Application of Pressure Cycling Technology (PCT) in Proteomics: Increased Cell Lysis and Protein Yields from Model Organisms

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Abstract

Sample preparation is critical to the success of two-dimensional gel electrophoresis (2DGE) and other analytical methods. The Pressure Cycling Technology (PCT) Sample Preparation System (SPS) was used to effectively release protein from a variety of organisms. The PCT SPS uses alternating cycles of high and low pressure to induce lysis. Maximum pressure, the time sustained at each pressure level, and the number of cycles in the pressure-generating instrument (Barocycler) are defined using the programmable logic controller interface of the Barocycler. In addition, temperature in the Barocycler can be controlled by use of an external, circulating water bath. Several organisms were used as model systems to demonstrate the programmable parameters available with the PCT SPS. These parameters were adjusted to improve cell lysis, which resulted in an increased yield of protein when compared to bead mill lysis or sonication. Bacterial cell suspensions were placed in specially designed PULSE Tubes and were subjected to 5-30 alternating pressure cycles. Cycles typically consisted of 20 seconds at 35,000 PSI followed by 20 seconds at ambient. For *Escherichia coli*, PCT extracted 14.2% more total protein than was extracted using a bead mill. Image analysis of two-dimensional gels revealed 801 protein spots in the PCT lysate, compared to 760 protein spots in the bead mill lysate. For the bacterium *Rhodopseudomonas palustris*, PCT isolated 5% and 17% more protein than probe sonication and enzymatic lysis, respectively. Further, the temperature in the Barocycler remained constant at 22º ± 0.6º C throughout 30 pressure cycles. In contrast, samples processed by the bead mill required manually chilling the sample vessel in shaved ice repetitively to maintain temperature below 40˚C and, with sonication, maintaining an open sample on ice during processing. In addition to bacteria, parameters were adjusted to improve the yield of protein from yeast suspensions and rat tissues. These findings suggest that by varying the processing parameters with the PCT SPS, a safer and improved release of proteins from human tissue should be possible, as compared to other currently used methods.

Pressure Cycling Technology (PCT)

PCT uses alternating cycles of high and low pressure to induce cell lysis. Cell suspensions or tissues are placed in specially designed single-use processing containers and are subjected to alternating cycles of high (up to 35,000 PSI) and ambient pressure in a pressure-generating instrument (Barocycler Model NEP3229). Maximum and minimum pressures, the time sustained at each pressure level, and the number of cycles is defined using a computer or programmable logic controller interface. The Barocycler instrument reaction chambers are 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 [1].

Methods

Comparison of PCT and Bead Mill Oscillation

Sixty milligrams of lyophilized *E. coli* K12 was reconstituted in 10 mL of 7M urea, 2M thiourea, and 25 mM 3-(4-heptyl) phenyl 3-hydroxypropyl dimethylammonio propanesulfonate (C7BzO) [2]. Twenty-five microliters of 200 mM tributylphosphine were added and 1.5 mL of this cell suspension was transferred to each of two PULSE Tubes. PCT was performed as described in **Figure 1**.

Alternatively, 1.5 mL of the cell suspension was transferred to each of two 2 mL polypropylene tubes. For the bead mill procedure, samples were placed in tubes and subsequently loaded into the adapter rack of a Retsch MM 301 mixer mill with tungsten carbide grinding balls provided by the manufacturer (Retsch GmbH, Haan, Germany). The tubes were cycled three times for one minute at 1,800 oscillations per minute. After each cycle, the temperature of the samples reached 40º C requiring that the tubes be removed from the adapter and placed on ice for several minutes between cycles. (A potentially precarious practice since the solubility of urea, thiourea, and the C7BzO detergent is compromised below 18º C.) The contents of the two tubes were combined and centrifuged at 24,000 RCF for 10 minutes to remove cellular debris.

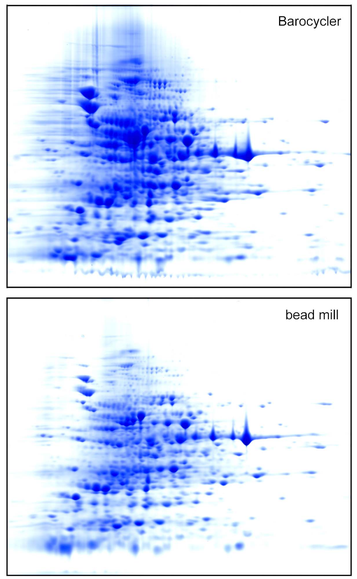

Figure 1. *E. coli lysates produced by PCT (top) or bead mill oscillation (bottom). Each cycle consisted of 20 seconds at 35,000 PSI followed by 20 seconds at ambient pressure. From image analysis, the sum of integrated spot volumes in the PCT lysate was 14.2% higher than the total spot volumes in the bead mill lysate. 801 proteins were revealed in the PCT lysate, compared to 760 proteins in the bead mill lysates. IPGs were pH 3-10.*

Three milliliters of each lysate were alkylated for two hours following the addition of 10 mM acrylamide and 40 mM Tris. Proteins were precipitated with 80% acetone at room temperature for 30 minutes. The flocculent was pelleted by centrifugation at 24,000 RCF for 10 minutes. Pellets were redissolved in 3 mL of ion-exchanged 7M urea, 2M thiourea, and 65 mM CHAPS.

Comparison of PCT to Sonication or Enzymatic Lysis.

Rhodopseudomonas palustris cells 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 (~200 mg cells) was again pelleted by centrifugation. One pellet was suspended in 1.5 mL of C7BzO reagent and processed by PCT as described in **Figure 2**.

