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Application of peptide chromatography for the isolation of antibodies from bovine skim milk, acid whey and colostrum

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ABSTRACT

Protein A mimetic peptide ligands have several benefits over conventional Protein A/G ligands, namely that they are small in size, have low production costs, are stable over a wide range of pH values and can withstand cleaning by harsh sanitization agents such as sodium hydroxide. In this paper, a hexamer peptide (HWRGWV) affinity matrix was used for the isolation of bovine immunoglobulins from various dairy streams (skim milk, acid whey and colostrum). Bound immunoglobulins were recovered in elution buffer (0.2 M sodium acetate buffer, pH 4.0) fractions with a purity of >85% in a single step. The peptide resin has achieved a maximum equilibrium adsorption capacity of $23 \pm 0.58\ mg\ mL^{-1}$ of resin for bovine IgG and had a dynamic binding capacity of $11.8 \pm 0.03\ mg\ mL^{-1}$ at residence time of 2 min. These results suggest that the hexamer peptide chromatography could potentially be used for the selective purification of bovine immunoglobulins from dairy streams. This method has promise as an alternative to conventional Protein A/G chromatography for direct capture of immunoglobulins from streams containing relatively high immunoglobulin concentrations such as colostrum, transgenic or hyper-immune milk.

Keywords: Protein A mimetic; Peptide ligands; Affinity chromatography; Bovine immunoglobulins; HWRGWV hexamer peptide; Colostrum

1. Introduction

The growing importance of bovine immunoglobulins as therapeutics and functional foods has provoked the need for innovative process technologies to isolate immunoglobulins (Igs) from dairy fluids. To date, most antibodies available on the market have been purified by affinity chromatography using either Protein A or Protein G ligands (Dancette et al., 1999; Farid, 2006; Fuglistaller, 1989; Hober et al., 2007). However, these ligands pose several inherent problems for process development because proteinaceous ligands are expensive and lose activity during the sanitization conditions generally applied during the column regeneration process. To overcome some of these drawbacks, a synthetic peptide ligand was used

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as an alternative affinity ligand and a chromatographic process for selective isolation of IgG from bovine milk fluids was developed.

Synthetic peptide based affinity chromatography processes have been successfully applied for the isolation of antibodies from human serum and cell culture media (D'Agostino et al., 2008; Fassina et al., 1996; Linhult et al., 2005; Menegatti et al., 2012). Small ligands such as peptides have potential advantages in chromatography processes because they can be more stable and less immunogenic than protein ligands, can be constructed with a wide variety of bio-specificity and their production cost is low (Fassina et al., 1996; Yang et al., 2005). Although several Protein-A Mimetic peptide ligands have been synthesized, screened and evaluated for their suitability in chromatography process development (Fassina, 2000; Roque et al., 2004; Yang et al., 2005), only a few have been extensively studied. For example, PAM peptide TG19318 (Fassina et al., 1998), a tetrameric peptide ligand, was successfully investigated for the isolation of various classes of human Igs (IgG, IgA, IgE, IgM) (Huse et al., 2002) from human serum and bacterial cell culture broths. Short linear hexameric peptide ligands HWRGWV, HYFKFD and HFRRHL were identified and developed by Carbonell's group through screening of combinatorial libraries on a solid resin and its selectivity towards human Igs through the Fc region was characterized (Yang et al., 2005, 2009). Unlike Protein A/G ligands, these hexameric peptides have a binding spectrum for all subclasses of human Igs (hIgG, IgD, IgE, IgM and, to a lesser extent, IgA) (Liu et al., 2012). They have binding interactions with IgG of different species such as bovine, rat, goat and mouse for which Protein A shows weak or no binding. Using the hexameric peptide ligands two commercial monoclonal antibodies were purified from CHO cell culture supernatants with good log reduction values for host cell proteins and residual DNA (Naik et al., 2011).

Systems such as milk and transgenic plants offer a potential economic alternative to CHO supernatants. However, the impurities encountered in these systems are different from those of CHO supernatants and therefore there is a need to study and develop purification strategies for these systems. Recently, a process comprising of a pretretament step and affinity chromatography using HWRGWV ligand was reported for purification of monoclonal antibody from transgenic *Lemna* plant extract (Naik et al., 2012). A few studies (Billakanti, 2009; Menegatti et al., 2012) have reported applications using hexameric peptide ligand for the isolation of IgG from dairy streams, which contain not only high levels of casein, whey proteins and lactose but also fat globules. Affinity chromatography processes using such challenging feed streams, where the value of the IgGs (polyclonal antibodies) is likely to be considerably lower than for recombinant therapeutic monoclonal antibodies, will require the advantages described above for small-molecule affinity ligands.

