

Purification of Proteolytic Activity - Vivaspin Ultrafiltration Devices: Tools for Concentrating and Washing Histidine-Tagged Recombinant Proteins Eluted with Imidazole from a Ni-metal Chelate Adsorbent

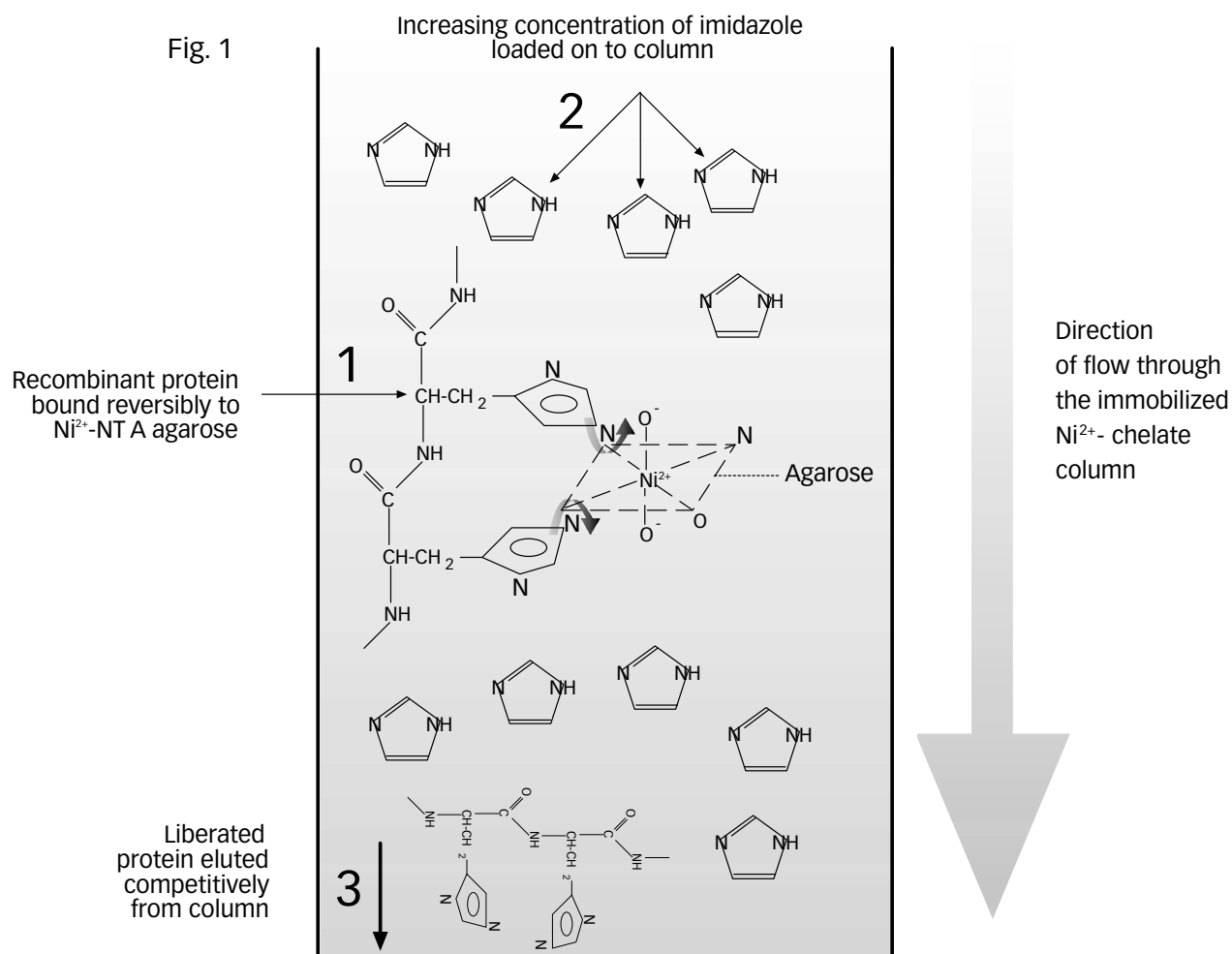
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Background

