The Evolution of Sample Preparation in Proteomics: Applications of Pressure Cycling Technology (PCT) Spanning from *Bacteria* to *Homo sapiens*

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1 Abstract

The fast, efficient, and accurate release of proteins from cells and tissues is a critically important initial step in most analytical processes, and is essential to reliable proteomic analyses. Two-dimensional gel electrophoresis (2DGE) can be an accurate representation of a proteome only if the entire protein constituency of cells is recovered during the sample preparation process. The use of chaotropes and new surface-reactive agents has substantially improved the capacity of 2DGE to produce more comprehensive maps of cellular and subcellular proteomes, but this increased stringency frequently complicates downstream analyses such as electrophoresis, chromatography, and mass spectrometry. Further, the disruption of cells and tissues usually requires coupling these chemistries with mechanical disruption methods, which may contribute to deleterious effects including the modification of proteins. 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 2DGE and other analytical or preparative methods.

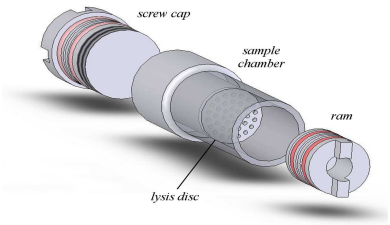

Figure 1. *Exploded view showing the components of the PULSE Tube FT-500. Under high pressure, the ram forces tissue and fluid through the perforated lysis disc. Upon return to ambient pressure, the ram retracts pulling in solvent from the other chamber.*

2 Materials and Methods

The NEP 3229 and 2320 Barocycler instruments, PULSE Tubes, ProteoSolve$_{LRS}$ Kit for lipid-rich samples, and ProteoSolve$_{CE}$ Lysis Reagent were from Pressure BioSciences (West Bridgewater, MA, USA).

2.1 Pressure Cycling Technology (PCT)

Pressure Cycling Technology (PCT) uses alternating cycles of high and low hydrostatic pressure to induce cell lysis. Rapid cycling between high and low pressure has been demonstrated to be more disruptive than sustained high pressure [1,2]. Typically, samples were loaded into PULSE Tubes and processed in the Barocycler NEP3229 for 40 cycles. Each cycle consisted of 10 seconds at 35,000 psi, followed by rapid return to atmospheric pressure held for 5 seconds.

2.2 IEF and 2DGE

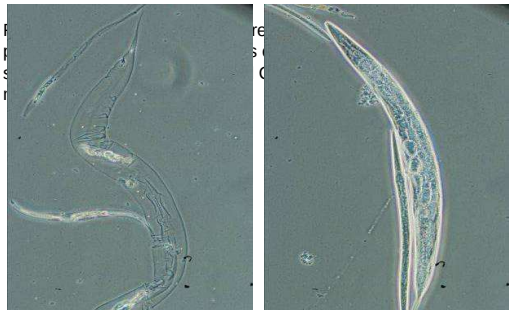

Figure 2. *Phase contrast microscopy (100X) showing nematode "ghosts" produced by PCT of Caenorhabditis elegans using ProteoSolve CE Lysis reagent (left). Undisrupted nematodes are shown (right) using modified RIPA buffer (10 mM Tris, 200 mM NaCl, 2.5 mM $MgCl_2$, 0.5% Triton X-100) for lysis.*

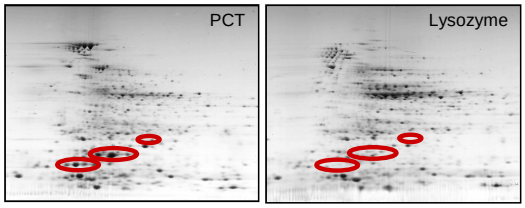

Figure 3. *Comparison of PCT and enzymatic lysis of Gram-negative bacteria. Rhodopseudomonas palustris lysates produced by PCT (left) or enzymatically using lysozyme (right). Several proteins were isolated by PCT that were not isolated by enzymatic lysis (red ellipses), which appears to enrich cytosolic proteins.*

