Continuous highly efficient protein purification using simulated moving bed chromatography

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Abstract

Most common methods for purifying proteins use one or more liquid chromatographic steps in which single columns are employed in a linear process. Whereas these methods are often sufficient for analytical scale separations, they can become cumbersome and expensive when larger amounts (hundreds of milligrams to multiple grams) of purified protein are required. The inherent limitations of single column methods, including inefficient use of the solid phase and linear protocol, are addressed by simulated moving bed chromatography (SMBC; 1, 2). Large scale SMBC systems have been well established for industrial production of a variety of chemicals, including sugars and pure enantiomer APIs (1). SMBC emulates countercurrent separation where the mobile phase flows in the opposite direction of the solid phase. The SMBC process is up to 20-fold more productive than single column elution methods due to the dramatic increase in utilization of the solid phase and continuous operation (1).

We have developed a bench top SMBC instrument, the Semba Octave™ Chromatography System, designed for milligram-to-gram scale purification of chemical and biological compounds. Here we demonstrate the utility of bench top SMBC for continuous IMAC purification of several human 6xHis fusion proteins expressed in *E. coli*. A comparison with single column purification revealed that the SMBC process resulted in significant gains in purity. In addition, the continuous SMBC process produced several hundred milligrams of purified protein from eight 1-ml cartridges in a few hours of unattended operation.

Fig. 1. Semba Octave Chromatography System

- A versatile bench top 8-column system
- Capable of performing SMBC and other continuous automated separation protocols
- Suitable for milligram-to-gram scale purification
- 100% biocompatible flow path
- SembaPro™ software controls multiple protocols

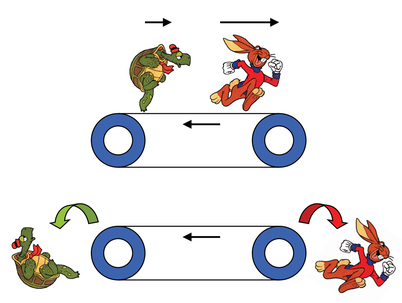


Fig. 2. Principle of countercurrent separation

In countercurrent systems the fluid phase flows in the opposite direction from the solid phase. Two species having different mobilities on the solid phase effectively separate if the transit time of the solid phase is greater than the residence time of the slower (more retained) species and less than the faster (less retained) species. In simulated moving bed systems the solid phase is represented by a series of columns and movement of the fluid and solid phases is achieved by switching the flow streams between consecutive columns at defined intervals.

