Automated Three-Dimensional Purification Method for Histidine-Tagged Proteins Using the BioLogic DuoFlow Maximizer™ Chromatography System

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Introduction

Protein purification can involve challenging separations of complex mixtures that may not resolve target proteins in a single chromatographic step. Such cases may require the use of multidimensional chromatography. Multidimensional chromatography combines a series of columns, each employing different separation mechanisms, into a single separation. Most common protein purification techniques can be incorporated into a multidimensional chromatography scheme, and such schemes provide great benefit for proteins purified on a routine basis. Typically, a multidimensional chromatographic scheme consists of an affinity purification step based on binding to a protein tag, such as multiple histidine (His) or glutathione-S-transferase (GST), followed by one or more additional resolution steps using ion exchange, size exclusion, or hydroxyapatite columns. To streamline the combination of such disparate chromatographic methods, an automated chromatography system with a flexible design and versatile control software is essential.

In this study, we describe an automated three-step (three-dimensional) chromatographic method for purification of a recombinant 100 kD His-tagged protein from a crude *E. coli* lysate. The method involved initial affinity capture of the tagged protein using Ni^{2+}-charged Profinity™ IMAC support, intermediate desalting with Bio-Gel® P-6DG desalting gel, and final purification on an UNO® Q1 anion exchange column. Using the high valve capacity provided by the BioLogic DuoFlow Maximizer chromatography system, we optimized the flow path with parallel connection of the three columns using one UV detector and one conductivity monitor (Figure 1).

Using the queuing feature of BioLogic DuoFlow™ software, the three-dimensional method was written as a series of individual one-dimensional method runs. The BioLogic DuoFlow multimodule hardware and software facilitated the routine running of this complex purification process, which required three columns and nine buffers, without operator attendance. This automated method yielded reliable, efficient His-tagged protein purification.

Methods

System Components and Buffers

The BioLogic DuoFlow Maximizer 20 chromatography system was used in this study. Components were connected to the F10 workstation and controller as illustrated in Figure 1 and included the following: three columns, a UV detector, a conductivity monitor, a BioFrac™ fraction collector, three AVR7-3 injection valves, two SV5-4 buffer select valves, two AVR9-8 stream-select valves, and two SV3T-2 valves, one for loop selection and the other for flow diversion. Nine buffers were used, and all were filtered, degassed, and connected to the SV5-4 valves as shown in Figure 1: A1, affinity equilibration buffer (50 mM potassium phosphate, 300 mM NaCl, pH 8.0); A2, affinity elution buffer (A1 buffer with 500 mM imidazole); A3, ion exchange buffer A (20 mM Bis-Tris, pH 6.0); A4, desalting buffer (20 mM Bis-Tris, pH 6.0); B1, ion exchange buffer B (20 mM Bis-Tris, 1 M NaCl, pH 6.0); B2, discharging buffer (50 mM EDTA, 300 mM NaCl, 50 mM potassium phosphate, pH 7.5); B3, charging buffer (100 mM $NiSO_4$, pH 4.0); B4, cleansing buffer (50 mM KOAc, 300 mM NaCl, pH 4.0).

