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Centrifugal Methods and Devices for Rapid In-Gel Digestion of Proteins

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Abstract

Modern proteomic research frequently relies upon separation of proteins in a polyacrylamide gel matrix followed by in-gel enzymatic digestion and extraction of peptides for subsequent analysis by mass spectrometry. In this work, we propose a novel semi-automated method of mechanical processing of gel bands by passing these bands through a specially designed centrifugal device termed a Gel Shredder prior to digestion and extraction of peptides. Such a device allows integrated washing, destaining and shredding of gel bands into uniform blocks of controlled size, approximately 150–300 μm , prior to the enzymatic digestion and extraction of peptides. Shredding into uniform blocks increases the surface area of the gel pieces and promotes improved gel rehydration, allowing the proteolytic enzymes and solvent with improved penetration of the gel lattice. We demonstrate that the new method substantially reduces the time spent on tedious manual handling of gel bands, while minimizing the risk of sample contamination. The performance of the Gel Shredder has been compared to a conventional in-gel digestion protocol using several standard proteins and a complex proteomic sample in terms of relative quantitation by either MALDI-TOF/TOF or nanoLC-ESI ion trap-FTICR mass spectrometry. It is shown that significant time savings and improved peptide recovery can be obtained for many proteins using the Gel Shredder as compared to the traditional in-gel digestion protocol.

Keywords

gel electrophoresis; proteomics; protein in-gel digestion; mass spectrometry; peptide recovery; centrifugal devices

Introduction

Separation of proteins with polyacrylamide gels is one of the most important methods used in proteomic and biochemical studies today. While considerable attention is currently being paid to the development of automated multi-dimensional chromatographic techniques for protein identification and quantitation, a significant step of sample preparation still uses polyacrylamide gel separations. Introduction of the pre-cast two-dimensional gels and immobilized pH gradient media have considerably simplified 2D gel protein separations. Methods based on various combinations of chromatography and one-dimensional gel separations are popular, particularly capitalizing on the fact that SDS-PAGE separation is, perhaps, one of the most efficient ways to remove protein-bound detergents.

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With the wide availability of high-end mass spectrometric instrumentation and the growing popularity of stable isotope labeling techniques, it has become clear that conventional in-gel procedures suffer from both incomplete digestion of proteins and problems related to the recovery of specific peptides out of the gel (1). Previous reports describe attempts to analyze the nature of the losses and eliminate their causes by using optimization of the procedures (2–9), detergents (10,11), organic solvents (12–15), microwave radiation (16), ultrasound (17), or an immobilized enzyme in an on-line (18) or off-line (19) reactor format; however, in comparison to in-solution digestion, the underlying problems still remain, as illustrated, for example, using SILAC isotopic labeling techniques (20).

A common trend, employed by many groups to increase recovery, is to cut the gel bands manually with the razor blade into cubes approximately 1 mm³ in size. However, the risk of protein loss by diffusion from smaller gel pieces during the washing and destaining steps precludes the use of this technique immediately after gel excision. On the other hand, manual cutting of the gel plugs after they have been washed and destained inevitably leads to errors due to loss of gel plugs, practically invisible in their rehydrated state.

A wide variety of robotic gel processors are commercially available. However, such commercial robotic in-gel digestion systems often exceed throughput requirements of many laboratories, frequently requiring a user to work in a 96-well format, regardless of the number of samples being processed. The growing popularity of the Gel-LC-MS workflow reduces throughput requirements even further.

In this study, we have developed a novel approach for moderate throughput in-gel digestion applications. This approach relies on mechanical disintegration of gel bands to small pieces of uniform size by passing destained gel bands through centrifugal device followed by digestion and extraction of peptides. The main purposes of the novel device are to reduce the time necessary for sample preparation and minimize manual handling of the gel bands throughout the in-gel digestion process. Importantly, unlike the in-gel digesting device reported in (19), the Gel Shredder is suitable for processing of larger segments of SDS-PAGE gels typically utilized in Gel-LC MS methods. As part of this study, we compared the performance of the novel device to the traditional methods of in-gel digestion and peptide extraction using mixtures of standard proteins as well as proteins extracted from mouse adipose tissue.

