Low Energy Mechanical Shredding: A New Approach for a Wide Range of Sample Preparation Needs

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Abstract

Tough tissues such as muscle and lung generally require extensive mechanical disruption, or chemical or enzymatic treatment of the samples, to recover and adequately analyze their proteome. Mortar and pestle grinding, pulverization in liquid nitrogen or homogenization with a Dounce or rotor-stator homogenizers are some of the classical methods that can be used for the disruption of tissues prior to protein extraction. However, these manual methods are inherently labor-intensive, time consuming and prone to sample-to-sample variability and cross-contamination. In addition, these traditional methods, while effective, can not be easily adapted for use with hard samples such as blood vessels and connective tissue, or for more gentle extraction, as is required for the isolation of intact organelles.
Here we demonstrate that a wide variety of sample types were efficiently disrupted using The PCT Shredder or The Shredder SG3, for diverse applications including proteomic profiling, isolation of nucleic acids and even extraction of intact mitochondria. We applied the methodology to a diverse range of sample types, including elastic tissues such as lung and muscle, tough samples such as leaves, hard samples such as seeds, as well as hard-to-disrupt organisms such as *C. elegans* and arthropods. In some protocols the shredder-disrupted samples were subjected to additional extraction using pressure cycling technology (PCT).
Using this adaptable new methodology, we were able to successfully isolate protein from rat skeletal muscle and human ovarian tumor tissue, as well as from *C. elegans* and dry rice grains. We were also able to obtain good yields of DNA from spinach leaves, apple seeds and whole ticks, as well as RNA from frozen rat lung tissue. In addition, we demonstrate that the Shredder can be used for initial tissue disruption for isolation of intact and functional mitochondria from fresh skeletal muscle, suggesting that brief disruption in the Shredder is compatible with isolation of other intact organelles.

Introduction

The PCT Shredder and the Shredder SG3

The PCT Shredder allows the user to rapidly grind samples directly in Shredder PULSE Tubes. The extraction of proteins, DNA, RNA, lipids, organelles and small molecules from tissues and organisms is often enhanced by the synergistic effects of Shredding and PCT. However, for some samples, Shredding alone provides adequate extraction.
The new SG3 differs from the original PCT Shredder (Figure 1B) by the addition of a three position force setting lever that enables the operator to select and apply reproducible force to the sample during the shredding process and eliminates the need for the operator to press down on the SG3 Driver for long periods when processing multiple samples.

Shredder PULSE Tube Configurations

There are 2 PULSE Tube configurations for the Shredder. The all-plastic design (FT 500-S, Figure 1C) works better with fibrous or sticky samples such as muscle and lung tissue. The FT 500-SM configuration incorporates a perforated metal disk into the PULSE Tube design that improves shredding of hard or brittle samples such as cartilage, seeds and grains.

Pressure Cycling Technology (PCT)

PCT destabilizes molecular interactions by rapidly and repeatedly raising and lowering pressure in the reaction vessel from ambient to high pressures (up to 45,000 psi [310 MPa, 3000 Atm]). At pressures in the 35,000-45,000 psi range, PCT can be used to disrupt cellular structures in order to release proteins, DNA, RNA and other analytes [1, 2]. At lower pressures, e.g. 10,000 - 20,000 psi, PCT can gently lyse cells and release their intracellular contents, including intact organelles [3]. PCT is also used to accelerate enzymatic activity by a wide range of enzymes including trypsin for proteomic studies [4] and Proteinase K for DNA isolation.

Results and Conclusions

We demonstrate convenient extraction of proteins, RNA, DNA and intact mitochondria from a variety of tough-to-disrupt sample types, including muscle (Fig. 7), cartilage (Fig. 6), tumor (Fig. 4) and lung tissues (Fig 10), *C. elegans* (Fig. 3), whole ticks (Fig. 9), as well as leaves (Fig 8) and grains (Fig. 2). Sample disruption in either type of Shredder can be combined with pressure cycling (Fig 5), but can also be used as a standalone technology for rapid single-tube sample grinding.
Sample disruption by shredding is gentle enough for recovery of intact and functional mitochondria, but can also be used for efficient extraction of intact genomic DNA, as well as proteins from a wide variety of sample types. Traditional manual methods for extraction from solid tissues are time consuming and often prone to cross-contamination due to the need to wash and re-use equipment such as mortars and pestles and other non-disposable homogenizers. In addition, most manual methods rely heavily on operator experience and skill to prevent undesirable side-effects like DNA shearing and mitochondrial damage.
The two configurations of the Shredder PULSE Tube (with and without the metal disk) can be used to optimize extraction from different sample types. The all-plastic FT 500-S works best with fibrous and sticky samples, such as muscle tissue. The FT 500-MS with the metal insert works best with hard but non-sticky samples, such as cartilage and plant seeds/grains.

