Large Volume Pelleting Using the Thermo Scientific Sorvall LYNX 6000 Superspeed Centrifuge and the Thermo Scientific Fiberlite F9-6x1000 LEX Carbon Fiber Rotor

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Application Note: ANCFGLYNXECOLI 1012

Key Words

Large-Volume Pelleting, Bacterial Pelleting, Protein Purification, Proteomics, Structural Biology, Superspeed Centrifuges, Carbon Fiber Rotors

Summary

Batch centrifugation is an important tool for large volume cell culture processing in academia, pharmaceutical and biotechnology research environments. We offer a variety of large-volume rotors and centrifuges that allow for the efficient pelleting of yeast, bacterial, mammalian, insect, and plant cells. By using the high capacity Thermo Scientific Fiberlite F9-6x1000 LEX carbon fiber rotor in a Thermo Scientific Sorvall LYNX 6000 superspeed centrifuge, 6 liters of cell culture can be pelleted in as little as 10 minutes depending on the cell type. These two centrifugation products are useful for functional proteomics, research involving protein-protein interaction studies, protein biochemical studies, large-scale protein folding, and 3-dimensional protein structure determinations.

Introduction

Proteomics research aims at analyzing the total protein profile of a given cell, organelle, or tissue. Currently this research is addressed by quantitative and functional approaches. While classical proteomics addresses quantitative differences between complete protein profiles of samples (e.g. normal vs. diseased) using 2-dimensional gel electrophoresis and mass spectrometry, functional studies are more focused on elucidating the important interactions between proteins. Functional proteomic studies focused on protein-protein interactions can take the form of protein chip or arrays, biochemical characterizations, and x-ray crystallography-based or NMR spectroscopy protein structure determinations. All these methodologies provide valuable tools for clinical, pharmaceutical, and basic research environments. Although the end product, such as a protein chip, may only use a small amount (micrograms) of protein, initial testing and optimization of this technology requires large quantities of proteins to be purified. Centrifugation remains a vital tool for harvesting of cells expressing proteins of interest. Described below are procedures for using the Sorvall® LYNX 6000 superspeed centrifuge and the Fiberlite® F9-6x1000 LEX carbon fiber rotor for pelleting of bacterial cells for further protein purification and characterization.

Figure 1. Thermo Scientific Sorvall LYNX 6000 superspeed centrifuge with Fiberlite F9-6x1000 LEX rotor.

Procedures

PROTOCOL 1: Pelleting of *Escherichia coli* for Purification of the Edema Factor, Adenylyl Cyclase

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Protein-protein and protein-ligand interactions form the molecular basis for complex cell-to-cell communication and intercellular signaling that control diverse physiological

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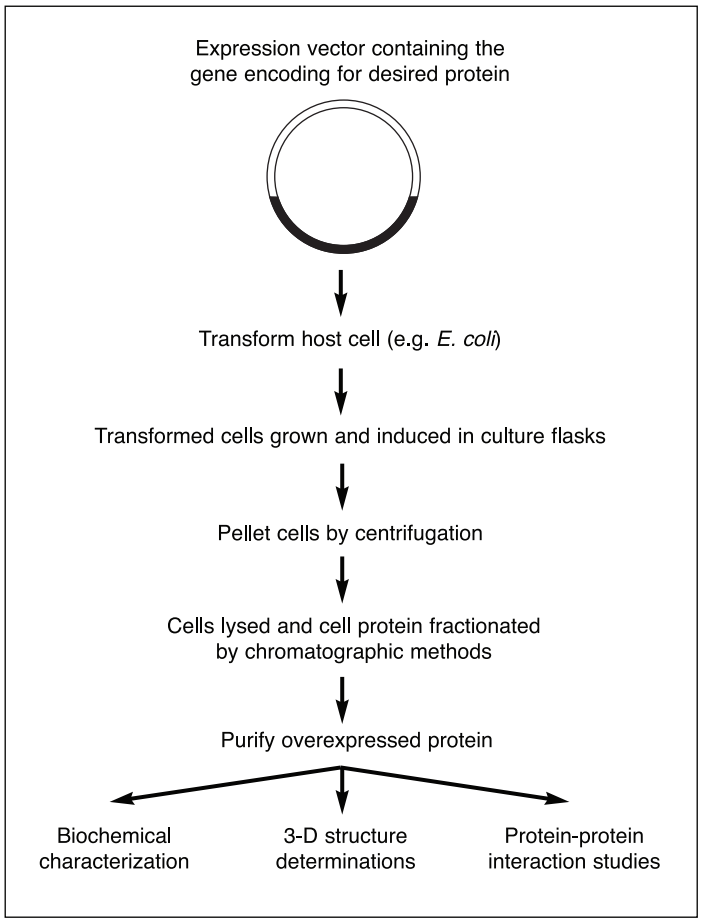

Figure 2. Overall scheme for protein expression and purification for proteomics.