A second pellet was suspended in 1.5 mL of C7BzO reagent and sonicated for six 30 second repetitions. The sample was placed on ice for one minute after every two sonication intervals to cool the sample. A third pellet was suspended in BugBuster® Plus reagent containing recombinant lysozyme and benzonase (EMD Biosciences, Madison, WI, USA). 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 of 7M urea, 2M thiourea, 65 mM CHAPS, 40 mM Tris, 5 mM tributylphosphine, and 10 mM acrylamide and was incubated for two hours. The reaction was terminated by ultrafiltration in an Ultrafree 0.5 mL centrifugal filtration device (Millipore, Danvers, MA, USA) by exchange into ion-exchanged 7M urea, 2M thiourea, and 65 mM CHAPS [3].

Comparison of PCT to Tissue Grinding

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**. For grinding in a ground glass tissue grinder, 250 mg of tissue was placed in a beaker containing 2 mL of lysis reagent, thoroughly minced with surgical scissors, and transferred to ground glass tubes for homogenization. Other rat liver samples were sonicated in 2 mL of lysis reagent. The lysates were collected and cellular debris was removed by centrifugation at 20,000 RCF for 10 minutes.

PCT of yeast cell suspensions

To demonstrate that it is the cycling of pressure (rather than high pressure alone) has a profound effect on lysis efficiency, lyophilized yeast were resuspended in TBS and subjected to PCT were the number of pressure cycles (n) varied, but the total time elapsed at high pressure remained constant. A benign buffer was used to limit autolysis and clearly differentiate mechanical disruption. Results are shown in **Figure 4**.

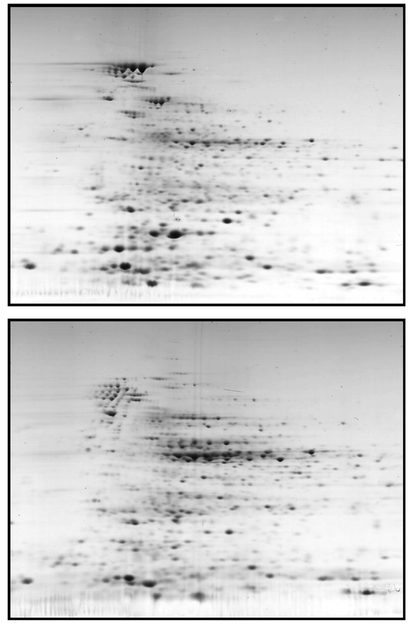

Figure 2. *R. palustris lysates produced by PCT (top) or enzymatically using lysozyme (bottom). PULSE Tubes were subjected to 30 pressure cycles. Each cycle consisted of 20 seconds at 35,000 PSI and 20 seconds at ambient pressure. Using PCT in combination with the chaotropic C7BzO reagent yielded 17.1%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

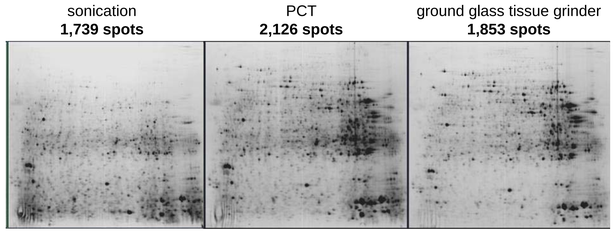

Figure 3. *2DGE of mouse liver lysates illustrating the loss of higher MW proteins that occurs with sonication. Overall protein spot detection was significantly higher in PCT processed samples. PCT was performed for 10 pressure cycles; each cycle consisted of 20 seconds at 35,000 PSI followed by 20 seconds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

Results and Discussion

For *E. coli*, PCT extracted 14.2% more total protein than was extracted using a bead mill. Two-dimensional gel electrophoresis (2DGE) revealed 801 protein spots in the PCT lysate, compared to 760 protein spots in the bead mill lysate. For *Rhodopseudomonas palustris*, PCT isolated 5% and 17% more protein than probe sonication and enzymatic lysis, respectively.

For 2DGE of rat liver, the overall number protein spots that were detected was significantly higher in PCT processed samples. While the use of high pressure to extract proteins from cells has been previously described [4], the ability to rapidly cycle between high and low pressures using PCT has a profound effect on lysis efficiency.

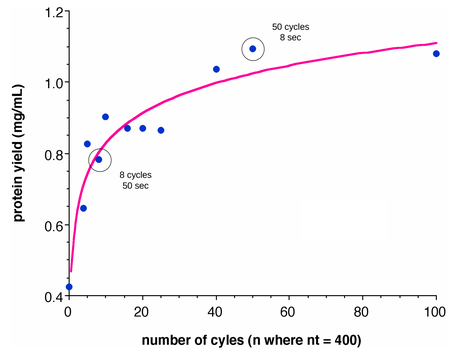

Figure 4. *Evidence that the number of pressure cycles (n), rather than the total time (t) elapsed at high pressure, has a profound effect on lysis efficiency. Relative protein yield from yeast shown as a function of n, where the product nt is constant.*

Summary

The PCT Sample Preparation System (PCT SPS) Extraction Method:

- Employs a new approach for the extraction of biomolecules from cells and tissues, including difficult-to-lyse materials.
- Functions by different mechanisms to release biomolecules compared to other commonly used mechanical and chemical extraction methods
- Is rapid, safe and reproducible.
- Is fully contained to minimize cross contamination and the exposure of operators to toxic or infectious agents
- Is capable of releasing proteins small molecules, and nucleic acids into buffers suitable for downstream purification or direct analysis

References

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