A Comparison of Pressure Cycling Technology (PCT) to Other Methods of Protein Extraction

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Abstract

Following the completion of the human genome project, many researchers have been focusing their efforts on developing a better understanding of the human proteome. It is expected that the elucidation of the proteome will lead to better diagnosis and treatment of disease through the identification of clinically important biomarkers. The quality of proteins extracted during initial sample preparation is critical to the success of downstream separation techniques and detection technologies. Here we present an evaluation of conventional physical and chemical methods used to extract proteins from a variety of biological samples, and we compare these data to a novel, biophysical mechanism for the release of proteins from cells and tissues that uses alternating cycles of high and ambient pressures called Pressure Cycling Technology (PCT). Human *stratum corneum*, murine liver, *Escherichia coli, Caenorhabditis elegans, Rhodopseudomonas palustris,* and *Daphnia pulex* were processed using PCT and conventional methods, such as sonication, homogenization, and enzyme digestion. Following extraction, total protein concentrations were determined by quantitative protein assays. Protein spots were defined on 2D gels and quantified using image analysis software.

Pressure Cycling Technology (PCT)

PCT uses alternating cycles of ultra-high and ambient pressures to induce cell lysis. Cell suspensions or tissues are placed in specially designed PULSE Tubes (**Figure 1**) and are subjected to cycles of high and low pressures in a pressure-generating instrument (Barocycler Model NEP3229). Maximum and minimum pressures (14 - 35,000 PSI or greater), the time sustained at each pressure level, and the number of cycles are defined using a programmable logic controller interface. The Barocycler instrument's reaction chambers are temperature controlled using a peripheral circulating water bath. Safety features built into the PCT System's design significantly reduce the risk of exposure to the researcher to pathogens [1].

Figure 1. *Specially designed PULSE Tubes (left) for disrupting cells and tissues by PCT using a Barocycler (right). At high pressure, the movable ram compresses the sample against the lysis disk. The perforated lysis disk macerates the sample, increasing the surface area that is exposed to the extraction buffer.*

Methods and Results

Comparison of PCT to Sonication or Enzymatic Lysis

Cells of *R. palustris,* a bacterium, were pelleted by centrifugation at 12,000 RCF for five minutes and suspended at a density of 0.35 g cells/mL in distilled water. For each analysis, 0.57 mL of this suspension was again pelleted by centrifugation. One pellet was suspended in 1.5 mL of ProteomIQ Suspension Reagent C7BzO (Proteome Systems, Woburn, MA, USA, ref. 2) and processed by PCT as described in **Figure 2**. A second pellet was suspended in 1.5 mL of the C7BzO reagent and sonicated for six 30 second intervals. The sample was cooled on ice for one minute after each sonication interval. A third pellet was suspended in BugBuster Plus (EMD Biosciences, Madison, WI, USA) reagent containing recombinant lysozyme and benzonase. The resulting lysates were centrifuged at 12,000 RCF to pellet cellular debris. One hundred microliters of each lysate was mixed with 0.4 mL 7M urea, 2M thiourea, 65 mM CHAPS, 40 mM Tris, 5 mM tributylphosphine (TBP), and 10 mM acrylamide. The lysates were incubated for two hours. The reaction was terminated by ultrafiltration in an Ultrafree 0.5 mL centrifugal ultrafiltration device (Millipore, Danvers, MA, USA) in the Urea/CHAPS reagent as previously described [3].

