Applications of Pressure Cycling Technology (PCT) to Sample Preparation for Two-Dimensional Gel Electrophoresis

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1. Introduction

Pressure Cycling Technology (PCT) uses alternating cycles of high and low hydrostatic pressure to effectively induce the lysis of cells and tissues in preparation for two-dimensional electrophoresis (2DGE) and other analytical or preparative methods. Rapid cycling between high and low pressure is more disruptive than high pressure alone, as evidenced by the increased protein yields from *Saccharomyces cerevisae* correlating to the number of pressure cycles rather than the total elapsed time at high pressure [1]. Similarly, proteins of increasing molecular mass correlating to the number of pressure cycles were released from the chitin exoskeletons of the arthropod *Daphnia pulex* [2].

Previously, Geiser *et al.* [3] reported the PCT release 37% more protein from the nematode *Caenorhabditis elegans* than sonication. From gram-negative bacteria, PCT reportedly yielded 14.2% more protein from *Escherichia coli* than bead mill [4] and 17.1% more protein from *Rhodopseudomonas palustris* than enzymatic lysis with lysozyme [5].

For plant tissues, PCT extracted more protein from several components of the *Strelitzia reginae* inflorescence than a centrifugal homogenizer [2]. For mammalian tissues, PCT also isolated more protein from liver, including unique proteins which were not isolated in Polytron or douncer homogenates [6]. From adipose tissue, PCT extracted more protein than pulverization under liquid nitrogen and detergent extraction of the triturate.

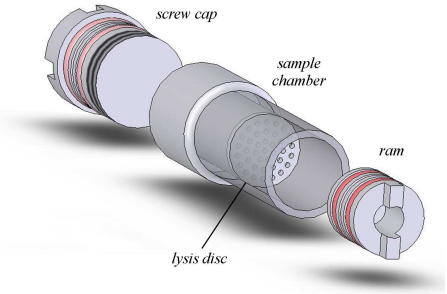

Figure 1. *Exploded view showing the components of the PULSE Tube FT-500. Under high pressure, the ram forces tissue and fluid through the perforated lysis disc. Upon return to ambient pressure, the ram retracts pulling in solvent from the other chamber.*

2. Materials and Methods

2.1 Pressure Cycling Technology

PCT uses alternating cycles of high and low pressure to induce cell lysis. Cell suspensions or tissues are placed in specially designed single-use PULSE Tubes (**Figure 1**) and are subjected to alternating cycles of high and ambient pressure in a pressure-generating instrument (Barocycler NEP3229). Maximum and minimum pressures (0-35,000 psi), the time sustained at each pressure level, and the number of cycles is defined using programmable logic controller interface. The Barocycler pressure chambers is temperature controlled using a peripheral circulating water bath. Safety features in the PCT system design significantly reduce risk of exposure to the researcher to pathogens. Recently, two researchers from the U.S. Center for Disease Control were infected with West Nile Virus when tubes ruptured during bead mill operation [4].

2.2 Sample Preparation

Unless otherwise specified, samples were processed in ProteoSOLVE CE Lysis Reagent (Pressure BioSciences, West Bridgewater, MA, USA) supplemented with 100 mM DTT and protease inhibitor cocktail P-2714 (Sigma-Aldrich Chemicals, St. Louis, MO, USA). For PCT, samples were typically subjected to 20 pressure cycles; each cycle consisting of 20 seconds at 35,000 psi followed by 20 seconds at ambient pressure. Following PCT, the samples were centrifuged for 10 minutes at 25,000 RCF and 500 uL of the sample was transferred to an Ultrafree Centrifugal Filtration Device (Millipore, Danvers, MA, USA) and centrifuged until a retentate volume of 100 uL was obtained. Four hundred microliters of ion-exchanged ProteoSOLVE IEF Reagent was added (reducing the DTT concentration to 20 mM) and the sample was alkylated for two hours following the addition of 40 mM acrylamide and 40 mM Tris. The alkylation reaction was terminated by resuming centrifugation in the ultrafiltration device [5].

2.3 SDS PAGE, IEF, and 2DGE

IEF and 2DGE was performed as described by previously by Smejkal *et al.* [5]. Extracts from adipose tissues were analyzed by SDS PAGE on NuPAGE Novex 4-12% Bis-Tris gels using MOPS-SDS running buffer (Invitrogen, Carlsbad, CA, USA).

3. Results and Discussion

3.1 PCT vs. Enzymatic Lysis

PCT used in combination with the chaotropic ProteoSOLVE Lysis Reagent yielded 17.1% more protein than enzymatic lysis with recombinant lysozyme from the gram-negative bacterium *Rhodopseudomonas palustris* (**Figure 2**). PCT isolated several putative membrane proteins from *R. palustris* were not isolated by enzymatic lysis, which appears biased for cytosolic proteins.

