Addition of imidazole during binding improves purity of histidine-tagged proteins

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The purity of histidine-tagged proteins* purified by metal chelate affinity chromatography can often be improved by optimizing the imidazole concentration in the sample and binding buffer to achieve a balance between purity and yield of the protein of interest. We determined a concentration range of imidazole that minimized the nonspecific binding of untagged proteins to Ni Sepharose™ High Performance, thereby greatly improving target protein purity. We demonstrate this approach using histidine-tagged protein kinase G ([His]$_6$-PknG) from *Mycobacterium bovis*.

Introduction

The (histidine)$_6$ tag is one of the most common tags used to facilitate the purification of recombinant proteins. However, the presence of surface-exposed histidine residues or other complex-forming amino acids can lead to nonspecific binding of untagged host cell proteins to purification media. These untagged proteins elute with the target protein and must be subsequently removed. In general, the binding affinity of these contaminants is lower than that of the tagged recombinant proteins, allowing their separation from the protein of interest by using more stringent conditions.

There are several ways to reduce the binding of contaminating proteins to Ni Sepharose media. On one hand, the amount of Ni Sepharose can be adapted to the expected amount of protein to be purified. On the other hand, imidazole can be used as a competitive agent. In this article, we demonstrate the importance of imidazole as a useful tool to enhance the purity of (His)$_6$-PknG purified with Ni Sepharose High Performance.

Determination of the optimal imidazole concentration

To determine the optimal imidazole concentration to use during the binding of (His)$_6$-PknG to Ni Sepharose High Performance, a cell lysate containing (His)$_6$-PknG was loaded on a column with 10 mM imidazole in the binding buffer. After additional washing with binding buffer, (His)$_6$-PknG was eluted using a linear gradient of up to 50% elution buffer (250 mM imidazole) within 20 column volumes (CV). By analyzing the collected fractions, it was found that most of the contaminants eluted in the range of about 40 to 70 mM imidazole (data not shown).

Improved purity

Based on this finding, 45 mM imidazole was used in the sample and binding buffer during the final purification procedure. To achieve a higher protein concentration, the protein was eluted in a two-step gradient (Fig 1). To demonstrate the advantageous effect of imidazole, an additional purification was performed under the same conditions except that imidazole was omitted from the sample and binding buffer (Fig 2). SDS-PAGE of the pooled elution fractions indicated a large improvement in purity when 45 mM imidazole was included in the sample and binding buffer (Fig 3).

Conclusion

Using a linear gradient, we determined a concentration range of imidazole that resulted in the removal of a majority of contaminating proteins, while retaining (His)$_6$-PknG. The addition of 45 mM imidazole to the sample and binding buffer resulted in much higher purity of the desired protein.

To obtain the Ni Sepharose High Performance data file (18-1174-40) visit www.amershambiosciences.com/promo_dm204

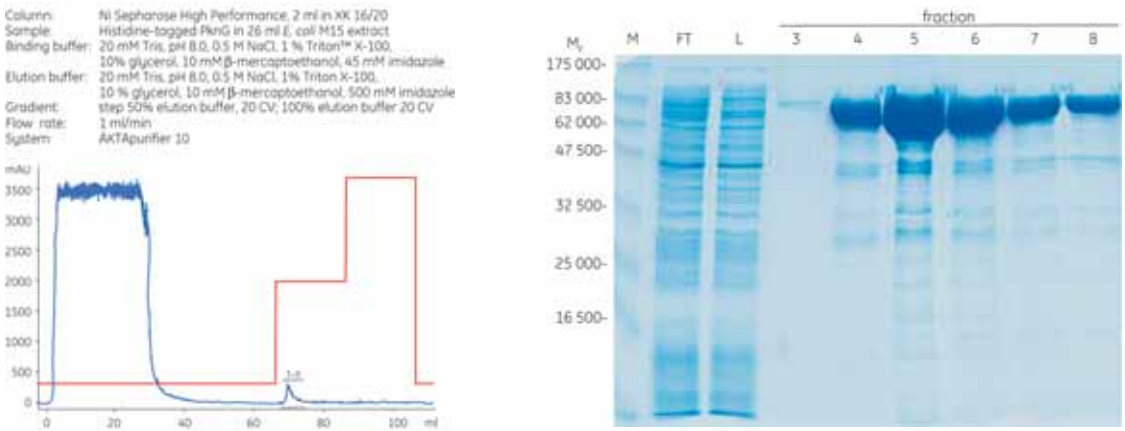
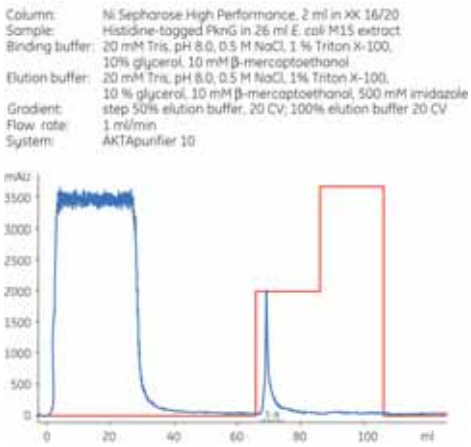