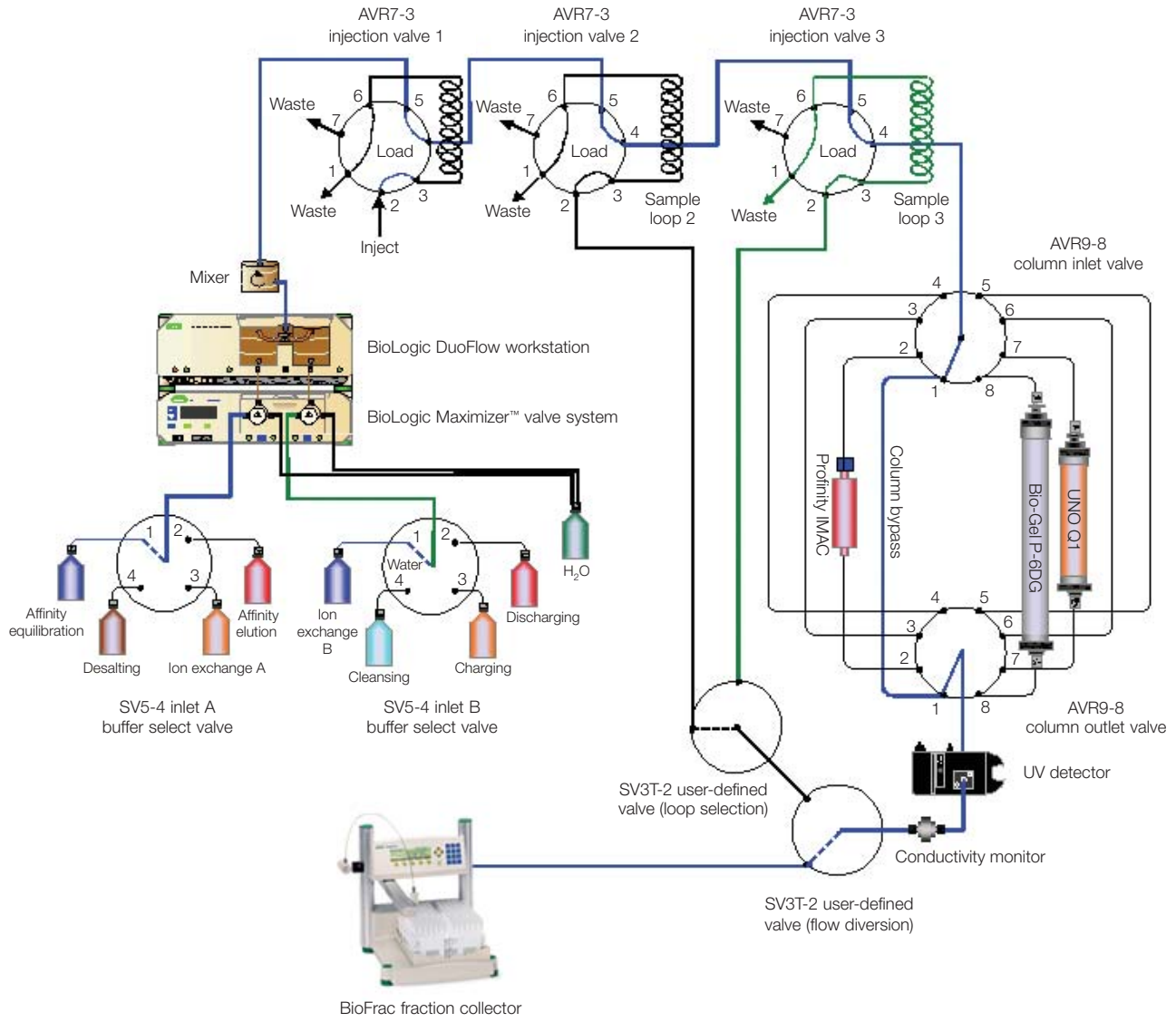

Fig. 1. Plumbing diagram of the BioLogic DuoFlow Maximizer system used in this study. This system can be expanded to incorporate up to seven columns for each step in the protocol. Alternatively, this scheme can be modified to allow processing of multiple samples on the multicolumn setup using a switching valve (AVR9-8) and an Econo™ gradient pump for sample injection.

Purification Protocol

The following columns were used: a 1 ml Tricorn column (0.5 x 5 cm) (GE Healthcare) manually packed with Ni^{2+}-charged Profinity IMAC support, a 10 ml Amicon column (1.2 x 8.8 cm) (Millipore) manually packed with Bio-Gel P-6DG desalting gel, and an UNO Q1 column (0.7 x 3.5 cm) prepacked with 1.3 ml anion exchange matrix.

Methods specific to each column type or step (described below) were created independently, linked using the queuing function of BioLogic DuoFlow 5.1 software, and executed sequentially. During the process, as a fraction containing the target 100 kD His-tagged protein eluted from one column, it was automatically transferred to the next column through an intermediate loop.