In this paper, therefore, we have investigated the application of the hexamer peptide, HWRGWV as an affinity ligand for the isolation of polyclonal bovine IgG (bIgG) from bovine milk streams.

2. Materials and methods

2.1. Reagents and instrumentation

The HWRGWV ligand was synthesized by Peptides International (Louisville, KY) on a Toyopearl AF-Amino-650M resin (particle size 65 μm) (Tosoh Bioscience, Inc., Montgomeryville, PA, USA) at a ligand density of 0.11 meq g^{-1}. Individual bovine milk proteins, including α-lactalbumin (L5385), β-lactoglobulin (L3908), Bovine Immunoglobulin (bIgG, I5506), BSA (A7906) and caseins were purchased from Sigma–Aldrich New Zealand Ltd, Auckland, New Zealand. Lactoferrin and lactoperoxidase were obtained from the Tatua Co-operative Dairy Company, Morrinsville, New Zealand. The AgResearch Group, Hamilton, New Zealand, kindly gifted bovine immunoglobulin A (bIgA). All other reagents, including sodium phosphate, sodium acetate and guanidine–HCl were of analytical grade and purchased from Sigma Aldrich New Zealand Ltd, Auckland, New Zealand.

For surface plasmon resonance (SPR) experiments, amine coupling reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl (EDAC, 0.4 M), sulfo-*N*-hydroxysuccinimide (NHS, 0.1 M), ethanolamine–HCl (1.0 M, pH 8.5), sodium acetate buffer (10 mM, pH 5.0) and GLC biosensor chips were purchased from Bio-Rad Laboratories New Zealand Ltd, Auckland, New Zealand. Glycine–HCl buffer (10 mM, pH 1.5 and 1.75) and HBS-EP running buffer (containing 10 mM 4-2-hydroxyethyl piperazine-1-ethanesulfonic acid (HEPES), 150 mM NaCl, 3.4 mM ethylenediaminetetraacetic acid (EDTA), 0.005% P20 (Tween 20) surfactant at pH 7.4) were prepared in the laboratory using analytical grade chemicals purchased from Sigma–Aldrich New Zealand Ltd, Auckland, New Zealand. Affinity purified sheep anti-bovine IgG (A10-118, 1 mg mL^{-1}) was purchased from Bethyl Laboratories (Montgomery, TX, USA). Skim milk was purchased from a local retail store and whey was prepared from this as described below. Spray dried colostrum powder was purchased from a local health and nutrition store. A ProteOn XPR36 SPR system (Bio-Rad Laboratories New Zealand Ltd, Auckland, New Zealand) was used to determine the IgG concentrations as described by Billakanti et al. (2010). RP-HPLC was performed on an AKTA*explorer* 10 (GE Healthcare Life Sciences, Uppsala, Sweden).

For SDS-PAGE experiments, an Xcell4 *SureLock*™ Midi-Cell system (WRO100), NuPAGE Novex 4-12% Bis-Tris midi-gels (Lot No. 9081482, 1 mm × 20 wells), NuPAGE LDS sample buffer (4×) (Cat. No. NP0007), NuPAGE MES SDS running buffer (20×) (Cat. No. NP0002) and Novex Sharp pre-strained protein markers (P/N 57318) were purchased from Life Technologies New Zealand Ltd, Auckland, New Zealand.

2.2. Methods

2.2.1. Peptide chromatography column preparation

The hexamer peptide, supplied as a dry resin, was stored at 4 °C for short term (<7 days) and was kept at −18 °C for longer storage. Peptide resin (280 mg, dry weight) was swollen in 10 mL of 20% (v/v) methanol in water in a 15 mL falcon tube for 1 h in an end-to-end shaker. The swollen wet resin was then carefully packed without trapping air bubbles into an HR 5/10 column (5 mm i.d. × 10 cm length, GE Healthcare Technologies, Uppsala, Sweden) (total resin volume of 1 mL) and 20% aqueous methanol solution was passed through the column at a flow rate of 0.2 mL min^{-1} for 20 h. The peptide resin was then washed and equilibrated with 20 mM phosphate buffer saline (equilibration buffer) containing 1 M NaCl (20–30 column volumes (CV) prior to first use and 6 CV before all subsequent runs) at a flow rate of 1 mL min^{-1}. Bovine IgG samples prepared in the equilibration buffer at pH 7.4 were injected onto the peptide column at a flow rate of 1 mL min^{-1}, followed by washing