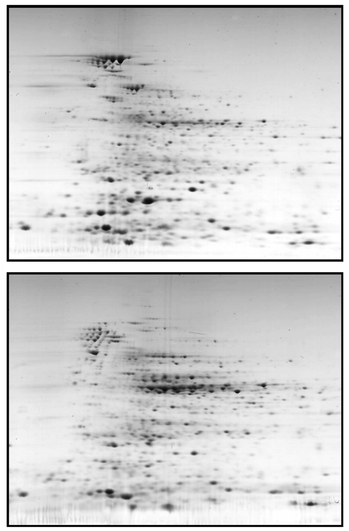

Figure 2. *R. palustris lysates produced by PCT (top) or enzymatic lysis using lysozyme (bottom). PULSE Tubes were subjected to 30 pressure cycles. Each cycle consisted of 20 seconds at 35,000 PSI and 20 seconds at ambient pressure. PCT used in combination with the chaotropic C7BzO reagent yielded 17.1% more protein than enzymatic lysis and yielded 5.3% more protein than sonication (sonication data not shown). IPGs were pH 3-10.*

Comparison of PCT to a Ground Glass Tissue Grinder or Polytron Homogenizer

Two hundred and fifty milligrams of saline-perfused rat liver sections were placed in PULSE Tubes with 1.25 mL of lysis reagent containing 9M urea, 4% NP-40, 0.5% ampholytes pH 3-10, 1% DTT and were subjected to PCT as described in **Figure 3**.

To process liver in a ground glass tissue grinder, 250 mg of tissue was placed in a beaker containing 2 mL of the same lysis reagent as above, and thoroughly minced with surgical scissors; the tissue was then transferred to ground glass tubes for manual homogenization. Other rat liver samples were sonicated in 2 mL of lysis reagent. For Polytron homogenization, 250 mg of tissue was pulverized under liquid nitrogen using a mortar and pestle and homogenized in 3 mL lysis reagent.

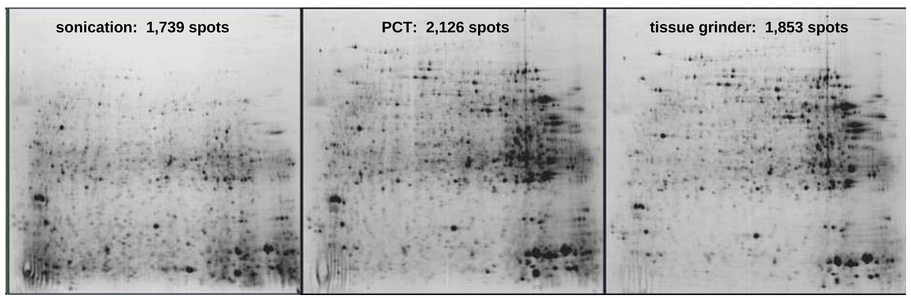

Figure 3. *2DGE of mouse liver lysates illustrating the loss of higher MW proteins that occurs with sonication. Overall protein spot detection was significantly higher in PCT processed samples. PCT was performed for 10 pressure cycles; each cycle consisted of 20 seconds at 35,000 psi followed by 20 seconds at ambient pressure. IPGs were pH 4.5-6.5.*

Comparison of PCT and Bead Mill Oscillation

Two hundred and fifty milligrams of lyophilized *C. elegans,* a nematode, were reconstituted in 6.4 mL of ProteoSOLVE CE Lysis Reagent, and 1.4 mL of this suspension was transferred to each of two PULSE Tubes. PCT was performed as described in **Table 1**. Alternatively, 1.4 mL of the cell suspension was transferred to 2 mL tubes containing 100 mg of polydisperse glass beads (0.5-1.0 mm) for bead mill oscillation. Tubes were cycled five times for 15 second intervals at 2500 oscillations/min on a Mini-Beadbeater (BioSpec Products, Bartlesville, OK, USA). The negative control consisted of an aliquot of the same nematode suspension in ProteoSOLVE CE without mechanical agitation.

