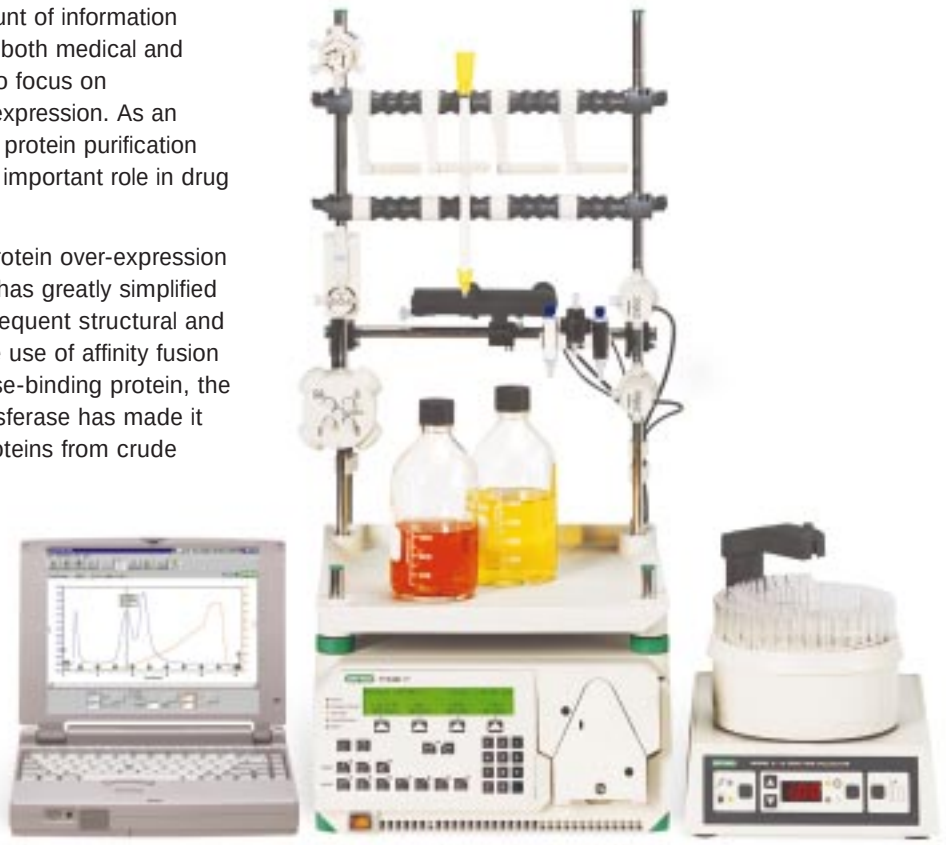
Purification and On-Column Renaturation of Recombinant Proteins Using the BioLogic LP System

Efforts to capitalize on the vast amount of information contained in genomic databases for both medical and agricultural purposes are expected to focus on proteomics-based analysis of gene expression. As an integral part of proteomics research, protein purification and analysis will play an increasingly important role in drug and pesticide discovery.

The advent of efficient vectors for protein over-expression in eukaryotic and prokaryotic hosts has greatly simplified the task of protein isolation for subsequent structural and functional analyses. In particular, the use of affinity fusion "tags" such as poly-histidine, maltose-binding protein, the Flag peptide and glutathione-S-transferase has made it possible to obtain highly-purified proteins from crude samples by simple affinity chromatography steps using a bound ligand specific for the fusion "tag."



The technology of recombinant protein expression and purification continues to advance via improved gene constructs and affinity chromatography media. However, many over-expressed proteins in bacteria often aggregate as insoluble inclusion bodies and require solubilization with chaotropes such as urea and guanidine-HCl followed by purification and re-folding or renaturing protocols to obtain active protein. While many initial purification experiments are run using simple gravity-flow equipment, low pressure chromatography systems capable of automating the on-column renaturing and purification steps offer significant advantages to the researcher.[1]

One such chromatography system is the BioLogic LP system available from Bio-Rad Laboratories. This system is an integrated purification workstation consisting of a peristaltic pump, UV and conductivity detectors, an injection valve, a column rack, and a choice of fraction collectors. Method programming and run control is performed via an integral keypad and screen. Chromatograms, Methods and Notes are recorded on a PC using LP Data View software (a chart recorder is optional).

