Regeneration of Prepacked IMAC Cartridges on the Profinia™ Protein Purification System

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Introduction

Immobilized metal affinity chromatography (IMAC) is the most common affinity method used to isolate recombinant proteins. Prepacked IMAC cartridges charged with nickel can often be used several times. However, regeneration of the column may be necessary or desirable if: 1) some or all of the bound metal ion is lost, resulting in decreased protein binding activity, 2) the resin needs to be stripped and sanitized prior to purification of a different protein, or 3) a metal ion other than nickel (such as cobalt) is needed to enhance the binding of a particular protein and tag combination.

The Profinia protein purification system performs automated and fast purification of recombinant proteins and antibodies by affinity chromatography (Berkelman and Urban 2006, Bernardini et al. 2008, Hui and Usinger 2006, Ngo et al. 2008). One of the applications for the Profinia system is metal affinity chromatography using prepacked IMAC cartridges of either 1 ml or 5 ml size. Here, we demonstrate that prepacked cartridges can be easily regenerated with nickel and cobalt directly on the Profinia system and that the newly regenerated IMAC cartridges function with performance similar to a brand new cartridge.

Methods

Materials and Instrumentation

Profinia control lysate (*E. coli*), prepacked Bio-Scale Mini™ Profinity™ IMAC cartridges (1 ml and 5 ml), native IMAC purification kit, and Profinia protein purification system were all from Bio-Rad Laboratories, Inc.

Regeneration of Prepacked Cartridges

The cartridges were cleaned with 20 column volumes (CV) of 0.5 M NaOH and rinsed with 10 CV deionized (DI) water. Metal ions were stripped with 1 CV of 0.1 M EDTA followed by a rinse with 10 CV DI water. The cartridges were then recharged with 5 CV of 0.1 M nickel sulfate or cobalt sulfate, pH 4.5, rinsed with 10 CV of water, and then rinsed with 7 CV of 2% benzyl alcohol for storage.

The buffer port positions on the Profinia system and buffer concentration used for cartridge regeneration were: B1, 150 ml 0.5 M NaOH (1x); B2, 100 ml DI water; B3, 100 ml 0.5 M EDTA, pH 8.0 (5x); B4, 100 ml DI water (not used); B5, 100 ml DI water; B6, 100 ml 0.1 M $NiSO_4$ (1x); B7, 100 ml storage solution (4% benzyl alcohol) (2x); B8, 125 ml 20% ethanol. Sample positions S1 and S2 were both 50 ml DI water.

The IMAC regeneration method on the Profinia system can be programmed starting from the home screen. Select Program Methods, IMAC, then press Next. On the Select Method Type & Options screen, select Native IMAC, 1 Sample, and 1 ml or 5 ml Cartridges (depending on the cartridge size to be regenerated), then press Next. Enter a method name (for example, 1 ml IMAC regeneration) and a username. Press Edit Methods at the bottom of the screen and use the down arrow on the right side of the screen to scroll from steps 1 to 12. At each step, modify only the CV and buffer concentration (Conc) to match the values in Table 1. Press Save. From the home screen, select Saved Methods, IMAC, and then the newly saved regeneration method. Make sure to enter a sample volume of 1 ml before starting the program.

Table 1. Custom program steps to regenerate an IMAC cartridge using the native IMAC methods template (for 1 ml and 5 ml cartridges). Step values specific for 5 ml cartridges are shown in parentheses.

Step Number	Step	Flow Rate, ml/min	CV	Time, min	Step Conc	Fraction
01	Water wash	—	—	—	DI	—
02	Equilibrate DI C1	2 (10)	2	1	DI	W
03	Equilibrate C1	2 (10)	20	10	B1, 1x	W
04	Load S1 to C1	2 (10)	0	0	S1	1A
05	Wash 1 C1	2 (10)	0	0	B1, 1x	1B
06	Wash 2 C1	2 (10)	10	5	B2, 1x	1C
07	Elute 1 C1	2 (10)	—	3.1	B3, 5x	W
08	Elute 2 C1	2 (10)	1	0.5	B3, 5x	1D
09	Clean 1 C1	2 (10)	10	5	B5, 1x	W
10	Clean 2 C1	2 (10)	5	2.5	B6, 1x	W
11	Clean 3 C1	2 (10)	10	5	DI	W
12	Store C1	2 (10)	7	3.5	B7, 2x	W

SDS-PAGE Analysis

The fractions from each purification were loaded along with unstained Precision Plus Protein™ standards on a Bio-Rad Criterion™ Tris-HCl gel (4–20% acrylamide), run for 60 min (200 V), fixed, and stained with Bio-Safe™ Coomassie stain.

Results and Discussion

We tested the regeneration program on the Profinia system using prepacked Bio-Scale Mini Profinity IMAC cartridges. A 1 ml and 5 ml cartridge were previously used for polyhystidine tag protein purifications (four cycles) prior to regeneration. We also took an unused, uncharged IMAC cartridge and charged it with nickel sulfate using the regeneration procedure. After regeneration, these IMAC cartridges were used to purify the 51 kD protein from the Profinia control lysate according to the Profinia native IMAC purification kit instructions and using the preprogrammed Bio-Rad native IMAC method on the Profinia system. A control purification of the 51 kD protein was carried out with a new (unused) 1 ml IMAC cartridge.