Table 1: Protein Isolated from *C. elegans* by PCT or Bead Mill

Method	Total Protein Recovered [a]	% Increase
negative control	6.94	-
bead mill	6.91	-
PCT, 10 cycles [b]	7.52	8.8
PCT, 20 cycles [b]	7.66	10.8

[a] mg protein recovered from 39 mg/mL nematode suspension
[b] 30 seconds at 35 kpsi, 10 seconds at ambient pressure

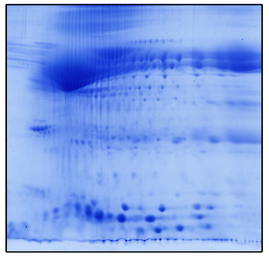

Figure 4. *Proteins released from psoriatic exfoliates by PCT. Five cycles were performed. Each cycle was for 30 seconds at 35 kpsi followed by 30 seconds at ambient pressure. The two most predominant proteins were putatively identified as keratin and IgG. Numerous other proteins of lesser abundance are currently being investigated as potential biomarkers.*

PCT Extraction of Proteins from Human Psoriatic Cells

Stratum corneum was sampled using adhesive films from a psoriasis patient undergoing treatment with 1% topical hydrocortisone. Psoriatic lesions were cleansed thoroughly with 70% isopropanol and dried before exfoliation with adhesive films. Two films were placed in a PULSE Tube with 1.5 mL of C7BzO reagent supplemented with 5 mM TBP and protease inhibitors. PCT was performed as described in **Figure 4.** The adhesive films were removed from the PULSE Tube and replaced with two unprocessed films, and PCT was repeated. The lysates were alkylated with 40 mM Tris and 10 mM acrylamide. After two hours, the alkylation reaction was terminated by ultrafiltrative exchange into CHAPS reagent. The lysates were concentrated six fold for 2DGE.

PCT Extraction of Proteins from *Daphnia pulex*

Fifteen milligrams of dry *D. pulex,* a crustacean, were placed in a PULSE Tube with 1.4 mL ProteoSOLVE CE Lysis Reagent with protease inhibitors added. The sample was treated with iterations of PCT that consisted of 10 cycles; each cycle was 35 kpsi for 40 seconds followed by 10 seconds at ambient pressure. After each iteration, the lysis reagent was removed and replaced with 1.4 mL of fresh reagent. Each fraction was precipitated in 80% acetone, and the proteins were solubilized in 50 mM Tris acetate pH 7.0 containing 2% SDS prior to electrophoresis in a 6%-15% gradient gel. The proteins extracted after each iteration are shown in **Figure 5**.

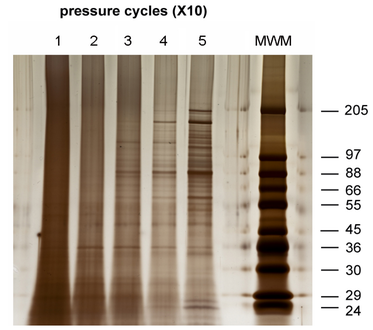

Figure 5. *Proteins of increasing molecular mass extracted from D. pulex by sequential iterations of ten pressure cycles. Protein molecular mass is indicated on right (in kDa). Gel was silver stained.*

Discussion

While the uses of high pressure to extract proteins from cells, such as the extraction using a French Press, have been previously described, we discovered that the ability to rapidly cycle between high and low pressures using PCT has a profound effect on lysis efficiency in a wide variety of cells and tissues, including difficult-to-lyse materials. When analyzed by 2D gel electrophoresis, it was found that various samples, including mouse liver, extracted by PCT in conjunction with chaotropic detergent solutions (ProteoSOLVE Lysis Reagent), consistently yielded more protein spots compared to the other extraction methods described here. Even greater differences were observed when PCT was compared to enzymatic lysis, which is commonly employed for the preparation of yeast and gram-negative bacteria. A greater number of protein spots on 2D gels of PCT lysates of *E. coli* was obtained compared to the bead beating method [4].

Our data suggest that extraction by PCT may elicit more, and sometimes different, proteins for further study than current sample preparation methods. We hypothesize that because PCT uses a different biophysical process than conventional methods, both more and a greater variety of proteins are released. We propose that PCT is an important, alternate extraction strategy to current methods to reveal the proteome, as well as to find new biomarkers.

References

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[3] Smejkal, G.B. *et al.* (2006). *J. Proteomic Res.,* in press.
[4] Smejkal, G.B. *et al.* (2006). *J. Biomolecular Techniques,* **17**, 159-161.