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Purification of a human immunoglobulin G1 monoclonal antibody from transgenic tobacco using membrane chromatographic processes

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Abstract

Efficient purification of protein biopharmaceuticals from transgenic plants is a major challenge, primarily due to low target protein expression levels, and high impurity content in the feed streams. These challenges may be addressed by using membrane chromatography. This paper discusses the use of cation-exchange and Protein A affinity-based membrane chromatographic techniques, singly and in combination for the purification of an anti-*Pseudomonas aerugenosa* O6ad human IgG1 monoclonal antibody from transgenic tobacco. Protein A membrane chromatography on its own was unable to provide a pure product, mainly due to extensive non-specific binding of impurities. Moreover, the Protein A membrane showed severe fouling tendency and generated high back-pressure. With cation-exchange membrane chromatography, minimal membrane fouling and high permeability were observed but high purity could not be achieved using one-step. Therefore, by using a combination of the cation-exchange and Protein A membrane chromatography, in that order, both high purity and recovery were achieved with high permeability. The antibody purification method was first systematically optimized using a simulated feed solution. Anti-*P. aeruginosa* human IgG1 type monoclonal antibody was then purified from transgenic tobacco juice using this optimized method.

Keywords: Membrane chromatography; Transgenic; Tobacco; Monoclonal antibody; Affinity; Ion-exchange

1. Introduction

Monoclonal antibodies (mAbs) are traditionally produced by mammalian cell culture [1,2], which is technically demanding, difficult to scale-up and expensive due to high capital and operating costs. Also, products synthesized by mammalian cell culture have the potential of being contaminated by animal viruses and other pathogens so their removal is needed to comply with regulatory requirements [3,4]. Molecular farming, which refers to the production of recombinant proteins in genetically modified plants, provides a new option for monoclonal antibody production [5–8]. The potential for this technology was first demonstrated by the expression of a functional antibody in tobacco [9]. More recently, antibody fragments such as an anti-picloram single-chain Fv Ab and an anti-botulinum toxin A neutralizing single-chain Fv Ab were also expressed in tobacco [10,11]. Some of the problems associated with mammalian cell culture can be solved using plants. For example, expression of proteins in transgenic plants is much safer than by CHO cell culture or expression in transgenic animals since plants are not known to carry any human or animal pathogens. Transgenic tobacco is considered safer than using edible crop plants since the transgenic protein will not end up in the food chain. Furthermore, protein production based on large-scale transgenic plant agriculture should be economically viable because of the low cost of plant growth and harvest as well as the large-scale of biomass production [11–15].

Separation and purification is a major challenge facing the manufacturers of plant-derived antibodies. The expression level of a mAb in transgenic tobacco is normally about 0.1% of total soluble protein (TSP) [5]. Moreover, tobacco leaves contain chemicals such as cellulose, alkaloids, pigments, polyphenols and a plethora of plant proteins from which the antibodies have to be selectively purified. Therefore, column chromatographic techniques used for purifying mAbs from CHO cell culture supernatant may not be suitable for purifying tobacco-based

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mAbs. The major limitations with column chromatography are high back-pressure, low product throughput and scale-up problems. These limitations result mainly from the use of soft gel-based chromatographic media within which diffusion-based mass transport predominates.

Membrane chromatography is a fast growing bioseparation technique which combines the advantages of membrane technology and chromatography, thereby leading to the possibility of developing high-throughput and -resolution purification methods. Due to the predominance of convective mass transport, the binding capacity of membranes is generally independent of flow rate [16]. Higher productivities and easier scale-up relative to column-based processes are, therefore, possible with membrane chromatography. Membrane chromatography is particularly well suited for application where large volumes of dilute feed solutions need to be processed. For example, Protein A membrane chromatography (PAMC) has been used for purification of human immunoglobulin [17]. Monoclonal antibody purification from CHO cell culture supernatant has also been studied using membrane chromatography [18–21]. To the best of our knowledge the use of membrane chromatography for mAb purification from transgenic plant has not yet been reported. Membrane chromatography is expected to be suitable for this application because large volumes of tobacco juice containing very low concentrations of mAb are used, which makes conventional packed bed chromatography unsuitable for mAb purification.