Materials and Methods

Gel Shredder

The Gel Shredder device was constructed from 1.0 mL and 0.6 mL Eppendorf tubes (Thermo Fisher, San Jose, CA). Two angled slots were cut in the bottom of the tubes using an in-house machined holder with an attached razor blade to cut shredding blades in the bottom of Eppendorf tube. The slots were cut in such a way that the tube could still retain aqueous solutions during regular manipulation. When a low centrifugal force ($900 \times g$) was applied, the solution could be removed from the tube while gel pieces were retained. At high centrifugal force ($10,000 \times g$), the slots opened and allowed shredding of the gel. See Fig. 1 and the Results and Discussion Section for more details.

Protein standards

Purified lyophilized protein standards were obtained from Sigma-Aldrich (St. Louis, MO). Stock solutions of equine heart myoglobin, bovine serum albumin, and chicken ovalbumin were prepared in water at 33, 33 and 16 pmol/ μ L, respectively, in a chaotropic sample solubilization solution, containing 7 M urea, 2 M thiourea, 2% CHAPS and 40 mM Tris-HCl (Invitrogen, Carlsbad, CA). The resulting solution was simultaneously reduced and alkylated

by addition of 5 mM tributylphosphine (TBP) and 10 mM acrylamide, both from Sigma, with incubation at room temperature for 90 minutes. The resulting protein standard stock solution was kept at -20°C prior to separation using SDS-PAGE. The partially purified sample of human cannabinoid receptor CB-2 (SwissProt accession P34972) was kindly provided by Dr. N. Zvonok (Center for Drug Discovery, Northeastern University, Boston, MA).

Complex proteomic sample

Samples of Swiss Webster mouse abdominal adipose tissue were obtained from Pel-Freez Biologicals (Rogers, AR). 100 mg aliquots of tissue were extracted using Barocycler NEP3229 (Pressure BioSciences, West Bridgewater, MA) in $2\times$ Laemmli buffer (0.5 M Tris, 3.84 M glycine and 2% SDS), according to a previously published protocol [21]. Subsequently, extracts were reduced and alkylated using TBP/acrylamide [22], desalted and concentrated using 10 MWCO Amicon Ultra 4 ultrafiltration devices (Millipore, Billerica, MA) to a final volume of 50 μL .

Electrophoresis, staining and protein spot excision

Analysis of protein standards—The protein standards were serially diluted to the desired final concentrations with a sample buffer (2% SDS, 20% glycerol, 50 mM DTT, 62 mM Tris-HCl pH 6.8 with bromophenol blue added as a tracking dye) from Invitrogen and loaded onto triplicate lanes of (NuPage 3–8% pre-cast gels, Invitrogen, Carlsbad, CA) at the protein levels per lane ranging from 6.8 to 105 ng per lane. The gels were run under a constant current of 40 mA/gel. The electrophoresis was stopped when the dye front was within 3 mm of the bottom of the gel.

Analysis of mouse adipose tissue—Twenty microliter aliquots of reduced and alkylated murine adipose tissue extracts were separated on Criterion SDS-PAGE 8–16% gradient pre-cast gels (Bio-Rad Laboratories, Hercules, CA). Gels were fixed and stained with either SYPRO Ruby (Invitrogen) or ProteomIQ Blue (Proteome Systems, Woburn, MA) using protocols supplied by the respective vendors. Gel bands were excised from the GeneCatcher spot picking tips (The Gel Company, San Francisco, CA) or a sterile disposable scalpel. To ensure reproducibility of sample processing, each selected band was cut into two identical pieces that were processed using either the Gel Shredder or a standard in-gel digestion procedure.

Washing, destaining and enzymatic digestion

Gel plugs or lanes were washed and destained in the Gel Shredder device using low centrifugal force (ca. $900\times g$) in an Eppendorf 5415 C microcentrifuge to assure complete solvent removal from the device without losing the gel samples. The gel plugs were rinsed once with 100 μL of 50 mM ammonium bicarbonate (ABC) and three times with 100 μL of 50% acetonitrile in 50 mM ABC. The gel plugs were then dehydrated with 100 μL of 100% acetonitrile following each wash cycle. Control gel plugs were washed and destained in regular centrifuge tubes. The gel plugs were subsequently centrifuged at $900\times g$ before solvent removal was performed using gel loading pipette tips. Following the last rehydration in ABC, every duplicate set of gel plugs was gently crushed into a slurry with the clean pipette tip or cut into uniform blocks using high speed centrifugation ($10,000\times g$) through the blades of the Gel Shredder device (Fig. 2). Washed and rehydrated control gel bands were cut into three to four blocks approximately 1mm^3 each using a clean surgical scalpel blade and placed into standard 1 mL centrifuge tubes. Gel samples in both sets of tubes were dried in a SpeedVac (Thermo Fisher) for 5 minutes. Twenty microliters of fresh trypsin solution (12.5 ng/ μL) in 50 mM ABC buffer were added to each tube on ice. Excess enzyme