Figure 1C. The FT500-S Shredder PULSE Tube. As the tissue is disrupted, the serrated Ram of the PULSE Tube forces the homogenate through the holes of the Lysis Disk into the upper compartment, significantly reducing the risk of sample over-homogenization, DNA shearing and mitochondrial damage. The **FT 500-MS** has a metal lysis disk to facilitate shredding of hard and brittle samples.

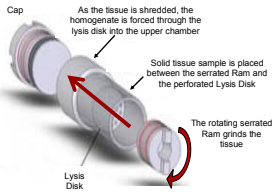


Figure 1A. Shredder ±PCT Workflow

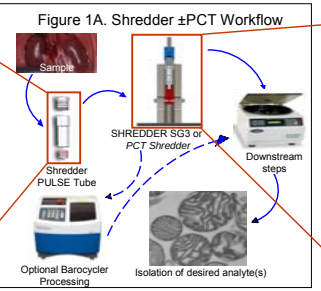
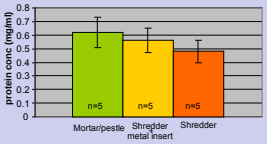


Figure 1B. *The* SHREDDER SG3 and The PCT Shredder

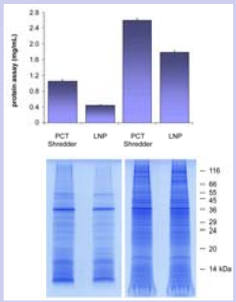
Figure 2. Protein Extraction from Rice Grains



Raw rice was homogenized in PBS. Samples (n=5 per group) were processed in The Shredder SG3 using either all-plastic FT 500-S PULSE Tubes or FT 500-MS PULSE Tubes with the metal disk insert. All shredder samples were shredded for a total of 90 seconds at lever setting #2.

For comparison, control samples were ground with mortar and pestle. Protein concentration was measured by Bradford assay.

Figure 3. Protein Extraction from *C. elegans*



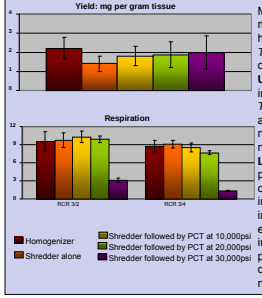
Extraction of protein from *C. elegans* using *The PCT Shredder* and The ProteoSolve-CE Native or The ProteoSolve-CE Stringent Kits (Pressure BioSciences, Inc) results in greater yield of protein than extraction by grinding in iquid Nitrogen with mortar and pestle (LNP). All Shredding was performed in FT 500-S PULSE Tubes. SDS-PAGE was run on 8-16% gels and stained with coomasie.

Figure 4. Protein Extraction from Human Ovarian Metastatic Tumor Tissue

Target Protein	Standard method* yield	Shredding with PCT** yield
FAS Ligand (single transmembrane segment protein)	3±0.5 pg/mg tissue	4.0±0.05 pg/mg tissue
AKT1 (soluble protein)	0.24 ng/mg tissue	0.35 ng/mg tissue

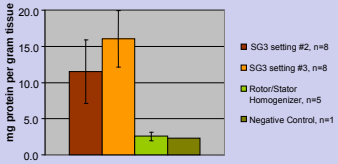
*Standard method extraction was performed by mortar and pestle grinding in liquid nitrogen (LMP).
** Shredding was performed by *The PCT Shredder* in all-plastic FT 500-S PULSE Tubes. Processing was performed as described in the ProteoSolve-TD2 protocol using silicon carbide abrasive, followed by pressure cycling at 35,000 psi.

Figure 5. Isolation of Intact and Functional Skeletal Muscle Mitochondria.