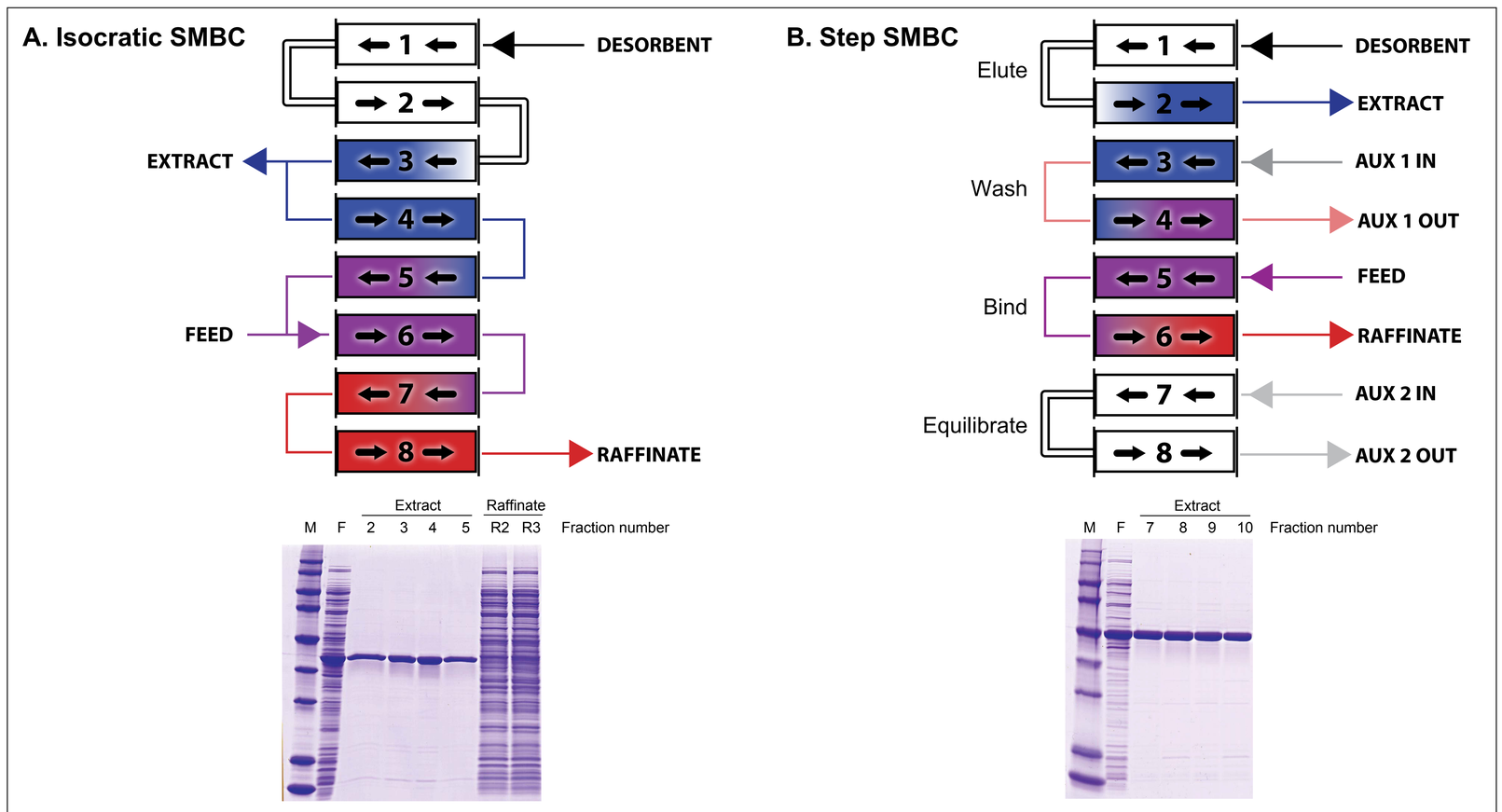
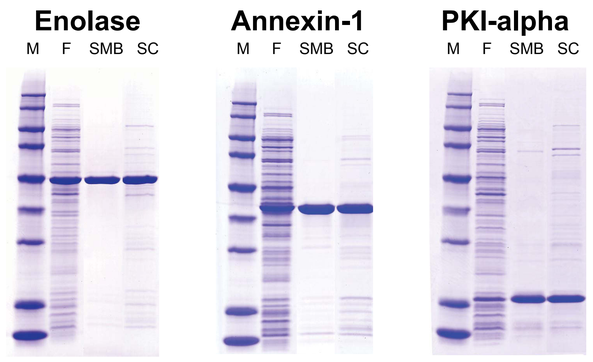


Fig. 3 Isocratic and Step SMBC purification of histidine-tagged proteins from bacterial lysates

Panels A and B show the column arrangement in the Octave System in isocratic and step flow configurations, respectively. In Isocratic SMBC (A), Feed and Desorbent buffers are at the same imidazole concentration. In Step SMBC (B), four independent zones are established via shut-off valves between appropriate columns, which enables a protocol using Bind, Wash, and Elution buffers containing different imidazole concentrations. Histidine-tagged human annexin-1 (A) and enolase (B) were expressed in *E. coli*. Bacterial lysates were prepared by standard methods and applied to Ni-chelate columns on the Semba Octave System in a 3-2-3 Isocratic (A) and Step (B) SMBC configurations. A, Isocratic Mode fractions. M, Markers 10-225 kDa; Feed, *E. coli* lysate containing recombinant His-annexin 1; 2-5, Extract fractions containing purified annexin-1; R2-R3, Raffinate. B, Step Mode fractions. M, Markers 10-225 kDa; Feed, *E. coli* lysate containing recombinant His-enolase; 7-10, Elute fractions of purified enolase.



