied for 10 CV. Elution was performed with 10 CV of equilibration buffer adjusted with NaCl at the adequate conductivity determined by the elution optimization. The sorbent was finally regenerated using the same conditions as for the elution optimization.

Evaluation of performance

α-Amylase yield and purity in elution fractions of the different chromatographic runs were calculated according to Eqs. (2) and (3), respectively.

$$Y_\alpha = \frac{A_E \times 100}{A_L} \quad (2)$$

$$P_\alpha = \frac{A_E \times 100}{C_E + A_E} \quad (3)$$

where Y_α is the yield of α-amylase in elution fraction according to load (%), A_E is the amount of α-amylase in the elution fraction (mg), A_L is the amount of α-amylase in the loaded CCS (mg), P_α is