Application of Pressure Cycling Technology (PCT) in Proteomics: Increased Yield of High Molecular Weight Proteins in Mouse Liver Lysates

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ABSTRACT

Two-dimensional electrophoresis (2-DE) analysis of differential protein expression is limited by the number of proteins detectable on a single gel. One technological area that directly addresses this limitation is improved sample preparation and protein solubilization. This presentation describes the application of various sample preparation techniques aimed at increasing the protein analytical power of 2-DE. Pressure Cycling Technology (PCT) uses alternating cycles of high and low pressures to induce cell lysis.  Tissues placed in specially designed PULSE Tubes were subjected to alternating cycles of maximum (15,000 PSI) and minimum (ambient) pressures in the Barocycler™ [1]. Mouse liver lysates produced by sonication or with a ground glass tissue grinder were compared to lysates produced by PCT.  PDQuest analysis of two-dimensional gels of the PCT lysates revealed 2,126 protein spots compared to 1,832 protein spots in duplicate gels of the tissue grinder lysate. The results produced by sonication yielded 1,730 protein spots with a reproducibility towards diminished recovery of high molecular weight proteins. The corresponding with a general increase in the spot intensities of low molecular weight proteins suggesting that some protein degradation might be occurring during sonication.  Supported in part by AFOSR Grant F49620-03-1-0089 (FAW).

INTRODUCTION

Two-dimensional electrophoresis (2-DE) is a powerful protein analytical tool whose major strengths include semi-global quantitation and charge separation of complex protein mixtures, enabling the analysis of variable post-translational modification. One of 2-DE’s limitations relates to its limited dynamic range and consequently the number of proteins expressed that can be analyzed on a single gel. In an attempt to improve the yield of detectable proteins during sample preparation, we applied a novel extraction technique, Pressure Cycling Technology (PCT), PCT uses alternating cycles of high and low pressures to induce cell lysis. Our hypothesis contends that tissue samples subjected to PCT in the presence of a suitable lysis buffer will liberate a greater abundance of cellular proteins for subsequent 2-DE and improve the utility of this proteomic approach.

METHODS

250 mg of fresh, ice-cold saline-perfused mouse and rat liver samples were excised and frozen.

Ground-glass (GG) Homogenization

Eight (8) volumes (2 mL) of lysis buffer (9M urea, 4% NP-40, 0.5% ampholytes pH 3-10, 1% DTT) were added to the samples in a 50 mL beaker; the samples were thoroughly minced with surgical scissors [2].  Mince liver was transferred to GG tubes for homogenization (at RT).  Lysates were stored in microcentrifuge tubes until 2-DE.

Sonication (Son)

Eight volumes (2 mL) of lysis buffer (9M urea, 4% NP-40, 1% ampholytes pH 3-10, 1% DTT) were added to the liver sample and sonicated.

Pressure Cycling Technology (PCT)

Mouse liver (250 mg) or rat liver samples (250 mg) were placed in specially designed PULSE Tubes (Figure 1) with 8 volumes (2 mL) of the lysis buffer described above and subjected to alternating cycles of high and low pressures in a pressure-generating instrument (Barocycler Model NEP3229, Figure 2).  PULSE Tubes were subjected to 10 pressure cycles; each cycle consisted of 20 seconds at 35,000 PSI followed by 20 seconds at ambient pressure.  Following PCT, the lysates were collected and cellular debris was removed by centrifugation at 20,000 RCF for 10 minutes.

2-DE

Protein concentration was determined using an Amido black assay.

RESULTS

1. In the initial experiment, duplicate mouse liver samples (n=2) were solubilized as described. PDQuest detected the following in each preparation group (see Figure 3):

- PCT: 2,280 ± 173
- GG: 1,630 ± 137
- GG/Son: 1,735 ± 144
- GG/Son/2X: 1,682 ± 165

2. A second experiment compared rat liver samples (n=5) preparation by PCT, GG, GG with sonication, and GG/sonication in twice the lysis buffer volume (16 volumes). PDQuest detected the following in each preparation group (see Figure 5):

- PCT 2,280 ± 173
- GG 1,630 ± 137
- GG/Son 1,735 ± 144
- GG/Son/2X 1,682 ± 165

CONCLUSIONS

1. This study confirms and extends previous results with E. coli where PCT sample preparation for 2-DE improved protein spot detection by 14.2% compared to standard bead mill treatment [4].

2. In mouse liver samples, PCT improved overall protein extraction from tissue as indicated by a significantly higher number of protein spots detected by 2-DE.

3. In rat liver samples, PCT demonstrated an even greater improvement in protein extraction.

4. To overcome some of 2-DE’s limitations (with respect to sensitivity), it is beneficial to include PCT whole tissue sample preparation protocols.

5. To better understand the mechanism of PCT’s utility, ongoing experiments are aimed at identifying proteins where relative abundance on 2-DE is selectively enhanced (or decreased) by PCT in lysate buffer.

6. Additional studies are necessary to optimize the PCT conditions (duration, number of cycles, etc.) for unique tissue and cell culture samples.

REFERENCES


