Protein Extraction from Microsomal Preparations Using Pressure Cycling Technology

The Endoplasmic Reticulum (ER) is a sub-cellular membrane network that spans the cytosol and connects the nuclear membrane to the plasma membrane. Critical cellular functions (such as protein biosynthesis, oxidation of xenobiotics, and protein transport) occur in the ER. Since ER-derived proteins account for only a small fraction of the total cellular proteome, and since the majority of ER-associated proteins are membrane proteins, which are especially difficult to purify, proteomic analysis of the ER has been a challenging endeavor.

To facilitate the study of the ER proteome, an ER-like fraction of small vesicles, termed microsomes, can be isolated from cell homogenates by differential centrifugation. However, efficiently extracting proteins from this membrane-rich fraction is difficult, making a comprehensive proteomic analysis of lipid-rich microsomal samples difficult [1]. Here we describe a method for the efficient extraction of proteins from rat liver microsomes, using Pressure Cycling Technology (**PCT**) and the novel chemistry of the ProteoSolve$_{LRS}$ Kit.

ProteoSolve$_{LRS}$ and PCT

Pressure BioSciences, Inc. (PBI) has developed a detergent-free sample preparation technique that allows for the isolation of proteins from lipid-rich samples, such as adipose tissue and microsomes. This novel method takes advantage of a synergistic combination of sample disruption using alternating hydrostatic pressure (pressure cycling technology, or PCT), and a reagent and protocol provided in the ProteoSolve$_{LRS}$ Kit that dissolves and partitions proteins and lipids into separate fractions.

PCT destabilizes inter-molecular interactions by rapidly and repeatedly raising and lowering pressure in the reaction vessel between ambient and high levels (up to 35,000 psi [240 MPa]). High hydrostatic pressure acts preferentially on the more compressible constituents of the sample, such as lipids. Thus, selective energy distribution results in destabilization of molecular interactions in the lipid bilayers and other cellular components, but not in the disruption of covalent bonds [2].

Methods

Comparison of ProteoSolve$_{LRS}$ and Traditional Detergent-based Extraction Buffer

Microsomal fractions were prepared from rats treated with either phenobarbitol or with clofibrate. Aliquots (80 µL each, 18-20 mg/mL) of the liver microsomal preparations were processed for total protein extraction by PCT with either detergent-based buffer (1% SDS Laemmli buffer) or with the ProteoSolve$_{LRS}$ Kit.

All samples were processed in either a Barocycler NEP3229 or NEP2320 for 20 cycles. Each cycle consisted of 20 seconds at 35,000 psi followed by 20 seconds at atmospheric pressure.

For detergent-based extractions, 80 µL of each sample were brought up to 1 mL with Laemmli sample buffer, transferred to PULSE Tubes, and brought to a final volume of 1.4 mL with mineral oil. After PCT treatment, samples were centrifuged, the mineral oil layer was removed, and DTT (50 mM final concentration) was added to the protein extract.

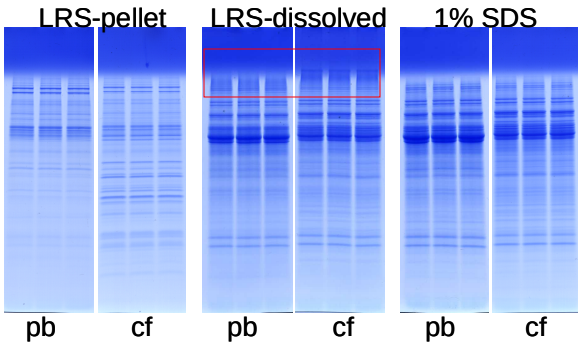

Figure 1. SDS-PAGE analysis of proteins extracted from phenobarbitol (pb) or clofibrate (cf) treated rat liver microsomes. Microsomal suspensions were processed by PCT in either Laemmli buffer (1% SDS), or using the ProteoSolve$_{LRS}$ Kit (LRS). After extraction, the LRS-pellet fraction was dissolved in a final volume of 0.3 mL SDS-PAGE loading buffer. The other samples were dissolved to a final volume, equivalent to 1 mL per sample, and were then diluted 1:10 to prevent overloading on the gel (note that the pellet lanes therefore represent a 30-fold greater fraction of starting material, compared to the other samples). All samples were loaded in triplicate. Boxed region shows high molecular weight proteins extracted by ProteoSolve$_{LRS}$ which are absent in the SDS-extract.

For extraction with the ProteoSolve$_{LRS}$ Kit, 80 µL of each sample was combined with 1 mL Reagent A, transferred to a PULSE Tube and brought up to 1.4 mL with Reagent B. After PCT, samples were centrifuged for 10 minutes at 12,000 g to separate the three fractions: the lipid phase, the solvent phase (containing the dissolved proteins), and the pellet, which is enriched in a sub-fraction of sample proteins. The lipid phase was removed; the solubilized proteins in solvent were transferred to clean test tubes, and the pellet was dissolved in 0.3 mL Laemmli sample buffer with 50 mM DTT. A 10% aliquot (0.1 mL) of the solubilized protein solution was dried under vacuum to remove the solvent and was re-dissolved in 0.1 mL Laemmli buffer with DTT. Each sample was loaded onto 8-16% Tris-glycine polyacrylamide gradient gels (Bio-Rad, Hercules, CA.)

Results and Discussion

PCT-mediated extraction, used in combination with the unique chemistry of the ProteoSolve$_{LRS}$ Kit, has previously been demonstrated to be an efficient detergent-free method for protein extraction from lipid-rich samples such as adipose tissue [3]. Here we expand the utility of this sample preparation system to the extraction of membrane-associated proteins from microsomes, a lipid-rich subcellular membrane fraction.

Previous methods for protein extraction from microsomes for electrophoretic analysis have relied upon detergents such as 1% SDS, 0.5-1.5% Triton X-100, or 1-4% CHAPS. While SDS-based extraction has given adequate protein recovery for analysis by 1D gel, it is not compatible with 2D PAGE. For 2D PAGE analysis, protein extraction using CHAPS/urea buffer is commonly used; however, this extraction method yields poor protein recovery from microsomes [2].

The data presented here show that protein extraction from microsomes by PCT, coupled with the detergent-free ProteoSolve$_{LRS}$ Kit, results in excellent recovery of microsomal proteins. Also, this process appears to extract high molecular weight protein more efficiently than SDS.

In addition, this PCT-dependent method is sensitive enough to allow detection of protein expression differences between samples treated with phenobarbitol and those treated with clofibrate. The small amount of protein that forms a pellet after PCT extraction in ProteoSolve$_{LRS}$ appears to be comprised of a sub-fraction of proteins and allows even more pronounced visualization of the differential protein expression in the two treatment groups.

ProteoSolve$_{LRS}$ is compatible with many common downstream applications. Following solvent removal by evaporation, protein pellets can be dissolved directly in a buffer-of-choice and subjected to further analysis.

The combination of PCT and ProteoSolve$_{LRS}$ is ideal for extracting proteins from lipid-rich samples such as whole adipose and brain tissue or membrane-enriched sub-cellular fractions such as microsomes.

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References

[1] Galeva N, and Altermann, MA, (2002). Proteomics 2, 713-722.

[2] Schumacher RT *et al.* (2002). *Am. Laboratory* 34, 38-43.

[3] Lazarev AV, *et al*., (2007). Journal of The American Society for Mass Spectrometry, p. 93S.

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