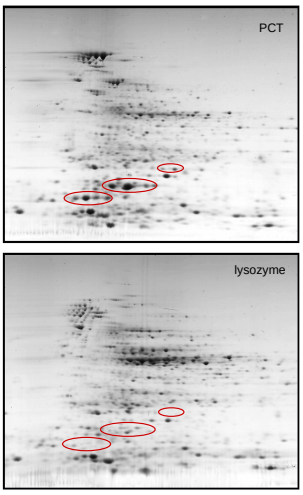

Figure 2. *R. palustris lysates produced by PCT (top) or enzymatically using lysozyme (bottom). Several proteins were isolated by PCT that were not isolated by enzymatic lysis (red elipses). IPGs were pH 3-10.*

3.2 PCT vs. Sonication

PCT was compared to sonication for the extraction of proteins from mouse livers macerated in a ground glass douncer. 2DGE of PCT lysates of mouse liver (250 mg) detected 2,126 protein spots, compared to 1,739 proteins spots detected in the sonication lysates. As shown in **Figure 3**, high molecular weight proteins were diminished in lysates produced by probe sonication. This was commensurate with an increase in low molecular weight proteins in the sonication lysates. It is hypothesized that the gradual deterioration of sonicator probes and the release of metal ions into the sample could facilitate reactivation of proteolytic enzymes or the oxidation of proteins.

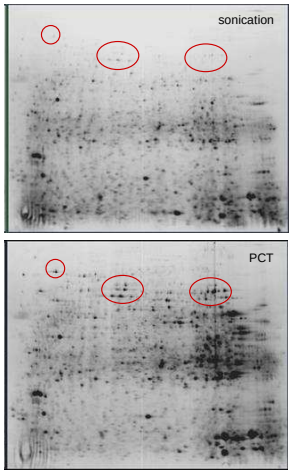

Figure 3. *2DGE of mouse liver proteins isolated by sonication (top) or PCT (bottom). PCT isolated more high molecular weight species (red elipses). IPGs were pH 4.5-6.5.*

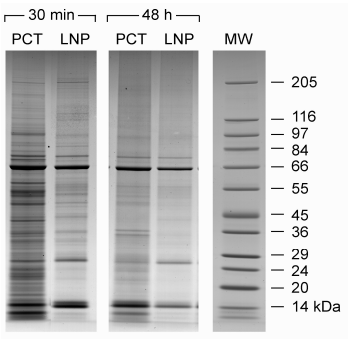

Figure 4. *Proteins extracted from adipose tissues by PCT or pulverization under liquid nitrogen (LNP). Samples were normalized to 8 mg initial tissue mass by dilution into lithium dodecylsulfate buffer supplemented with 60 mM DTT.*

3.3 PCT vs. Pulverization

PCT was compared to pulverization under liquid nitrogen followed by buffer extraction of the triturate for the isolation of proteins from mouse adipose tissue. Tissue samples (20-80 mg) were placed in a porcelain crucible and pulverized under liquid nitrogen. The triturates were transferred to polypropylene tubes containing 500 uL of NPB buffer (20 mm Tris, 150 mM NaCl, 60 mM KCl, 9% sucrose, 5% glycerol, 1% Triton X-100, 2 mM EDTA, pH 7.5) and incubated 30 minutes to 48 hours at 4º C.

PCT extracted more protein from adipose in 30 minutes than buffer extraction from pulverized triturates. "Longer is not better" as significant protein losses were observed commensurate with extended incubation times of PCT and pulverized lysates. SDS PAGE, shown in **Figure 4,** showed no accumulation of small molecular weight proteins suggesting proteolysis was not the culprit. It is hypothesized that this loss of protein was due to aggregation of hydrophobic proteins from adipose.

3.4 PCT vs. Glass Bead Beating

PCT was compared with bead beating for the disruption of nematodes. Approximately 50 mg of *C. elegans* nematodes were suspended in 500 uL water and combined with 500 mg of 1 mm glass beads. The tubes were vortexed for 8X 40 seconds during which time the temperature increased to 45º C. Alternatively, temperature remained constant at 22º C during PCT in the thermostated Barocycler. In **Figure 5**, micrographs compare nematodes processed by PCT and bead beating. When processed in combination with the ProteoSOLVE Lysis reagent, PCT yielded 10.8% more protein from *C. elegans* than bead beating [7].

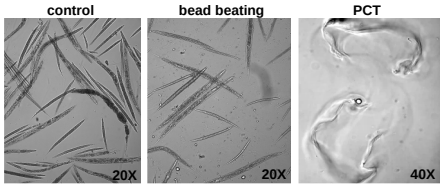

Figure 5. *Microscopy showing many unaffected nematodes after 320 seconds of bead beating (center) which resembles the untreated control (left.) Enlargement of remaining nematode "ghosts" following PCT (right).*

4. References

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