Automated on-column renaturing and purification is achieved using a five-port, four-position buffer select valve. A typical application is the purification of a His_6 tagged protein on a nickel ion chelating affinity column following solubilization of inclusion bodies with 8 M urea.

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Protocol

STEP 1

E. coli cells are harvested by centrifugation, broken by sonication, and insoluble material pelleted.

STEP 2

Inclusion bodies are dissolved in binding buffer (20 mM Tris base, 0.5 M NaCl, 5 mM imidazole, pH 7.8) containing 8 M urea (**A**).

STEP 3

The sample (**E**) is loaded onto a nickel ion chelate affinity column pre-equilibrated with binding buffer containing 8 M urea (**A**). The flow rate should be chosen for maximum binding of the solubilized protein.

STEP 4

The column is washed with 5 column volumes of binding buffer containing 8 M urea (**A**).

STEP 5

The column is washed with 5 column volumes of wash buffer (20 mM Tris base, 0.5 M NaCl, 20 mM imidazole, pH 7.8) containing 8 M urea (**C**). The higher concentration of imidazole removes non-specifically bound protein.

STEP 6

The immobilized protein is renatured with a gradient of 8-0 M urea in 80 column volumes (100% binding buffer **A** to 100% binding buffer **B**).

STEP 7

The column is further washed with 3 column volumes of binding buffer without urea (**B**).

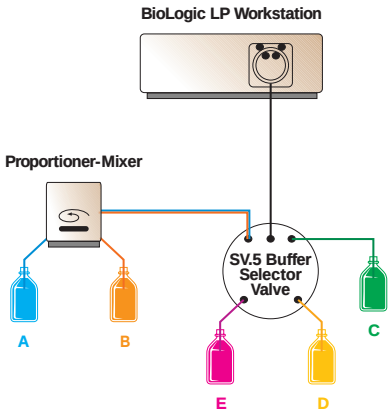
STEP 8

The renatured protein is eluted with 3 column volumes of elution buffer (20 mM Tris base, 0.5 M NaCl, 0.3 M imidazole, pH 7.8) (**D**).

STEP 9

The eluted protein is dialyzed against 20 mM Tris-HCl (pH 8.0) containing 50 mM NaCl to remove imidazole.

Note that the above steps are only guidelines and the actual chromatography conditions will vary according to the instructions provided by manufacturers of the chelating affinity resin.



Buffer plumbing scheme. ***A****. Binding buffer (20 mM Tris base, 0.5 M NaCl, 5 mM imidazole, pH 7.8) containing 8 M urea.* ***B****. Binding buffer (20 mM Tris base, 0.5 M NaCl, 5 mM imidazole, pH 7.8).* ***C****. Wash buffer (20 mM Tris base, 0.5 M NaCl, 20 mM imidazole, pH 7.8) containing 8 M urea.* ***D****. Elution buffer (20 mM Tris base, 0.5 M NaCl, 0.3 M imidazole, pH 7.8).* ***E****. Protein sample.*

Summary

The methodology described in this report is generally applicable to any affinity step or other chromatographic step (*e.g.* ion exchange, hydrophobic interaction) where the protein of interest is immobilized on the solid matrix. Renaturation may be achieved using either continuous or step gradients under the control of the BioLogic LP workstation, which provides extremely reproducible gradients. This is a significant advantage in developing protocols to obtain the maximum amounts of correctly folded and active proteins for further study.

Acknowledgments

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Reference

1 *Shi, P-Y, Maizels, N. and Weiner, A. M.,* BioTechniques, *23, 1036–1038 (1997).*

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