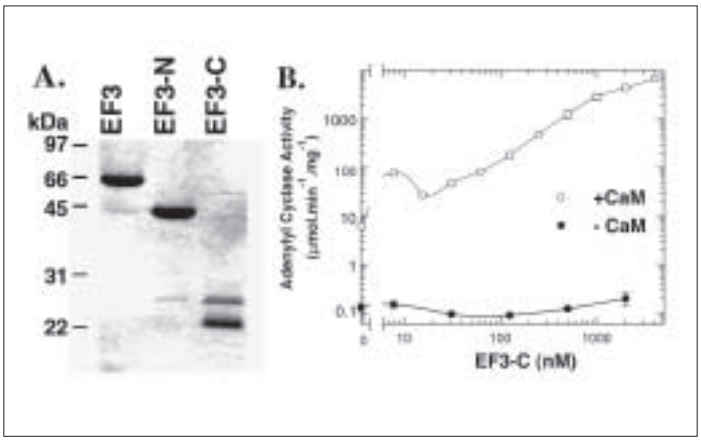

Figure 3. Characterization of the edema factor fragments expressed in *E. coli* (reproduced with permission from the Journal of Biological Chemistry).

A. Coomassie Blue-stained SDS polyacrylamide gels of purified edema factor fragments.
B. Activation of EF3-N in the presence or absence of calmodulin (CaM). Adenylyl cyclase activity was measured with 10 ng of EF3-N (+CaM) or 500 ng of EF3-N (-CaM).

activities. Adenylyl cyclases produced by certain bacterial species (also referred to as edema factors) are an important class of proteins that regulate several cellular activities through protein-protein interactions. Interaction of the edema factor with host cellular proteins such as calmodulin can initiate a series of biochemical changes.[1] A comprehensive understanding of the bacterial toxin's interaction with cellular proteins can provide a wealth of information on how to intervene these processes chemothera-peutically. To achieve this, it is critical to identify the catalytic domain(s) of the edema factor and its interaction with calmodulin.

This procedure describes the use of a Sorvall LYNX 6000 superspeed centrifuge and the Fiberlite F9-6x1000 LEX carbon fiber rotor to harvest large quantities of bacteria over-expressing adenylyl cyclase.

The pelleted cells can be processed to isolate the edema factor and this purified adenylyl cyclase can then be used for subsequent protein-protein interaction studies.

Materials and Methods

1. Transform *Escherichia coli (E. coli)* BL21(DE3) cells with appropriate plasmids containing the gene coding for the edema factor.
2. Grow *E. coli* cells in modified T7 medium with 50 mg/mL kanamycin at 25-30 °C to A_{600} of 0.4.
3. Induce by adding IPTG and harvested 12-19 hr post induction.
4. Harvest cells using the Fiberlite F9-6x1000 LEX rotor in the Sorvall LYNX 6000 superspeed centrifuge with the following parameters: 13,000 x g for 15 min at 4 °C.
5. Collect the cell pellet, lyse, and further process by column chromatographic methods for isolating the edema factor.

Results

Figure 2 provides a basic scheme for overexpressing recombinant proteins in cells. To isolate active proteins overexpressed in cells, multi-liter cell cultures must be harvested rapidly at optimum conditions after induction of cells and processed quickly for protein purification. Harvesting delays due to centrifugation capacity constraints can result in protease-inactivated proteins or cause expressed proteins to "fall out" as undesirable inclusion bodies.

The *E. coli* cells obtained by centrifugation were then lysed and the edema factor(s) was isolated after processing through several chromatographic procedures. Three different truncated edema factor fragments were purified using the above methods. Figure 3 illustrates the three different purified Edema Factors (EF) 3, 3-N, and 3-C resolved on a SDS polyacrylamide gel.

PROTOCOL 2: Pelleting of *Escherichia coli* for the Purification of the FAK Focal Adhesion Target (FAT) Domain and Structural Determination by NMR Spectroscopy

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NMR spectroscopy can be used to study the structure and function of proteins that are involved in intracellular signal transduction.[2-4] One such protein is focal adhesion kinase (FAK), which plays an important role in integrin-mediated signaling and in the modulation of processes

such as cell growth, cell differentiation, wound healing, and tumor metastasis.[5, 6] Although the precise function of FAK is currently undefined, some recent observations strongly suggest that FAK suppresses apoptosis of certain types of cancer cells.[7] For this reason, FAK is considered a possible target for anticancer therapy.

This procedure describes the use of the Sorvall LYNX 6000 superspeed centrifuge and the Fiberlite F9-6x1000 LEX carbon fiber rotor to efficiently produce large quantities of FAK's focal adhesion target (FAT) domain, whose structure was subsequently determined by NMR spectroscopy.[4]

Materials and Methods

Expression of the FAT domain of FAK

Chicken FAK-related non-kinase (FRNK) cDNA, which encodes the FAT domain, was kindly supplied by J. Thomas Parsons (University of Virginia).