Many recombinant proteins are engineered to contain at least 6 adjacent histidine residues at the N or C terminus, referred to as the His-Tag, that facilitates their rapid purification based upon the selective affinity of proteins with polyhistidines for an immobilized metal chelate adsorbent such as Ni²⁺(1,2). This binding occurs in physiological buffers that are suitable for the purification of recombinant protein and can be

performed under native or denaturing conditions (3). Strong denaturants such as urea can be used for efficient solubilization and purification of receptors, membrane proteins and proteins of limited solubility found in inclusion bodies. The interaction between the Histidine residues and the Ni²⁺ ions is reversible and the bound protein can be eluted under mild conditions with increasing amounts of imidazole (up to 500 mM imidazole) or by lowering the pH. The

former elution protocol tends to be the method of choice. Soluble recombinant proteins can be purified from expression systems such as mammalian and insect cells, yeast, filamentous fungi, bacteriophage, bacteria etc by either batch purification using Ni²⁺-NTA agarose pellets or spin columns/ gravity-flow columns through eluting the bound fusion protein with concentrations up to 500 mM imidazole (Fig. 1).



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The eluted protein can be washed by either overnight dialysis in 50 mM Tris pH 8.0 or concentrated/de-salted rapidly by 10 kDa MWCO ultrafiltration.

Methods

His-tagged proteins are eluted typically with 200 mM NaCl in 50 mM Tris/HCl pH 7.6 containing up to 500 mM imidazole. Chemical tolerance to imidazole was, therefore, measured by challenging the Vivaspin (VS) 10 kDa MWCO devices with 0.05 mg/ml BSA (to mimic the recombinant protein) in 200 mM NaCl, 0-500 mM imidazole in 50 mM Tris/HCl pH 7.6 for 2 hours prior to centrifugation at 12,000 g for 10 min (Vivaspin 500; VS 500), 5,000 g for 10 min (Vivaspin 2; VS 2) in a 45° fixed-angle rotor and 3,000 g for 20 min (Vivaspin 15 or 20; VS 15 or VS 20) in a swing-out rotor.

In this research note, we describe the direct application of Vivaspin 500, 2, 15 and 20 ultrafiltration devices for concentrating or washing His-tagged recombinant proteins eluted with up to 500 mM imidazole.

The devices were inspected visually for any signs of plastic deformation. The performance of the Vivaspin devices was measured using estimations of protein concentration by the dye-binding method of Bradford (4). BSA (fraction V, essentially fatty acid free) was used as the protein standard. According to the BIO-RAD Technical Support Service (Hemel Hempstead, UK), concentrations up to 500 mM imidazole are compatible with the BIO-RAD protein assay based upon the Bradford method of protein determination.

Results

Total compatibility with 0.5 M imidazole was observed. No visible defects of the centrifugal devices were observed after 2 hour exposure to 500 mM imidazole.



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Table 1

Vivascience Centrifugal Concentrator	Initial volume (µl)	Final mean vol. (µl)	Fold Conc'n	Time of concentration (min)	% Mean recoveries at different imidazole concentrations ^a			
					0 mM	100 mM	250mM	500 mM
VS 500	500	5	100	10 @ 12,000 g	94 %	93%	92%	96%
VS 2	2,000	66	30	10 @ 5,000 g	95%	96%	92%	98%
VS 15	10,000	81	123	20 @ 3,000 g	98%	95%	96%	98%
VS 20	10,000	117	85	20 @ 3,000 g	97%	98%	95%	94%

^a % recoveries are based upon averages of eight replicate data values.

Rapid concentration of the protein sample was observed. The concentration factor is often a critical measure of performance. The concentration factor for all Vivaspin devices was independent of the imidazole concentration. Two ml protein sample loaded on to a Vivaspin 2 device centrifuged at 5,000 g for 10 min resulted in 30 fold concentration of BSA with tightly clustered final mean volumes of 66 µl ± 3.9 SD (Table 1).

High recoveries (in excess of 90 %) of protein were observed at all concentrations of imidazole (Table 1) further supporting the use of Vivascience centrifugal concentrators in the concentration and washing of imidazole-eluted histidine-tagged recombinant proteins from immobilized metal chelate adsorbents.

Discussion

Recombinant DNA technology has already started to shape our society with startling scientific progress achieved in disciplines ranging from

PCR-based diagnosis of genetic disease, engineering of proteins with improved characteristics to novel routes of vaccine development.

Recombinant proteins containing a His-Tag can be over-expressed in *E.coli* to form over 30% of the total protein of the cell. These proteins are purified with a single affinity chromatography step to achieve either the desired purity of the target protein, which would need to be washed in order to remove any residual imidazole, or concentrated prior to further chromatography-based purification.

Two hour exposure of Vivaspin devices to 500 mM imidazole had no deleterious effect upon both the speed of concentration and recovery of the target protein.

These ultrafiltration devices would, therefore, serve as ideal research tools for sample preparation/conditioning.

References

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