A 1 ml cartridge that has been regenerated (stripped and recharged) functions the same as a new one (see the Profinia chromatography profiles overlayed in Figure 1). The amounts of sample loaded for each separation varied from 5.5 to 5.8 ml, and the resulting chromatograms shifted by as much as 0.33 ml. Using the Profinia 2.0 software, elution peak values for the control and the used/recharged cartridge were compared. The beginning of each elution peak occurred after 5.4 and 5.5 ml of elution buffer was delivered, and the actual elution peaks nearly coincide at 5.9 and 6.0 ml of elution. Correcting the chromatograms for the slight difference in load volume, the elution peaks for the control and the used/recharged cartridge coincide to a high degree (see Figure 1 inset chromatogram overlay). A Bio-Scale Mini cartridge, hand-packed with uncharged Profinity IMAC resin and charged with nickel sulfate using the regeneration procedure, produces a purification profile (blue trace in Figure 1) very similar to the control chromatogram (green trace).

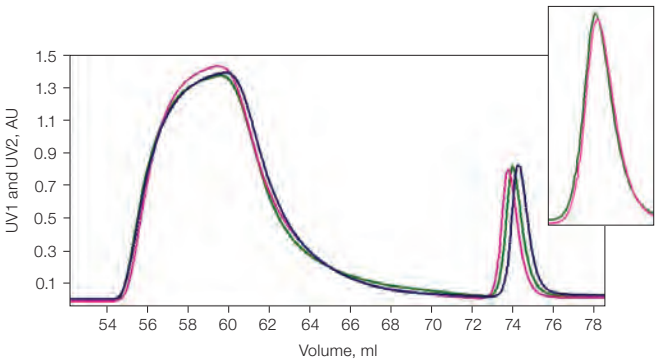

Fig. 1. Overlay of chromatograms from 1 ml native IMAC purifications of a 51 kD control protein. (—), new 1 ml cartridge (control); (—), cartridge that was regenerated with nickel sulfate (used/recharged); (—), uncharged resin cartridge that was charged with nickel sulfate (new/charged).

Gel electrophoresis of the chromatography fractions (Figure 2) shows that similar amounts of 51 kD protein were isolated in each separation and at similar purity levels. Purification using the regenerated 5 ml cartridge gave similar results but with higher overall protein yield (5.3 mg), as expected as a result of the increased amount of IMAC resin in that cartridge (data not shown). These results validate the regeneration procedure and show that both used and uncharged new IMAC resins give good performance, unchanged from a control cartridge, after recharging with nickel ion. The purification using resin recharged with cobalt generated the same conclusion (data not shown).

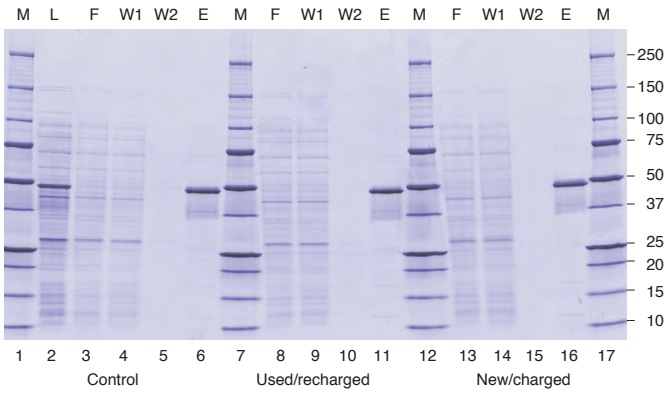

Fig. 2. SDS-PAGE gel showing chromatography fractions from the three purifications shown in Figure 1. Lanes: M, marker; L, load; F, flowthrough; W1 and W2, wash 1 and 2; E, elution-purified 51 kD protein. Lanes 3 to 6 are fractions from the purification with a new 1 ml IMAC cartridge (control); lanes 8 to 11 are from purification with the 1 ml IMAC cartridge that was regenerated with nickel sulfate (used/recharged); lanes 13 to 16 are from the purification with the 1 ml IMAC uncharged cartridge that was then charged with nickel sulfate (new/charged).

The protein concentration and yield for the different 1 ml IMAC chromatography runs are listed in Table 2. The amount of protein purified per ml of loaded lysate is almost the same for the three runs.

Table 2. Comparison of yield using 1 ml IMAC control and regenerated cartridges.

Type of 1 ml Cartridge	Loading Volume, ml	Total Protein Purified, mg	Protein Concentration, mg/ml	Total Protein Purified/ml of Lysate, mg	Total Collection Volume, ml
New Ni-charged cartridge	5.74*	3.2	0.83	0.55	4
Regenerated (Ni) after 4-time use	5.48*	3.0	0.75	0.55	4
Charged (Ni) from uncharged IMAC cartridge	5.81	3.3	0.83	0.57	4

* Samples for these purifications were from the same vial of Profinia control lysate (total volume 12 ml). Protein concentration shown was determined on the Profinia by UV absorbance at 280 nm using 1.33 absorbance units for a 1 mg/ml solution of 51 kD.

Conclusions

We have shown that in addition to performing fast and automated affinity purification, the Profinia protein purification system can also be used to sanitize and regenerate IMAC cartridges in an automated fashion. The regeneration of IMAC resin on the Profinia system should help reduce cross-contamination between runs on the same cartridge, help maintain reproducible results, and extend the life of the cartridge. This procedure gives users the flexibility to easily recharge a cartridge with the metal ion of their choice, which can be specific for their research needs.

References

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