Efficient Purification of a Recombinant Protein from *Escherichia coli* Fermentate with Nuvia aPrime 4A Resin

David Frisch,[1] Hyunsic Choi,[1] James M. Sulzberger Jr.,[2] William Rushton,[2] Daniel M. Yoshikawa,[2] Sharon Bola[2]
[1] Scarab Genomics, 1202 Ann Street, Madison, WI 53713
[2] Bio-Rad Laboratories, Inc., 6000 Alfred Nobel Drive, Hercules, CA 94547

Protein Purification

Bulletin 7302

Abstract

A purification protocol to purify a recombinant protein from *E. coli* was developed and optimized. Mixed-mode, ion exchange, and hydrophobic interaction chromatography (HIC) resins from various commercial vendors were screened for the capture step. Goals were to minimize fermentation dilution, determine acceptable binding conditions, and increase purity and recovery to >75%. A hydrophobic anion exchange mixed-mode resin was selected for the capture step. Optimal wash and elution conditions were determined using a design of experiment (DOE) approach. An elution buffer containing NaCl and $MgCl_2$ was found to efficiently purify the target molecule with high recovery and purity.

Introduction

The purification and manufacture of recombinant proteins, especially those lacking affinity handles, often requires multiple chromatography steps and complicated feedstream conditioning, resulting in high production costs and relatively low recovery. These issues may be exacerbated by low protein expression, pH sensitivity, and limited stability. Mixed-mode chromatography resins have become an important tool for the purification of such proteins. The objective of this study was to purify one such recombinant protein, EPA, from *E. coli* fermentate to >95% purity and recovery. EPA is the detoxified form of exotoxin A from *Pseudomonas aeruginosa* and is used as a carrier protein in conjugate vaccines. EPA enhances the immune response of weakly immunogenic antigens (either peptides or polysaccharides) conjugated to it. A number of conjugate vaccines made with EPA are in clinical trials. EPA is a single polypeptide ~80 kD protein with a theoretical pI of ~5.32 and is known to have stability issues at pH <6.0.

Methods

E. coli fermentate was produced and processed using Clean Genome and C-Flow Technologies, developed by Scarab Genomics, LLC. Initially, multiple mixed-mode, anion exchange, and hydrophobic interaction chromatography resins were screened using standard protocols: ascending salt gradients for ion exchangers at an appropriate pH, descending ammonium sulfate gradients for hydrophobic interaction at a neutral pH, and a combination of high salt and arginine gradients to evaluate ionic and hydrophobic effects on mixed-mode resins. While many resins were screened, only Bio-Rad's Nuvia aPrime 4A Resin satisfied all capture requirements (Table 1).

BIO-RAD

Results

Resins were screened based on binding, recovery, purity, and elution conditions. While many resins were screened, only Bio-Rad's Nuvia aPrime 4A performed well in all categories evaluated. The resins that did not bind effectively were not evaluated on other criteria. While Pall Biotech PPA HyperCel Resin performed well in terms of binding, neither good recovery nor favorable elution conditions could be established for it.

Table 1. Summary of resin screening results.

Chromatography Resins	Binding	Recovery	Purity	Elution Conditions
Bio-Rad Laboratories, Inc. Nuvia aPrime 4A	✔	✔	✔	✔
HyperCel STAR AX (Pall Biotech)	X	*	*	*
TOYOPEARL Butyl-650M (Tosoh Bioscience)	X	*	*	*
TOYOPEARL Phenyl-600M (Tosoh Bioscience)	X	*	*	*
CMM HyperCel (Pall Biotech)	X	*	*	*
HEA HyperCel (Pall Biotech)	X	*	*	*
PPA HyperCel (Pall Biotech)	✔	X	*	X

* Indicates criteria were not evaluated due to primary criteria (binding) not being satisfied by chromatographic media.

Initial Capture Purification Study

Capture chromatography was performed with Nuvia aPrime 4A Resin. At modest salt concentrations and neutral pH (Table 2), the majority of the impurities, such as host cell proteins, lipids, and nucleic acids, were eliminated in the column flow-through fractions (Figure 1).

Table 2. Initial purification protocol.

Step	Buffer Description	Volume, CV	Flow Rate, CV/min
Equilibration	50 mM MOPS + 130 mM NaCl (15 mS/cm), pH 7.0	3	0.3
Load	Crude fermentate diluted 1:1 with equilibration buffer (A_{280} = 900 mAU)	1.2	0.3
Wash 1	50 mM MOPS + 130 mM NaCl (15 mS/cm), pH 7.0	5	0.3
Elution 1	50 mM MOPS + 1 M NaCl (86 mS/cm), pH 7.0	5	0.3
Elution 2	750 mM Arg, pH 9.0	5	0.3
Strip	0.5 M acetic acid	5	0.3

