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Evaluation of commercial chromatographic adsorbents for the direct capture of polyclonal rabbit antibodies from clarified antiserum☆

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Abstract

We have carried out a rigorous evaluation of eight commercially available packed bed chromatography adsorbents for direct capture and purification of immunoglobulins from clarified rabbit antiserum. Three of these materials featured rProtein A (rProtein A Sepharose Fast Flow, Mabselect, Prosep rProtein A) as the affinity ligand, and differed from one another primarily with respect to the underlying base matrix. The remaining five matrices comprised various synthetic low molecular weight ligands immobilised on hydrophilic porous supports and these included: MEP HyperCel, MabSorbent A1P, MabSorbent A2P, FastMabsA and Kaptiv-GY. The general experimental approach taken was to sequentially challenge packed beds of each matrix with a series of different strengths of a clarified antiserum; beginning with the weakest and ending with the strongest. Marked differences in performance (principally evaluated on the basis of dynamic binding capacity, recovery, and purity) were obtained, which allowed clear recommendations concerning the choice of adsorbents best suited for antibody capture from rabbit antisera, to be made.

Keywords: Biomimetic ligands; Hydrophobic charge induction; Immunoglobulin purification; Mixed mode chromatography; Protein A; Lipoproteins

1. Introduction

Currently the large-scale purification of polyclonal antibodies is, for the most part, carried out by the plasma fractionation industry, with production being geared towards generation of a wide range of plasma proteins (including albumin, Factor VIII, Factor IX, Protein C and von Willebrand Factor) rather than single antibody products [1–3]. Since the first reports on the use of immobilised Protein A for the affinity purification of antibodies over 30 years ago [4,5]. Protein A affinity chromatography has become the industrial standard for the purification of clinical grade monoclonal antibodies [3,6–8], but is rarely applied in the plasma fractionation industry [1,2], largely for reasons of cost and reservations concerning Protein A's chemical stability, adsorbent shelf-life and cleaning [9]. For the production of diagnostic antibody products such concerns are less serious; thus Protein A chromatography currently represents one of most potent separation tools available to the diagnostic industry.

Not withstanding the success of Protein A chromatography in the purification of antibodies, adsorbents based on this biological ligand suffer numerous drawbacks (alluded to immediately above), which have stimulated the search for stable synthetic low molecular weight ligand alternatives to [10–12] or mimics of Protein A [13,14]. As adsorbents based on such ligands allow rigorous cleaning without loss in ligand or binding capacity, their use with mildly conditioned, or even unconditioned, feedstocks (so called 'dirty' feedstocks) would appear to offer highly attractive prospects for bioprocess intensification.

In parallel with the development of new synthetic alternatives to Protein A, others have sought to improve Protein A adsorbents, largely through applying recombinant DNA techniques to: improve the ligand's tolerance to cleaning with sodium hydroxide [15,16]; lower the binding affinity to enable use of milder elution conditions [17]; and allowing

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Table 1
Description of adsorbents employed in this study

Adsorbent	Immobilised ligand	Description of base particle	Manufacturer
rProtein A Sepharose FF	Recombinant Protein A[a] (lot no. 279973, 6 mg mL^{-1})	60–165 μm highly cross-linked 4% agarose	GE Healthcare, Uppsala, Sweden
MabSelect	Recombinant Protein A[a] (lot no. 286511, 5 mg mL^{-1})	40–130 μm (av. 85 μm) highly cross-linked rigid 'HF' 3.5% agarose base matrix[b]	GE Healthcare, Uppsala, Sweden
ProSep rProtein A	Recombinant Protein A[c] (lot no. 113112724)	75–125 μm controlled pore high silica glass	Millipore, Billerica, MA, USA
MEP HyperCel	4-mercapto-ethyl-pyridine (lot no. A112, 100 μmol mL^{-1})[d]	Av. 90 ± 10 μm cellulosic base matrix	Pall Life Sciences, BioSepra AS, Cergy-Saint-Christophe, France
MabSorbents A1P & A2P	Mimics of key dipeptide motif in Protein A[e,f] (lot no's. FA0415 & FA0485, respectively)	75–125 μm highly cross-linked 6% agarose base matrix	Prometic Biosciences Ltd., Cambridge, UK
Kaptiv-GY	'Peptidomimetic'-branched synthetic peptide of 15 amino acids[g] (lot no. 0005021150, 8–10 mg mL^{-1})	50–80 μm bis-acrylamide/azalactone copolymer beaded matrix	Tecnogen, Piana di Monte Verna (CE), Italy
FastMabsA	A 'mixed mode' ligand[h] (lot no. XAES-A12, 73 μmol g^{-1})	80–250 μm highly cross-linked agarose base matrix	UpFront Chromatography A/S, Copenhagen, Denmark