1. Subclone cDNA encoding the FAT domain (residues 916 to 1053) into a pET28a vector (Stratagene).
2. Transform *E. coli* strain BL-21 pLysS cells (Stratagene) with the pET28a vector.[8]
3. Grow cells expressing N-terminal His-tagged FAT domain in Luria-Bertani (LB) broth containing the antibiotics kanamycin and chloramphenicol, overnight at 37 °C with aeration.
4. Dilute culture 1:100 in 1 L of LB broth and allow to grow until the OD_{600} reaches 0.7.
5. Induce cells to express the protein by incubation in LB broth containing 1 mM isopropyl-ß-D-thiogalactoside (IPTG) at 37 °C with aeration for 4 hr.
6. Harvest *E. coli* cells from 6 L of LB broth by performing centrifugation in the Fiberlite F9-6x1000 LEX rotor with the following parameters: 5,000 x g for 12 min at 4 °C.
7. Label the proteins with isotopes [$^{15}NH_4Cl$ (1 g/L) and $^{13}C_6$-glucose (2.5 g/L) present in medium buffered with 3-(*N*-morpholino) propanesulfonate (MOPS)[9]].

Note: For most NMR studies, the protein of interest must be isotopically labeled.

8. Grow 10 mL of seed cells overnight in LB broth, use the resulting culture to inoculate 2 L of MOPS-buffered medium containing the isotopes and antibiotics.
9. Incubate and grow larger culture at 37 °C with aeration until the OD_{600} reaches 0.6.
10. Induce the cells to express protein by the addition of 1 mM IPTG and further incubation at 37 °C with aeration, for 6 hr.
11. After induction, harvest cells by performing centrifugation with the following parameters: 5,000 x g for 12 min at 4 °C.
12. Separate lysates of cells grown in the absence or presence of IPTG by 10-20% SDS-PAGE (Figure 4).

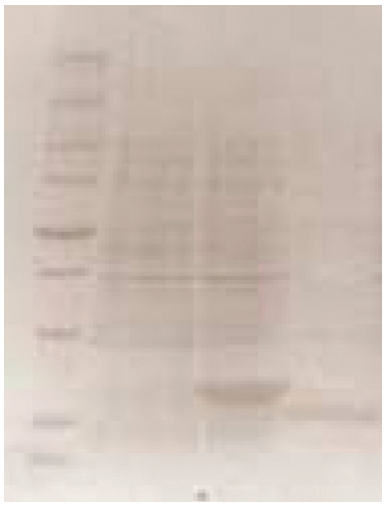

Figure 4. Induction of FAT domain expression with IPTG. Lane 1: Bio-Rad Precision molecular weight standards (from top: 250, 150, 100, 75, 50, 37, 25, 15, and 10 kDa). Lane 2: Crude extract of E. coli strain BL-21 (pLysS) cells before induction with IPTG. Lane 3: FATdomain expression in crude extract of E. coli strain BL-21 (pLysS) cells after induction with IPTG.

Figure 5. Ribbon diagram of the solution structure of the FAT domain. The FAT domain (dark) is bound to the LD2 motif of paxillin (light). The image was generated with MOLMOL software.[9]

Purification of the FAT domain of FAK

1. Incubate *E. coli* lysate at 4 °C, overnight with 6 mL of nickel-chelating resin.
2. Harvest resin and pack a chromatography column through which an imidazole gradient.
3. Run to elute the FAT domain.
4. Digest the protein with thrombin purified by size exclusion chromatography.

The FAT domain comprises four helices in an elongated "right-turn" bundle that is stabilized mainly by hydrophobic interactions. The presence of a bound peptide derived from paxillin further stabilizes the structure (Figure 5).[4]

Conclusion

Higher throughput in batch centrifugation is achieved using the high capacity Fiberlite F9-6x1000 LEX rotor with the Sorvall LYNX 6000 superspeed centrifuge. Comparing the processing capabilities in one batch run, the use of the Fiberlite F9-6x1000 LEX rotor is 2 to 4 times more productive than conventional 6 x 500 mL or 6 x 250 mL rotors. For the above described applications, a 15-min run at 13,000 x g, 4 °C resulted in efficient pelleting of *E. coli* cells expressing the edema factor and a 12-min run at 5,000 x g, 4 °C was sufficient to pellet *E. coli* cells expressing the FAT domain of FAK. Overall, the Sorvall LYNX 6000 superspeed centrifuge and Fiberlite F9-6x1000 LEX rotor meets the need for high throughput, efficient processing of samples for proteomics applications.

Acknowledgements

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