Breaking the Tough Cuticle of *Caenorhabditis elegans* Using Pressure Cycling Technology (PCT) and ProteoSOLVE Lysis Reagent: "Giving the Worms a Perm"

No other multicellular organism has been studied more extensively at the cellular and molecular levels than the nematode *Caenorhabditis elegans* [1]. However, the tough exterior cuticle makes the nematode resilient to lysis and impedes proteomic and glycoproteomic analyses. The cuticle is comprised largely of chitin and of a collagen that is particularly rich in *N*- and *C*-terminal cysteines, most of which are involved in disulfide linkages. Enzymes such as chitinase or collagenase could be used to weaken the cuticle. However, unless these enzymes are immobilized, they would contribute exogenous protein that would be present in downstream analyses. Further, enzymatically induced cell lysis has been shown to be biased towards the recovery of cytoplasmic and membrane proteins and other hydrophobic proteins, which results in a misrepresentation of the proteome in the lysate [2].

Inspired by earlier chemists who developed the "permanent wave" in which the cuticle of human hair is first softened in a chemical process that reduces protein disulfides, the ProteoSOLVE Lysis Reagent was designed specifically for maximal disruption of the *C. elegans* cuticle and increased protein yield. The reagent contains a proprietary detergent and reducing agent. Both the type and the concentration of reducing agent were optimized to maximize the efficiency of nematode cuticle disruption.

Use of the alternative reducing agent, tributylphosphine (TBP), was less effective than ProteoSOLVE when high disulfide concentrations are present. This is likely related to the instability of TBP, which oxidizes within minutes to Bu_3PO [3]. When used in combination with PCT, ProteoSOLVE produced higher protein yields than other disruption 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 (PULSE Tubes) 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 programmable logic controller interface. The Barocycler instrument reaction chamber 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 [4].

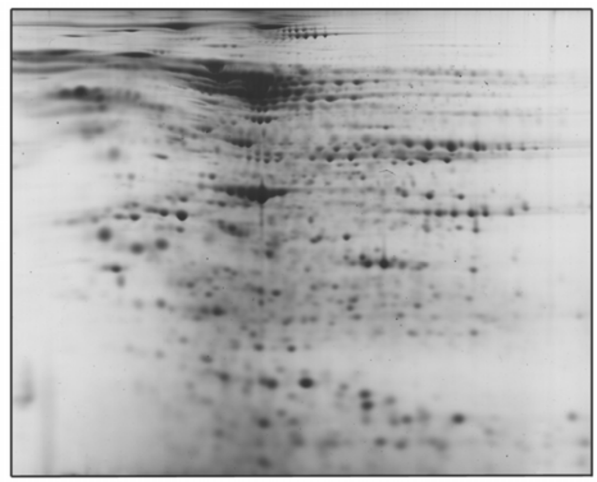

Figure 1. *2DGE of C. elegans Lysate Derived from 20 Cycles PCT. The IPG was hydrated with 0.2 mL of lysate that was diluted 1:6.*

Methods

Fifty milligrams of lyophilized *C. elegans* were suspended in 1.4 mL ProteoSOLVE Lysis Reagent in each PULSE Tube. Fourteen microliters of water-soluble protease inhibitor cocktail (Sigma, St. Louis, MO, USA) was added and pressure cycling was performed for 10 or 20 cycles. Each cycle consisted of 40 seconds at 35,000 PSI (or 235 MPa), followed by 10 seconds at ambient pressure. Alternatively, 1.4 of the *C. elegans* suspension, 14 μL protease inhibitor cocktail, and 100 mg of 1 mm glass beads (BioSpec, Madison, WI, USA) was added to a standard Eppendorf tube and processed for five 15 second cycles at 2,400 oscillations per minute on a BioSpec Mini-Beadbeater. The sample was placed on ice between cycles. As a negative control, 1.4 mL of the same nematode suspension was incubated 30 minutes at 25º C without mechanical agitation. The lysates were centrifuged 10 minutes at 10,000 RCF to pellet cellular debris. The supernatants were alkylated for two hours following the addition of 40 mM Tris and 200 mM acrylamide monomer. Protein concentrations were determined with the Bradford Reagent (BioRad, Hercules, CA, USA). The supernatants were then ultrafiltered and exchanged into 7M urea, 2M thiourea, and 65 mM CHAPS.

IEF was performed as described by Smejkal et al. [2]. Immobilized pH gradients (IPGs), ProteomIQ Equilibration Reagent, and ProteomIQ Blue Polyacrylamide Gel Stain were from Proteome Systems (Woburn, MA, USA). Following IEF, the IPGs were equilibrated twice for ten minutes in ProteomIQ Equilibration Reagent that was supplemented with an addition 0.5% SDS. Second dimension PAGE was performed on 12% polyacrylamide gels. Gels were stained with the ProteomIQ Blue reagent.

Results and Discussion

ProteoSOLVE, when used in combination with PCT, produced maximal protein yields, PCT performed for 10 and 20 cycles yielded 8.8% and 10.9% more total protein than was derived by bead mill oscillation as determined by Bradford assay (Table 1). The bead mill lysate yielded a similar amount of protein, as the negative control in which there was no mechanical agitation. This implies that *C. elegans* resistance to disruption is perhaps overstated, particularly when reagents that exploit structural characteristics of the cuticle are used to weaken the integument.

Table 1. Protein Isolated from *C. elegans* by PCT and Bead Mill Oscillation

Method	Total protein recovered*
Negative control	6.94
Bead mill	6.91
PCT, 10 cycles	7.52
PCT, 20 cycles	7.66

**mg protein recovered from 50 mg lyophilized C. elegans dry weight.*

References

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