M: markers 10-225 kDa
F: Feed (clarified *E. coli* lysate)
SMB: SMBC-purified
SC: single column-purified

Purity

	SMBC	Single Column
Enolase	96%	89%
Annexin	83%	66%
PKI-alpha	69%	44%

SMBC Yield (8 x 1-ml columns)

	Feed Input	Total Product	mg/h
Enolase	213 ml	246 mg	55
Annexin	162 ml	231 mg	68

Fig. 4. Comparison of Isocratic SMBC and single column purification of histidine-tagged proteins

The indicated human histidine-tagged fusion proteins were expressed in *E. coli*. Crude bacterial cell lysates were prepared by standard methods and applied to 1-ml Ni-chelate columns on the Semba Octave System in a 3-2-3 Isocratic SMBC configuration and to an identical single column. The single columns were processed manually per manufacturers' instructions. Sample of Feed and purified products were analyzed by SDS-polyacrylamide gel electrophoresis and Coomassie blue staining. Equivalent amounts of protein were loaded for each pair of purified samples. Lanes are indicated in the legend. Purities were determined by scanning densitometry of separate gels in which 3 different sample loads were run side by side in triplicate. Yield data were obtained from extended Isocratic SMBC runs of enolase and annexin-1.

Factors to determine SMBC conditions

Column properties
- Column static capacity

Sample properties
- Protein solubility, viscosity
- Approximate target protein concentration
- Approximate eluent concentration required to elute target protein

Parameters for isocratic SMBC conditions

- Switch time: t*
- External flow rates: $Q_{Desorbent} + Q_{Feed} = Q_{Extract} + Q_{Raffinate}$
- Internal zone flow rates
  - » $Q_1 = Q_{Desorbent}$
  - » $Q_2 = Q_1 - Q_{Extract}$
  - » $Q_3 = Q_2 + Q_{Feed}$

Discussion

The Semba Octave™ System has been used successfully to purify histidine-tagged human kinase substrate proteins from bacterial lysates. Continuous operation is made possible by the coordinated switching of inlet and outlet streams through eight columns. The system's versatility enables a variety of flow configurations, including the isocratic and step modes described here. The system can process milliliters to liters of sample by adjustment of flow parameters and cycle times.

The application of the isocratic SMBC mode to metal affinity chromatography of tagged proteins has two advantages over step (bind/wash/elute) protocols; (1) increased purity, and (2) simplicity. Increased purity is likely due to the resolution achieved by countercurrent separation performed at a high imidazole concentration. To achieve countercurrent separation the imidazole concentration must be high enough to enable movement of the target protein through the matrix, yet low enough to retain its differential mobility relative to untagged proteins. These conditions produce higher selectivity of the solid phase for the target protein, effectively removing weakly-binding proteins without sacrificing yield. We believe that this is the first demonstration of isocratic affinity purification of a recombinant protein, and this strategy may be applicable to other affinity systems, such as GST, Strep-Tag, MBP, Protein A/G, and target-specific antibodies. Countercurrent separation also has advantages for protein IEX, HIC, and SEC separations.

Conclusions

The combination of the SMBC process and Semba Octave Chromatography System allows:

- Unattended continuous purification of milligrams to grams of proteins
- Significant gains in purity as compared with the single column elution chromatography process
- More efficient use of chromatography media
- Preparation of concentrated highly purified proteins from dilute samples

References

1. Perrin, S.R. and Nicoud, R.M. (2001) *in* "Chiral Separation Techniques: A Practical Approach", Second Edition (ed. by G. Subramanian), pp. 253-285, Wiley-VCH Verlag GmbH.
2. Andersson, J. and Mattiasson, B. (2006) SMB technology with a simplified approach for protein purification. *J Chromatog A* **1107**, 88-95.