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RAPID PURIFICATION OF HISTIDINE-TAGGED GLUTATHIONE S-TRANSFERASE FUSION PROTEIN BY METAL CHELATE POROS® PERFUSION CHROMATOGRAPHY® MEDIA

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ABSTRACT

The use of POROS MC media allowed high speed column preparation, sample loading, and elution. Under these conditions the proteins of interest can be recovered under mild conditions to maintain maximum biological activity. The speed of the process also assures minimum degradation or post-extraction modifications of the protein of interest from exposure to naturally occurring proteases and other enzymes. It is evident from the analysis by polyacrylamide gel electrophoresis of the purified fusion protein and starting material that a high degree of purification is possible in a single high-speed separation on POROS MC.

The high flow rate capabilities of POROS media offer several other significant advantages over conventional media. A few of these include high speed and systematic method optimization, rapid column preparation and sample loading, and fast time-saving chromatographic separations.

INTRODUCTION

Metal chelate or immobilized metal affinity chromatography (IMAC) is a powerful technique for isolating recombinant fusion proteins under mild conditions (1, 2). A metal chelating group is first immobilized on a chromatographic medium, and a multivalent metal ion (usually Cu^{2+}, Ni^{2+}, Zn^{2+} or Co^{2+}) is bound in a way that leaves some coordination sites free for selective interaction with proteins. Typically, 5-6 histidine residues ("tag") are added to the C- or N-terminus of a target protein using recombinant techniques. The tag specifically interacts with the chelated metal ions, thereby holding these proteins on the medium. Other components bind weakly or not at all. Elution of the fusion protein is usually performed by increasing the concentration of a competitive eluting agent, such as imidazole, or by reducing the pH. POROS chromatography media have a unique structure that allows separation of biomolecules to be carried out significantly faster than on conventional media. Resolution and capacity are maintained at these high flow rates and the technique is known as Perfusion Chromatography (3).

This article describes the rapid one-step purification of a histidine-tagged recombinant fusion protein, glutathione 5-transferase, $GST(His)_6$, from bacterial lysate with POROS MC (Metal Chelate) media.

MATERIALS AND METHODS

Reagents

Water used in buffer preparation was deionized and free of organic impurities. All buffers and salts were analytical grade or higher, and solutions were degassed and filtered (0.45 mm) prior to use.

Determination of protein concentration

Protein concentration of the cell lysate and the purified fusion protein was determined spectrophotometrically using the Protein Assay ESL (Boehringer Mannheim) as described in the accompanying pack insert.

Preparation of bacterial lysate

Genetically engineered E. coli cells which express the fusion protein $GST(His)_6$, a kind gift from Dr. Thomas Emrich (Boehringer Mannheim), were suspended in 150 mM NaCl, 50 mM sodium phosphate, pH 6.8 buffer, and lysed with a French press. The lysate was then centrifuged to remove cell debris prior to the chromatographic separation. Total protein content in the starting sample was 9.0 mg/ml.

Column preparation

The column size, bed volume, and flow rate are given in Figure 1. The POROS MC/M column (Boehringer Mannheim) was prepared as described in the pack insert. Briefly, the column was cleaned with 30 column bed volumes (CV) of 50 mM EDTA in 1 M NaCl, washed with 10 CV of H_20, and finally charged with 30 CV of 50 mM $ZnCl_2$. Excess metal ions were washed out with 10 CV H_20, followed by 10 CV 0.2 M NaCl. The column was then equilibrated with 10 CV of starting buffer (see Figure 1).

If Ni^{2+} is used for charging the column, caution must be exercised since the metal is a known allergen and several nickel salts are carcinogenic.

RESULTS AND DISCUSSION

The column was charged with Zn^{2+} as described earlier and 1.8 mg (200 m1) of crude protein extract applied to it. As shown in the chromatogram in Figure 1, a large majority of the injected sample's components was not retained and eluted in 100% buffer A. Such conditions are very desirable since the bulk of the column binding capacity is reserved for binding the molecules of interest. When the stringency of the eluting buffer was

