Effect of flow rate, molecular weight of (His)_6-tagged proteins, and expression system on the performance of HisTrap HP columns

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(His)_6-tagged proteins were used in studies to evaluate the purification performance of HisTrap™ HP columns. (His)_6-tagged maltose-binding protein (MBP-(His)_6, Mr ~43 000) expressed in E. coli was purified on HisTrap HP 1 ml columns at flow rates of 1, 2, or 4 ml/min. The yield of MBP-(His)_6 was lower at the higher flow rates, but still satisfactory. Sample purity remained high and unaffected. A (His)_6-tagged mannanase (Mr ~100 000) and a hydrolase (Mr ~34 000), were expressed in E. coli and P. pastoris, respectively. The (His)_6-mannanase was purified using a two-step protocol with a HisTrap HP column followed by gel filtration on Superdex™ 200 10/300 GL; (His)_6-hydrolase was purified using a one-step IMAC protocol on a HisTrap HP column. High yield and purity of these (His)_6-tagged enzymes were observed. The results of this study show that the performance of Ni Sepharose™ High Performance, the prepacked medium in HisTrap HP columns, was not limited by flow rate, molecular weight of the three (His)_6-tagged proteins used, or the two expression systems employed.

Introduction

The (His)_6 tag is the most used affinity tag due to its small size, strong metal ion binding, and ability to bind under denaturing, as well as native conditions (1, 2). Immobilized metal ion affinity chromatography (IMAC) with nickel-charged media is a well-documented method for purifying histidine-tagged proteins (3). HisTrap HP columns, prepacked with Ni Sepharose High Performance, are effective and convenient tools for this application. The medium, supplied precharged with Ni²⁺ ions, strongly binds His-tagged proteins, which are then easily eluted from the column.

The aim of this study was to evaluate the performance of prepacked HisTrap HP columns 1) at different flow rates; 2) using (His)_6-tagged proteins of diverse molecular weight; 3) in one-step or two-step purification of (His)_6-tagged proteins expressed in different hosts.

High yield and purity at increased flow rate

C-terminally (His)_6-tagged maltose binding protein, MBP-(His)_6, was subcloned and expressed in E. coli. Prior to cell lysis, PMSF was added to a final concentration of 1 mM to prevent proteolysis. Sodium chloride and imidazole were added to the cell lysate to give final concentrations of 500 mM and 35 mM, respectively; the pH was adjusted to 7.4. The cell lysate was centrifuged and filtered prior to IMAC. Extracts with MBP-(His)_6 were loaded onto equilibrated 1-ml HisTrap HP columns at flow rates of 1, 2, or 4 ml/min. All steps in the purification procedure i.e. equilibration, sample application, and wash were performed at these flow rates. The imidazole concentration during equilibration, sample application, and wash was 35 mM, which reduced the amount of contaminating E. coli proteins and allowed selective binding of MBP-(His)_6. One-step elution was performed with buffer containing 500 mM imidazole and 1-ml fractions were collected. The concentration and yield of MBP-(His)_6 were estimated by measurement at 280 nm. Sample purity was analyzed by SDS polyacrylamide gel electrophoresis using ExcelGel™ SDS Gradient 8–18.

A decrease in yield of MBP-(His)_6 was observed in runs at the higher flow rates of 2 and 4 ml/min (Fig 1). This can, in many cases, be regarded as an acceptable loss. The loss at high flow rates might be minimized by washing at a lower concentration of imidazole than the 35 mM used here. MBP-(His)_6 purity was unaffected by changes in flow rate as indicated in Figure 2.

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Two-step purification of a high molecular weight (His)_6-tagged protein expressed in E. coli

A (His)_6-tagged mannanase (Man 26A from Cellulomonas fimi) was expressed in E. coli. Sample preparation was performed according to the procedures described for MBP-(His)_6. Initial purification was performed using Ni Sepharose High Performance. The buffer used for equilibration, sample application, and wash contained 30 mM imidazole, which optimized recovery of this particular protein. Extract with (His)_6-Man 26A was loaded onto an equilibrated HisTrap HP column at a flow rate of 1 ml/min. Elution was performed using a linear gradient of elution buffer and 1-ml fractions of eluted (His)_6-Man 26A were collected, analyzed, and pooled. One ml of the concentrated pool was loaded onto a Superdex 200 10/300 GL column for gel filtration. Sample purity was analyzed by SDS-PAGE as described previously.

One-step purification of a (His)_6-tagged protein expressed in P. pastoris

A (His)_6-tagged hydrolase (putative hydrolase from Saccharomyces cerevisiae) was expressed in Pichia pastoris. The methods used for IMAC of the clarified cell lysate were as described for (His)_6-mannanase except that 85 mM imidazole was used during equilibration, sample application, and wash. Sample purity was analyzed by SDS-PAGE as described previously.

Conclusions

The excellent binding properties of Ni Sepharose High Performance allow high flow rate purifications of MBP-(His)_6 with an acceptable loss of yield and unaffected purity. Moreover, effective purification of (His)_6-tagged proteins with different molecular weights obtained from different expression systems was possible using HisTrap HP columns.

References


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Fig 3. Two-step purification of (His)_6-Man 26A expressed in E. coli. (A) First-step IMAC using HisTrap HP. (B) The fractions between the black lines were pooled and further purified by gel filtration on Superdex 200 10/300 GL. (C) Purity analysis and confirmation of the apparent molecular weight of (His)_6-Man 26A. The purified (His)_6-Man 26A is indicated by the arrow. Note: Previous results have indicated that the contaminants present after IMAC include various truncated, His-tagged forms of the mannanase (data not shown).

Fig 4. Purification of (His)_6-hydrolase expressed in P. pastoris on HisTrap HP 1 ml. Running conditions were as shown in Figure 1 with the following exceptions: 50 ml of extract was applied; the binding buffer and extract contained 85 mM imidazole; the linear gradient employed was 25 ml 85–300 mM imidazole; and the flow rate was 1 ml/min.

Fig 5. SDS-PAGE results of the one-step purification of (His)_6-hydrolase. (His)_6-hydrolase in the extract is indicated by the arrow.