Process Biochemistry 49 (2014) 1005–1011

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Review

Negative chromatography: Progress, applications and future perspectives

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ARTICLE INFO

Article history:
Received 5 January 2014
Received in revised form 27 January 2014
Accepted 24 February 2014
Available online 7 March 2014

Keywords:
Negative chromatography
Flow-through chromatography
Protein purification
Adsorbent
Recombinant protein
Downstream bottleneck

ABSTRACT

In negative chromatography, the impurities bind on the adsorbent, and the product is allowed to flow through the chromatographic column. Negative chromatography is an alternative to positive chromatography under certain circumstances and has been used to purify various biomolecules. For this review, a detailed survey of the performance of reported studies on negative chromatography was conducted. The applications of negative chromatography in the capture and intermediate purification steps for biomolecules (e.g., plasmid DNA, antibodies, enzymes, hemoglobin, virus particles and cells) are reviewed. The negative chromatographic adsorbents adsorb the impurities through surface charge, hydrophobic interaction at specific sites on the surface, hydrophobic interaction, hydrogen bonding and functional groups. Examples of applications of negative chromatography according to the type of chromatography matrix used are summarized and discussed. In addition, the effects of operating conditions (initial protein concentration, buffer ions, pH and salt concentration) are discussed, and the criteria for choosing negative or positive chromatography are summarized. The literature survey showed that there will be future limitations and challenges ahead in implementation of negative chromatography. Possible solutions to the limitations and challenges of negative chromatography and future trends for developing negative chromatography are discussed.

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http://dx.doi.org/10.1016/j.procbio.2014.02.018
1359-5113/

1. Introduction

Liquid chromatography is still the dominant technique for improving the purity of a product. This method outstrips other bioseparation techniques in terms of process capacity, recovery, scalability and well-established setup. A typical recovery process for a recombinant protein involves three chromatographic steps: capture, intermediate purification and polishing [1]. The desired proteins remain on the stationary phase and are concentrated and stabilized during the capture step. The subsequent chromatographic step removes bulk impurities. The polishing step separates trace impurities from the desired protein. This sequence has become the industrial platform for monoclonal antibody purification [2].

The chromatographic technique can be divided into two modes depending on the type of adsorbate: positive chromatography retains the desired product on the stationary phase while the unbound impurities flow through the column, and vice versa for negative chromatography. In most cases, positive chromatography is applied in the capture and intermediate purification steps, whereas negative chromatography is mostly used in the polishing step. For the convenience of the purification process, positive chromatography can be perceived as easier, as it involves the product instead of impurities. To the best of the authors' knowledge, no previous review has exclusively focused on the features of negative chromatography. Only side notes on negative chromatography have been reviewed by Stellwagen [3] and Levison [4].

In the present review, the applications of negative chromatography in the capture and intermediate purification steps for biomolecules (e.g., plasmid DNAs, antibodies, enzymes, hemoglobin, virus particles and cells) are discussed. Primary focus is given to identifying the feasibility of the application of negative chromatography in the capture step. This review also reviews the progress of negative chromatography to identify why negative chromatography is less popular in the primary step. The limitations and challenges faced by negative chromatography were investigated, and possible solutions to improve its performance and the future prospects of negative chromatography are discussed.

2. Applications of negative chromatography

This section provides an overview of the progress of negative chromatography based on the available published studies. The mechanism of negative chromatography is illustrated in Fig. 1. In negative chromatography, adsorbents adsorb the impurities through surface charge [5], hydrophobic interaction at specific sites on the surface [6,7], hydrogen bonding and functional groups [8,9]. The examples of application of negative chromatography are shown in Table 1 according to the type of chromatography matrix used and are discussed in this section.

2.1. Types of chromatography

2.1.1. Dye affinity chromatography

The adsorption of proteins to immobilized dyes generally consists of specific and non-specific interaction. The specific interaction is known to be contributed by the nucleotide-specific interaction and biomimetic enzyme-coenzyme interaction. The non-specific interactions are contributed by their different functional groups where amine and sulfonate groups could contribute to electrostatic interaction while aromatic ring structure could contribute to hydrophobic interaction.

Dye affinity chromatography has been applied as the primary capture step to minimize fouling and improve the separation efficiency on subsequent chromatography columns for enzyme purification [10–15]. Among the dye ligands have been used are (generic name, commercial name) Purple A (generic name is not known) [10]; Navy HE-R (Reactive Blue 171) [11]; Scarlet MX-G (Reactive Red 8) [12]; Green H-E4BD (Reactive Green 19), [13,14] and Red H-E3B (Reactive Red 120) [15]. One should note that because the interaction of the dye with the enzyme is not clearly defined, empirical screening for a suitable dye is normally performed rather than simply adopting a commonly used dye [10,11]. The trial and error steps involve could be time consuming. Commercialized dye screening kit is available (e.g., PIKSI-M Mimetic screen kit from Prometic Biosciences, Ltd.) to ease the identification of the suitable dye ligand. The dye with the least product adsorption and highest removal of impurities is preferred. The dye with a higher removal efficiency should be chosen when there is a tradeoff between product recovery and the removal of impurities. Product loss is observed because many interactions could be involved in the co-adsorption of the product.

2.1.2. Immobilized metal affinity chromatography (IMAC)

IMAC performs adsorption through the metal affinity of histidine and cysteine protein side chains to the metal ions (e.g., nickel and copper ions) on the stationary phase. It is an unusual case when IMAC is chosen as the primary capture step in the negative mode. As IMAC has high specificity, it is not generally a practical means of removing heterogeneous impurities. Related studies have been performed on the purification of enzymes [16] and inhibitors [17].

Like other methods, in the purification of an enzyme, IMAC was used as a precolumn [16]. Unlike dyes, varieties of metal ions have more defined interaction with proteins [18]. Hence, screening for a suitable ligand is not required. IMAC was chosen because it was a non-adsorptive adsorbent for the enzyme (phosphoglycerate mutase in this case). When IMAC was used in the purification of an inhibitor (aprotinin), it was suspected to suffer from a competitive adsorption of the impurities and inhibitor through non-specific interaction [19]. Consequently, a preliminary purification step was designed to reduce the amount of impurities in the feedstock prior to the negative chromatography step.

2.1.3. Ion exchange chromatography

Ion exchange may be the most common type of negative chromatography for bioseparation. Viruses [20], IgG [5,21–25], enzymes [26], Hb (hemoglobin) [6,27] and cell surface proteins [28] have been purified in negative chromatography mode using ion exchange chromatography to capture the impurities. Proteins with charged amino acids tend to have electrostatic interactions with the ion exchange ligands. Because of the charged surface, ion exchange chromatography is robust to deal with varieties of impurities with oppositely charged surfaces. For enzyme purification, because negative chromatography was only mentioned as one of the purification steps, it will not be discussed here.