Fig 1. Purification of (His)$_6$-PknG with 45 mM imidazole in sample and binding buffer. (A) Chromatogram showing the purification of (His)$_6$-PknG. The lysate of a 2 l *E. coli* culture (sample volume: 26 ml; filtered through a 0.45-µm syringe filter) was loaded on a 2-ml Ni Sepharose High Performance column (XK 16/20 column) (GE Healthcare) using ÄKTApurifier™. The kinase was eluted in a two-step gradient with 50 and 100 % of elution buffer. (B) SDS-PAGE analysis (12% gel) of the purification of (His)$_6$-PknG. M: prestained protein marker, broad range (New England Biolabs); L: lysate; FT: flowthrough; (His)$_6$-PknG mainly eluted in fractions 4–8 (with an imidazole concentration of 250 mM).



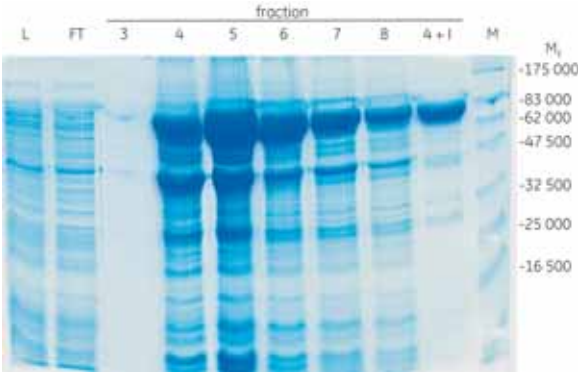


Fig 2. Purification of $(His)_6$-PknG without imidazole in sample and binding buffer. (A) Chromatogram showing the purification of $(His)_6$-PknG. Lysate and column as described in Fig 1. The kinase was eluted in a two-step gradient with 50% and 100% of elution buffer. (B) SDS-PAGE (12% gel) of fractions from the purification of $(His)_6$-PknG. M: molecular weight marker as in Fig 1; L: lysate; FT: flowthrough; 4 + I: fraction 4 from the purification with imidazole in sample and binding buffer (see Fig 1). $(His)_6$-PknG mainly eluted in fractions 4–8 (with an imidazole concentration of 250 mM).

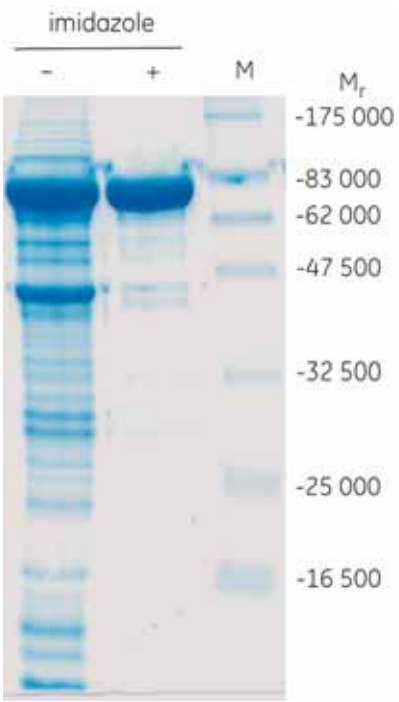


Fig 3. SDS-PAGE (12% gel) of $(His)_6$-PknG fractions. Comparison of the pooled $(His)_6$-PknG eluates purified without (-) or with (+) imidazole (45 mM) in the binding buffer. M: molecular weight marker as in Fig 1.

Ordering information

Product	Code number
Ni Sepharose High Performance (25 ml)	17-5268-01
Ni Sepharose High Performance (100 ml)	17-5268-02
XK 16/20 Column	18-8773-01
HisTrap™ HP (5 x 1 ml)	17-5247-01
HisTrap HP (5 x 5 ml)	17-5248-02
HisTrap HP (1 x 5 ml)	17-5248-01

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