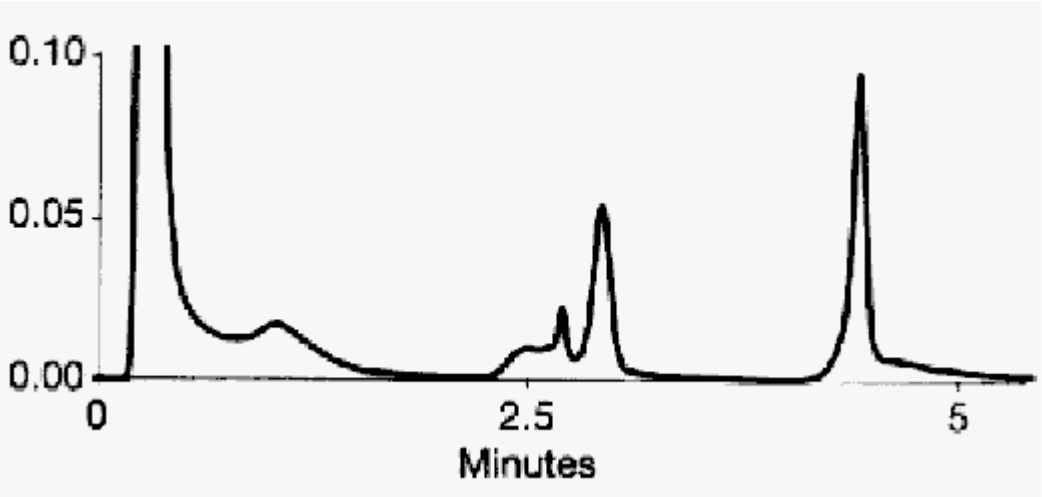

Figure 1. Purification of GST-$(His)_6$ on POROS MC/M, 4.6 mm x100 mm (Column volume = 1.8 ml). Sample: 200 ml centrifuged bacterial lysate containing rec. GST-$(His)_6$; Buffer A: 50 mM potassium phosphate, 500 mM NaCl, pH 7.8; Buffer B: buffer A + 500 mM imidazole, pH 7.8; Flow rate: 2500 cm/h (7 ml/min; Gradient: 0% B for 5 CV, 0-10% B in 1 CV, 10% B for 7 CV, 10-100% B in 2 CV, 100% B for 10 CV.

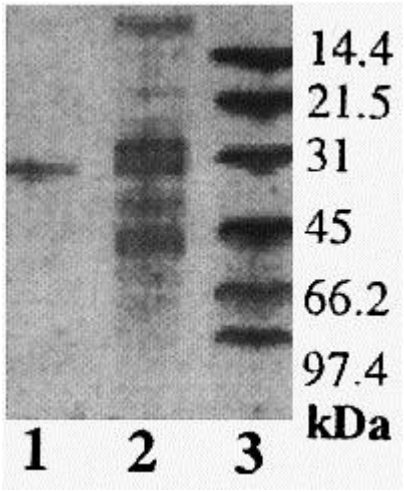

Figure 2. SDS electrophoresis of POROS MC-purified fusion protein and crude bacterial lysate. Lane 1: POROS MC-purified fraction; Lane 2: crude bacterial cell lysate; Lane 3: molecular weight marker.

increased (50 mM imidazole), several components were eluted. These probably represent proteins that naturally contain a few histidines or other amino acids on their surfaces that interact weakly with the separation media. These proteins are readily separated from the recombinant protein since the hexa-histidine tag binds much more tightly to the chromatographic media. Finally, upon increasing the imidazole concentration to 500 mM, a single chromatographic peak was observed. The whole separation process was complete in less than 5 min.

The peaks were collected and analyzed using denaturing polyacrylamide gel electrophoresis. The results of this analysis are shown in Figure 2. The starting material contained numerous bands. Peaks that eluted before the application of 50 mM imidazole did not show the characteristic band for recombinant GST fusion protein (31 kD, results not shown). The peak that eluted following application of 500 mM imidazole produced only a single band (Figure 2, lane 1) with the expected molecular weight. The total pooled protein content of this peak (0.025 mg) indicates that a 72-fold purification was achieved in the 5-minute separation.

REFERENCES

1. Lindner, P., *et al.* (1992) Purification of Native Proteins from the Cytoplasm and Periplasm of Escherichia coli

Using IMAC and Histidine Tails: A Comparison of Proteins and Protocols. *METHODS: A Companion to Methods in Enzymology* 4: 41-56.

2. Porath, J. (1992) Immobilized Metal Ion Affinity Chromatography. *Protein Expression and Purification.* 3: 263-281.

3. Afeyan NB, Gordon NF, Mazsaroff I, Varady L, Fulton SP, Yang YB, Regnier FE (1990) Flow-through particles for the high-performance liquid chromatographic separation of biomolecules: Perfusion Chromatography. *J Chromatogr* 519:1-29.