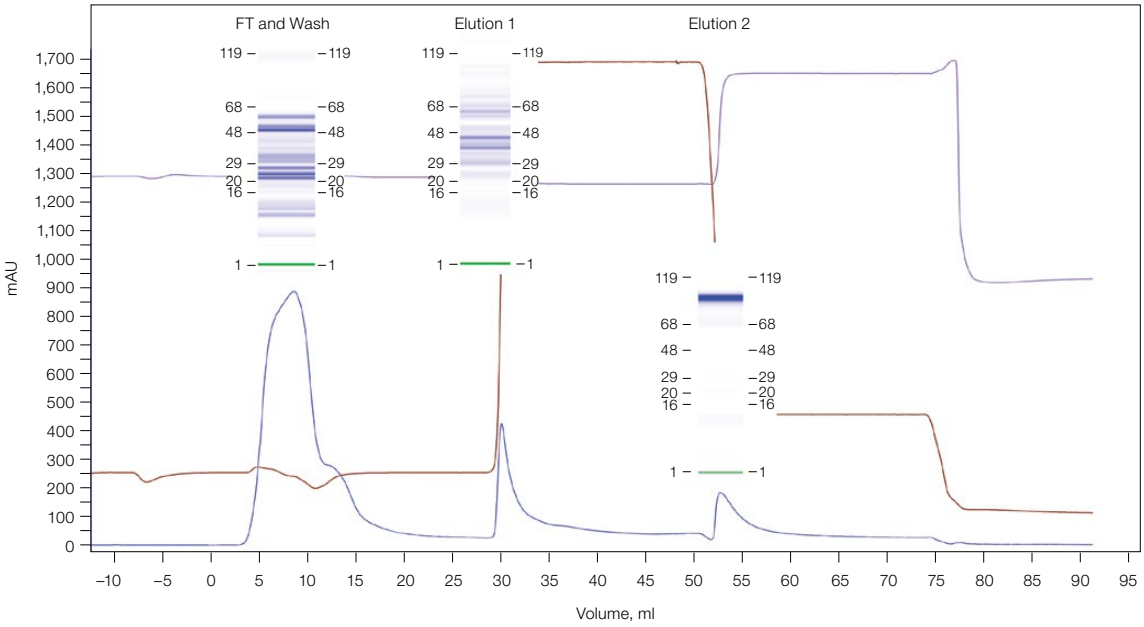

Fig. 1. Capture chromatography with Nuvia aPrime 4A Resin. Further optimization of elution conditions was needed to elute the target protein completely from the column. FT, flowthrough.

Optimization of the Capture Purification Conditions

Interestingly, the target protein was eluted from the column not by a high concentration of NaCl (Figure 1, Elution 1), but by high concentrations of arginine at pH 9.0. Finding an alternative to the use of arginine for elution was desirable due to its cost and its effect on subsequent columns. Divalent metal ion (Ca^{+2} and Mg^{+2})–containing buffers were found effective for recovery of the target. A DOE approach was used to determine the best conditions and, based on the purity and recovery results (Figure 2A), the final elution was performed. See Table 3 for elution conditions and Figure 2B for results.