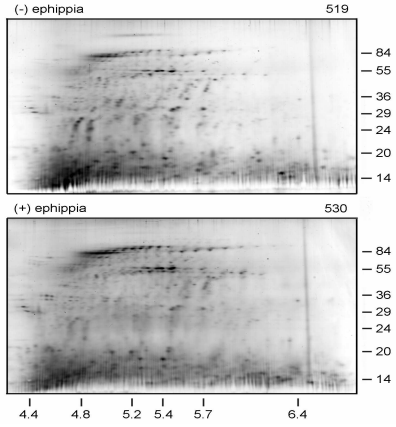

Figure 4. *Phenotypic differences between sexual (+) and asexual (-) Daphnia magna. Total protein derived from a single microcrustacean (0.23 ± 0.06 mg dry weight) of each phenotype processed by PCT. The number of protein spots in each gel is indicated (upper right).*

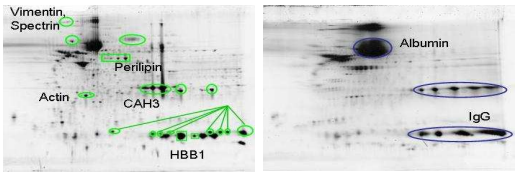

Figure 5. *Extraction of proteins from 100 mg of normal murine adipose tissue. Extraction in the conventional CHAPS-based 2D sample extraction buffer (right) results in a solution of predominantly blood plasma proteins, while tissue dissolution by PCT and ProteoSolve$_{LRS}$ (left) followed by removal of lipids and solvent and reconstitution in 2D electrophoresis sample buffer appears to produce a sample representing the entire proteome of the adipose tissue.*

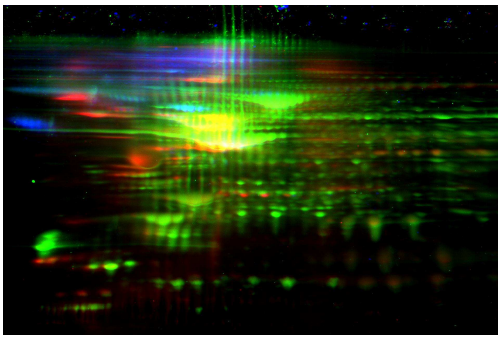

Figure 6. *2DGE of human atrium proteins isolated by PCT. Gel was sequentially stained for phosphoprotein (blue), glycoprotein (red), and total protein and the images were electronically superimposed. Pro-Q Diamond Phosphoprotein, Pro-Q Emerald Glycoprotein, and SYPRO Ruby Stains were from Invitrogen (Carlsbad, CA, USA).*

3. Results and Discussion

PCT has been used for the disruption of cells and tissues ranging from bacteria to *H. sapiens*. Data show that PCT can more effectively release proteins than current standard methods. PCT also facilitates precise control over sample processing conditions and helps ensure greater reproducibility. For example from the Gram-negative bacteria *R. palustris*, PCT yielded 17.1% more protein than by enzymatic lysis [3] and 14.2% more protein from *E. coli* than bead mill [7]. Furthermore, PCT effectively disrupted diazovesicles from the nitrogen-fixing bacteria Frankia that remained intact even following treatment by a French press [6]. PCT was also used to analyze protein expression for the microcrustacean *D. magna including the* phenotypes from single representative organisms. In another application PCT yielded 37% more protein from the nematode *C. elegans* than sonication [5]. PCT effectively lysed complex mammalian and plant tissues. PCT released more protein from liver [8] and adipose [9,10], both quantitatively and qualitatively, as demonstrated by the appearance of several proteins in 2DGE, which were not recovered by conventional homogenization techniques. Improved protein recoveries have also been demonstrated for plant tissues where PCT yielded three times more protein from *S. reginae* sepals and nearly ten times more protein from the American Beech (*F. grandifolia)* tree bark phloem th

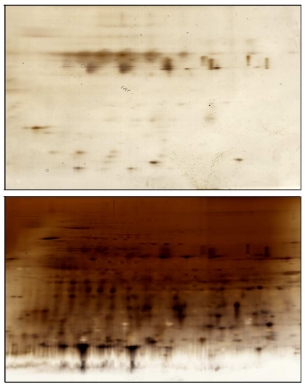

Figure 7. *Extraction of proteins from Fagus grandifolia tree bark by conventional methods (top) or PCT (bottom). For the top sample preparation, 450 mg tree bark suspended was in 60 mM Tris pH 6.8, 5 mM EDTA,125 mM BME, 10% PVPP and homogenized 2X 50 seconds at 24,000 rpm using an IKA-Labortechnik homogenizer. For the bottom sample, 450 mg tree bark was suspended in ProteoSolve$_{CE}$ Lysis reagent and subjected to PCT for 60 cycles at 35,000 psi maximum pressure.*

4. References

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