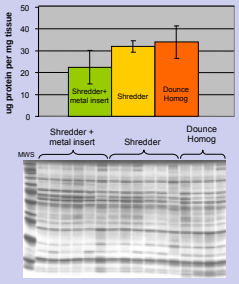
Mitochondria were extracted from rat muscle using a manual Potter-Elvehjem homogenizer method or by shredding in *The PCT Shredder* for 10 seconds with or without subsequent PCT.
Upper Panel: Yield of mitochondria is improved by PCT after disruption with *The PCT Shredder*, with yields that approach the control method (Control, n=15; Shredder alone, n=19; 10,000psi, n=18; 20,000psi, n=3; 30,000psi, n=2).
Lower panel: RCRs of mitochondria prepared using *The PCT Shredder* are comparable to homogenizer control, indicating that the mitochondria are intact and respire normally. Mitochondria exposed to 30,000psi (n=2) are severely impaired, while those treated at 10,000 psi (n=7) and 20,000 psi (n=3) are comparable to controls (homogenizer; n=5, Shredder alone; n=5).

Figure 6. Protein Extraction from Cartilage Tissue



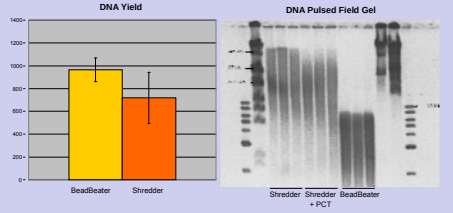
Protein from porcine cartilage (~30 mg per sample) was extracted in ProteoSolve-IEF buffer using the SG3 Shredder in FT 500-MS PULSE Tubes for 40 seconds at lever setting #2 or #3. Control samples were disrupted using a popular rotor-stator homogenizer for 1 minute. The negative control was generated by soaking a piece of cartilage in ProteoSolve-IEF without mechanical disruption. Protein concentration was measured by Bradford assay.

Figure 7. Protein Extraction from Skeletal Muscle



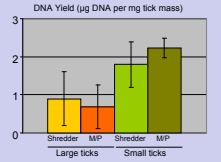
Rat skeletal muscle tissue was homogenized in ProteoSolve-IEF buffer supplemented with protease inhibitor cocktail. Samples (n=6 per group) were processed in The Shredder SG3 using either all-plastic FT 500-S PULSE Tubes or FT 500-MS PULSE Tubes with the metal disk insert. All shredder samples were shredded for 20 seconds at lever setting #2. For comparison, samples were disrupted in a glass/glass Dounce homogenizer by extensive homogenization. Protein concentration was measured by Bradford assay. SDS-PAGE was run on 8-16% gels and stained with coomasie.

Figure 8. Long Genomic DNA Extraction from Spinach



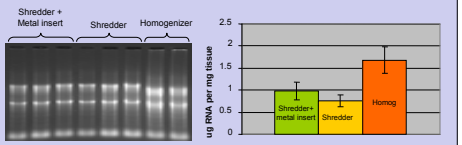
Total DNA was extracted from fresh spinach leaves in DNAzol reagent (Invitrogen). Samples were disrupted either in *The PCT Shredder* (20 seconds) or by BeadBeating (ten 10 second bursts, with chilling on ice to prevent overheating). After initial disruption, some shredded samples were subjected to PCT at 35,000 psi. DNA yield was quantified by Nanodrop and visualized by pulsed field electrophoresis.

Figure 9. DNA Extraction from Whole Ticks



Partially desiccated ticks (wild caught) were separated into two categories; small (<1.5-5 mg) and large (>12-31mg). Ticks (n=3 per group) were either ground to fine paste in a mortar and pestle, or were disrupted in *The* Shredder SG3 by shredding 3 times for 10 seconds (30 sec total) in FT 500-S PULSE Tubes. DNA was purified using a phenol/chloroform protocol with CTAB.

Figure 10. RNA Extraction from Lung Tissue



Total RNA was extracted from frozen rat lung tissue using Trizol Reagent (Invitrogen). Samples (n=6 per group) were processed using either all-plastic FT 500-S PULSE Tubes or FT 500-MS PULSE Tubes with the metal disk insert. Tissue was disrupted by shredding for 20 seconds in *The* Shredder SG3 (lever setting #2) or with a rotor-stator homogenizer for 1 minute (homogenizer tip was rinsed with DEPC-treated water between samples). DNA yield was quantified by NanoDrop and visualized by agarose gel electrophoresis.

References

[1] Gross et al. (2008) Biotechniques 45(1):99-100
[2] Schumacher RT *et al.* (2002). *Am. Laboratory* 34, 38-43.
[3] Gross *et al.* manuscript in preparation
[4] Lopez-Ferrer D., *et al.*, (2008). J. Proteome Res., 7(8):3276-81

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