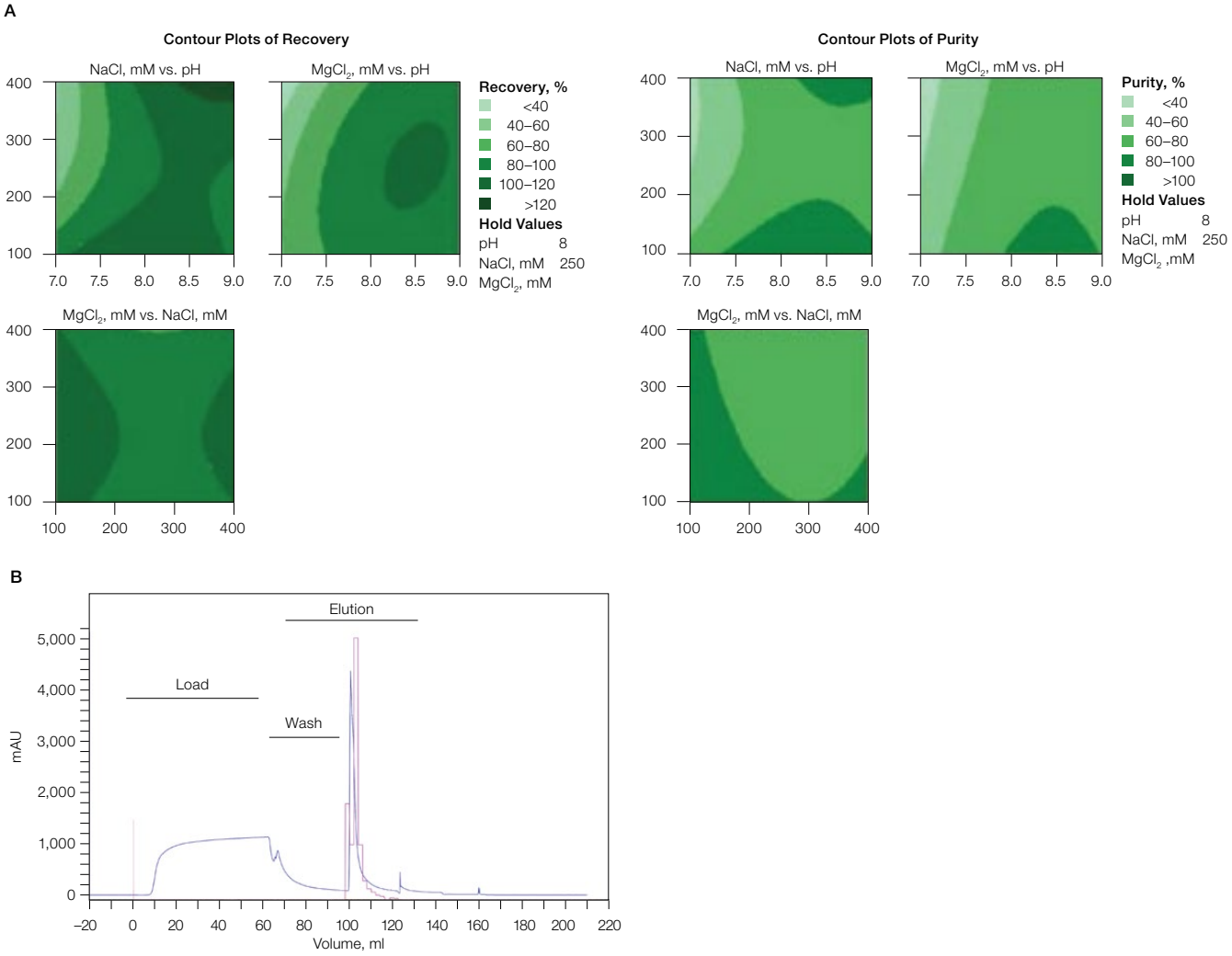

Fig. 2. DOE and final capture results. A, contour plots for purity and recovery with the various salt/additive concentrations and pH levels. **B**, optimized capture chromatography with Nuvia aPrime 4A Resin.

Table 3. Final elution conditions.

Step	Buffer	Volume, CV	Linear Velocity, cm/hr
Equilibration	25 mM Tris, pH 7.0	16	150
Load	Flocculated SCRB-981 fermentate diluted 1:1 with 25 mM Tris pH 7.0 (8 mS/cm, pH 7.0, A_{280} = 7.9)	16	150
Wash	25 mM Tris + 200 mM NaCl, pH 7.0	8	150
Elution	25 mM Tris + 400 mM NaCl + 250 mM $MgCl_2$, pH 8.5	4	150
Strip	0.5 M NaOH	4	150

Polish Purification

Following DOE studies, CHT Type I Media, 40 µm illustrated promise as a polishing step, resulting in greater than 95% purity and greater than 75% recovery of the target protein (Figure 3).

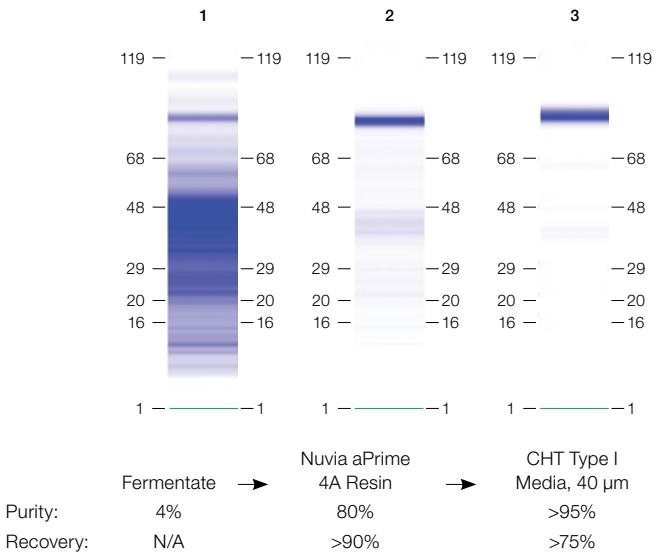

Fig. 3. SDS-PAGE analysis from the two-step purification workflow involving Nuvia aPrime 4A Resin.

Conclusions

This is an effective purification workflow option for purification of proteins that are expressed at low levels and lack affinity handles. Both mixed-mode chromatography resins, Nuvia aPrime 4A and CHT Type I, are macroporous particles, which offer efficient mass transfer in a wide range of operational flow rates. Moreover, the gentle chromatography conditions and the easy step transition allow maximum protection of the target protein integrity. The two-step workflow is robust and readily scalable for process production.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399* ***Canada*** *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23* ***France*** *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050* ***Italy*** *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23* ***Russian Federation*** *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23* ***Switzerland*** *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

Bulletin 7302 Ver B US/EG *20-0492 0820 Sig 0220*