[a] Engineered to include C-terminal cysteine allowing oriented coupling via thioether linkage through a stable 12-atom epoxide spacer arm to the base matrix (ensuring low ligand leakage).
[b] Rigid agarose support cross-linked with new technique (not disclosed) to allow high flow rates under process conditions.
[c] Engineering of rProtein A, the ligand density and coupling chemistry employed are proprietary to the producer. The stereochemistry of rProtein A immobilisation is designed to maximise antibody binding.
[d] Binding relies on salt-independent hydrophobic interaction, whereas elution is mediated by pH induced electrostatic repulsion, hence the term 'hydrophobic charge induction' [10,19–22].
[e] Ligands (structures not disclosed) comprise a triazine scaffold with two spatially oriented substituents that mimic the helical twist of the key dipeptide in Protein A [14].
[f] The ligand density is proprietary to the producer.
[g] Found by screening a peptide library by measuring ability to interfere with interaction of Protein A and biotinylated immunoglobulins [13].
[h] Low molecular weight synthetic ligand (structure not disclosed) having hydrophilic and hydrophobic functionalities within the same molecule [12].

for chemoselective oriented immobilisation to the base matrix through stable linkage chemistries [18].

Here, eight commercial adsorbents specifically intended for IgG purification were directly compared, for their ability to recover polyclonal antibodies from clarified rabbit antisera feedstocks of varying strength. Three of these materials were new improved rProtein A-linked chromatographic matrices, and the remainder comprised five low molecular synthetic ligand-based adsorbents (for descriptions of the ligands employed in this work the reader is referred to Table 1). The principal criteria evaluated in this work, of dynamic immunoglobulin (Ig) binding capacity, recovery and purity, provided the framework for selection of adsorbents well-suited to the task of antibody purification from complex antiserum feedstocks.

2. Materials and methods

2.1. Materials

The chromatography feedstocks (i.e. rabbit anti-human transferrin antiserum) and goat anti-rabbit serum used in the preparation of gels for crossed immunoelectrophoresis, were produced internally at DakoCytomation A/S (Glostrup, Denmark) following published procedures [23]. Pure rabbit albumin was prepared in house at DakoCytomation A/S using proprietary procedures, while rabbit Ig was available as a commercial product (X0903 concentrate and commercial product strength 20 g L^{-1}) at DakoCytomation A/S. The various chromatographic media employed, i.e.: MabSelect^{TM} and rProtein A Sepharose FF (GE Healthcare, Amersham Biosciences, Uppsala, Sweden); ProSep rProtein A (Millipore, Billerica, MA, USA); MEP HyperCel (Pall Life Sciences, BioSepra SA, Cergy-Saint-Christophe, France); MabSorbent A1P and MabSorbent A2P (Prometic BioSciences Ltd., Cambridge, UK); FastMabsA (UpFront Chromatography, Copenhagen, Denmark); and Kaptiv-GY^{TM} (Tecnogen, Piana di Monte Verna (CE), Italy), were obtained as gifts from the manufacturers (see Table 1 for detailed descriptions). High performance thin layer chromatography (HP-TLC) plates (Silica gel 60) and HAS 1000 protein grade agarose were acquired from Merck (Darmstadt, Germany) and Medinova Scientific A/S (Hellerup, Denmark), respectively. The staining reagents used on crossed immunoelectrophoretograms, i.e. Coomassie Brilliant Blue R250, Sudan Black and zinc acetate, were supplied by Kem-En-Tech (Copenhagen, Denmark), Sigma (St. Louis, MO, USA) and Merck (Whitehouse Station, NJ, USA), respectively, and Primuline stain for HP-TLC was purchased from Sigma (St. Louis, MO, USA). All reagents for reducing SDS–PAGE (NuPage® 10-well pre-cast '4–12%' polyacrylamide gradient gels, Mark 12^{TM} molecular weight marker, Simply Blue^{TM} Safe Stain, Novex Gel-Dry Solution, NuPage^{TM} sample and MOPS running buffer systems) were acquired from Invitrogen (Carlsbad, CA, USA). All other chemicals were of AnalaR or equivalent grade, and were obtained from registered suppliers.

2.2. Preparation of feedstocks for packed bed chromatography experiments

The chromatographic feedstock used in all chromatography experiments detailed within this paper, was a rabbit anti human