The current research explores the purification of mAbs from transgenic tobacco leaves using various membrane chromatographic techniques: PAMC, ion-exchange membrane chromatography (IEMC), and PAMC and IEMC in combination. The two mAbs used in this study were human (h)IgG1-CD4 (which is a CHO cell culture-derived antibody [22]), and anti-*Pseudomonas aeruginosa* O6ad hIgG1 (expressed in transgenic tobacco [23]); hIgG1-CD4 is a humanized IgG1 type antibody which has been shown to be quite promising in the treatment of refractory psoriasis and rheumatoid arthritis [22]; anti-*P. aeruginosa* hIgG1 is a fully human antibody with protective action against this pathogenic bacterium [23,24]. Wild type tobacco juice was first spiked with hIgG1-CD4, i.e., simulated feed solution. This solution was used to systematically optimize operating conditions for the membrane chromatographic purification processes. After establishing the separation and purification methodology for mAb purification, anti-*P. aeruginosa* hIgG1 mAb was purified from transgenic tobacco using this method.

2. Experimental

2.1. Materials

Humanized monoclonal antibody hIgG1-CD4 (batch 12) was kindly donated by the Therapeutic Antibody Center, University of Oxford, UK. The samples in vials were shipped in dry ice and used as-received. Chemical reagents, including sodium phosphate (mono- and di-basic), sodium citrate, citric acid and sodium chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). All buffers were prepared using ultra-pure water (18.2 MΩ-cm) obtained from a Diamond Nanopure water purification unit (Barnstead International, Dubuque, IA, USA) and vacuum-filtered using 0.45 μm cellulose acetate membrane (Nalgene Nunc, Rochester, NY, USA; order number DS0210-4045). Sartobind S cation-exchange membrane sheets (thickness = 275 μm, catalogue number 94IEXS42-001) for IEMC and Sartobind Protein A 75 membrane module (catalogue number 93PR-A06DB-12–V) for PAMC were purchased from Sartorius (Goettingen, Germany). A Protein A chromatographic column (1 ml rProtein A FF, catalogue number 17-5079-02) was purchased from GE Healthcare Bio-Sciences (Uppsala, Sweden). A homozygous transgenic tobacco line was bred for uniform production of the anti-*P. aeruginosa* hIgG1 by standard genetic breeding procedures from a primary transgenic plant (produced in Ref. [23]) identified with a single T-DNA locus (performed according to procedures outlined in Ref. [25]; data not shown). Transgenic and wild type tobacco (cultivar 81v9; Ref. [26]) were grown in a greenhouse at the University of Guelph, Ontario, Canada and the leaves were stored in plastic bags at −20 °C following their harvest.

2.2. Pretreatment of tobacco leaves

The overall bioseparation method for obtaining pure mAb from tobacco leaves was composed of two processing segments: pretreatment (i.e., recovery and isolation) and purification (see Fig. 1). Tobacco leaves were removed from the freezer and immediately crushed into small pieces within the plastic bag. The crushed leaves were mixed with extraction buffer (40 mM pH 7.0 phosphate buffer containing 50 mM ascorbic acid as antioxidant, 50 μM leupeptin as protease inhibitor, and 10 mM sodium EDTA), using 2 l buffer per kg of leaves, and ground in a 51BL32 blender (Waring Commercial, Torrington, CT, USA) for 3 min (three pulses of 1 min each with 30 s between each pulse). The grinding process reduced the particle size of the tobacco leaves to ca. 1 mm. The ground material was homogenized for 3 min (three pulses of 1 min each with 30-s intervals between each pulse) using a flow-through Ultra Turrax T25 Basic homog-

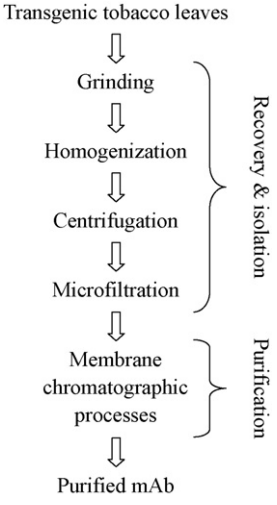

Fig. 1. Scheme for purifying mAb from transgenic tobacco leaves.