Affinity Capture — The Profinity IMAC column was equilibrated with 5–10 ml affinity equilibration buffer (A1) at a flow rate of 1 ml/min. Next, 1 ml crude *E. coli* lysate supernatant containing the 100 kD His-tagged protein was loaded on a 1 ml injection loop and injected through AVR7-3 injection valve 1 (Figure 1). The column was washed with 5 ml, or 5 column volumes (CV), of affinity equilibration buffer (A1) to remove host protein contaminants, the vast majority of which should not be retained by Profinity IMAC support. The 100 kD His-tagged protein was eluted with affinity elution buffer (A2) until all of it was removed. During elution, once the 100 kD protein peak was detected, the flow path was changed and directed the fraction containing the protein to AVR7-3 sample loop 2 (Figure 1). Finally, the column was washed with an additional 5 ml (5 CV) affinity elution buffer (A2) to eliminate residual proteins.

To regenerate the Profinity IMAC column for future runs, the column was discharged with 10 ml (10 CV) discharging buffer (B2) and then washed with 10 ml (10 CV) cleansing buffer (B4). The column was charged with 10 ml (10 CV) charging buffer (B3), washed with 10 ml (10 CV) cleansing buffer (B4), and then reequilibrated with 10 ml (10 CV) affinity equilibration buffer (A1).

Desalting — The 10 ml column containing Bio-Gel P-6DG desalting gel was equilibrated with 15–30 ml desalting buffer (A4) at a flow rate of 1 ml/min, and the 100 kD His-tagged protein was injected from AVR7-3 injection valve 2 (Figure 1). The His-tagged protein, salt, and imidazole were eluted until the UV and conductivity traces reached their baselines. During elution, the desalted 100 kD His-tagged protein was redirected to AVR7-3 sample loop 3 (Figure 1). Finally, the column was reequilibrated with 25 ml (2.5 CV) desalting buffer (A4).

Anion Exchange — The UNO Q1 anion exchange column was equilibrated with 10 ml (7.7 CV) ion exchange buffer A (A3) at a flow rate of 1 ml/min. The 100 kD His-tagged protein was injected from AVR7-3 injection valve 3 (Figure 1), and the column was washed with 10 ml (7.7 CV) ion exchange buffer A (A3) to remove unbound impurities. Next, the column was washed with 3 ml (2.3 CV) 10% ion exchange buffer B (B1) to remove weakly bound impurities. The 100 kD His-tagged protein was eluted from the column in 39 ml (30 CV) ion exchange buffer B (B1) at 10–60% strength. Finally, the column was washed with an additional 3 ml (2.3 CV) ion exchange buffer B (B1) at 100% strength to remove residual proteins from the column and then reequilibrated with 10 ml (7.7 CV) ion exchange buffer A (A3).

Fraction Collection and Analysis

The elution profile during each step of the purification was monitored at 280 nm, and a series of 2 ml fractions was collected. Fractions containing the 100 kD His-tagged protein were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on Criterion™ 4–20% gradient Tris-HCl polyacrylamide gels. Gels were analyzed using a GS-800™ densitometer and Quantity One® 1-D analysis software.

Results

A crude *E. coli* lysate supernatant containing a 100 kD His-tagged protein of interest was loaded onto the BioLogic DuoFlow Maximizer 20 chromatography system configured as shown in Figure 1. The chromatograms obtained following affinity capture, desalting, and anion exchange were plotted by BioLogic DuoFlow 5.1 software and are shown in Figure 2.

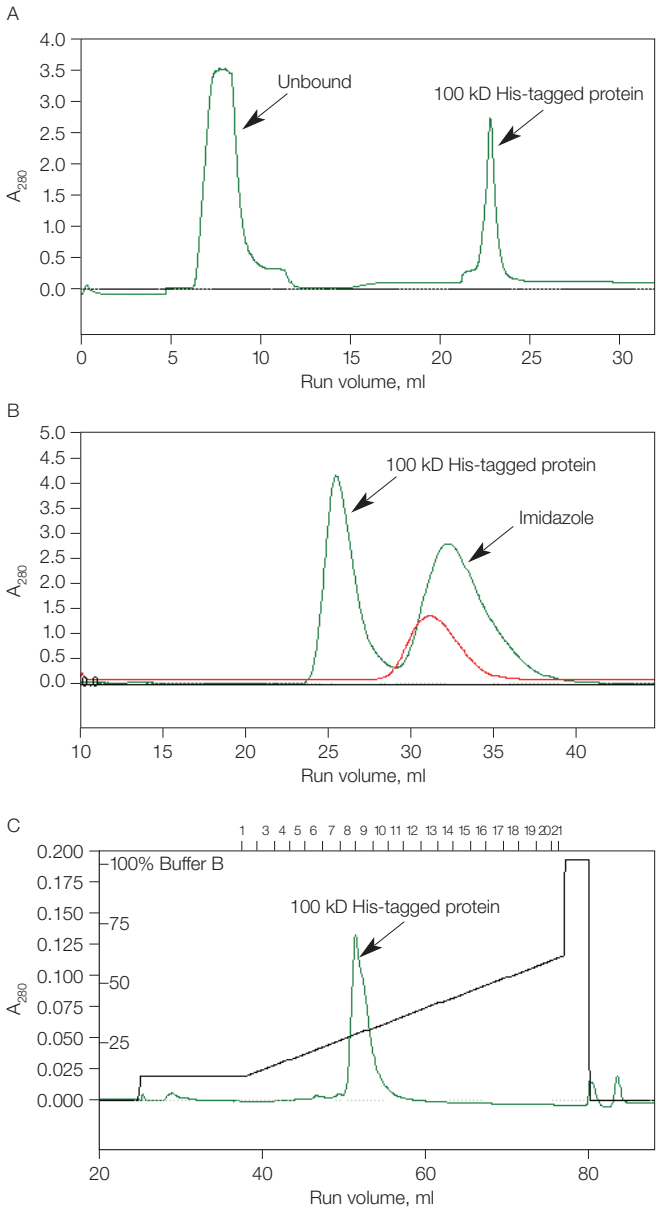

Fig. 2. Elution profiles of a 100 kD His-tagged protein purified from a crude *E. coli* lysate on the BioLogic DuoFlow Maximizer 20 chromatography system. Chromatograms plotted with BioLogic DuoFlow 5.1 software are shown following affinity capture on a Profinity IMAC column (A), desalting with Bio-Gel P-6DG desalting gel (B), and anion exchange on an UNO Q1 column (C).

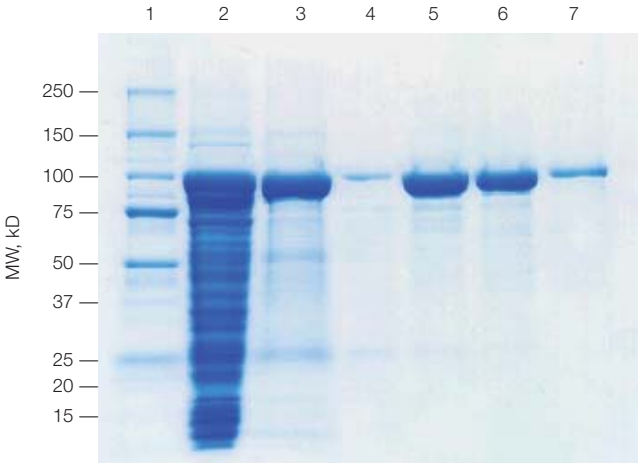

Fig. 3. SDS-PAGE analysis of fractions from three-dimensional purification of a 100 kD His-tagged protein. Lane 1, Precision Plus Protein™ standard; lane 2, crude *E. coli* lysate supernatant; lane 3, fraction eluted from the Profinity IMAC affinity column and containing the 100 kD His-tagged protein; lanes 4–7, four fractions containing 100 kD His-tagged protein eluted from the UNO Q1 anion exchange column.

SDS-PAGE analysis of the fractions containing the 100 kD His-tagged protein showed that the protein was substantially purified by this automated three-dimensional chromatographic process (Figure 3). Analysis of these gels revealed that the percent purity of the His-tagged protein following elution from the Profinity IMAC column was 78%, and that it reached 96% purity after anion exchange on the UNO Q1 column.

Conclusions

This study demonstrates that the BioLogic DuoFlow Maximizer 20 chromatography system, when used in combination with appropriate chromatographic media, is well suited to performing multidimensional chromatography. This automated system facilitates development of an efficient and robust protein purification process. Incorporating an automated multidimensional chromatographic process is especially advantageous for laboratories involved in structural or drug-target screening studies, where the automated purification of large amounts of pure and homogeneous protein can be a key factor in research success.

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