solution was discarded using the gel loading tips after 10 minutes of rehydration, and the solution was replaced with an equivalent volume of fresh 25 mM ABC buffer, followed by incubation for 12 hours at 36 °C. Tubes were kept in the humidified chamber to prevent sample evaporation during incubation. Digestion was stopped by addition of 10 µL of 1% trifluoroacetic acid (TFA) (in the case of MALDI MS analysis) or 5% formic acid (in the case of LC-MS/MS analysis).

Robotic liquid handling

The compatibility of the gel slurry with automated liquid handling robotic instrumentation was tested using an Xcise proteomic robot (Shimadzu Scientific Instruments, Columbia, MD). Washed, destained and dried aggregated gel slurry clusters produced by the Gel Shredder device and control gel bands manually cut into four pieces were transferred to polypropylene 96-well microtiter plates (Granier, Nürtingen, Germany) and placed on the Xcise platform. Dispensing of trypsin solution into wells, incubation of sealed microtiter plates, peptide extraction, ZipTip µC18 (Millipore, Billerica, MA) clean-up and spotting onto a MALDI-TOF target plate were programmed into the Xcise robot and performed in an unattended fashion. No plugging of ZipTips was observed when the gel slurry was used. Adequate sample spots were produced resulting in good MALDI-TOF/TOF spectra obtained as described below.

Peptide extraction for MALDI-TOF/TOF experiments

Digest supernatants were aspirated with gel-loading tips and stored in a new set of tubes. Peptides remaining in the gel plugs or slurry were extracted with 50 µL of aqueous 50% acetonitrile, containing 0.1% TFA. Gel debris was centrifuged down, and the extracted peptides were aspirated out and combined with the respective original digests. The gel plugs were shrunk by addition of 20 µL of 100% acetonitrile. After centrifugation, the acetonitrile extracts were aspirated out and combined with aqueous peptide solutions from the corresponding samples. The volume of the combined peptide extracts was reduced to roughly 5–10 µL in a SpeedVac, followed by the addition of 0.2% TFA to bring the final volume of the extract to 20 µL in order to compensate for possible loss of TFA in vacuum. Samples were purified by ZipTip and spotted onto a MALDI target for analysis. Standard peptides (angiotensin I, and ACTH fragment 18–39) were added to at the level of 100 fmol following tryptic digestion.

Samples were analyzed on an ABI 4700 MALDI-TOF/TOF (Applied Biosystems, Foster City, CA) mass spectrometer in the reflectron and MS/MS modes using α -cyano hydroxycinnamic acid (CHCA) purchased from Sigma as matrix (7 mg/mL in 50% ACN:water, 0.1% TFA solution). Relative quantitation was performed by averaging the ratios of isotope cluster areas, determined using Data Explorer ver. 4.6 (Applied Biosystems) for each of the several selected tryptic peptides to the average isotope cluster area of exogenous internal standard peptides. Assessment of proteolytic cleavage efficiency was performed by monitoring the number of peptides resulting from missed tryptic cleavages detected in the samples digested under various conditions.

Peptide extraction for LC-MS/MS experiments

Extraction was carried out as outlined above, except 1% formic acid was used instead of TFA to stop the digestion. Also, following the SpeedVac volume reduction, samples were diluted in 0.1% aqueous formic acid to a final volume of 20 µL and subjected to HPLC separation.

The LC-MS system was an Ultimate 3000 nanoflow LC (Dionex, Mountain View, CA) coupled to an LTQ-FT mass spectrometer (Thermo Fisher) equipped with a PicoView ESI

source (New Objective, Woburn, MA). The in-gel digested peptides were injected on a self-packed reversed phase column (10 cm 75 μ m i.d., Magic C18, 3 μ m, 200 Å pore size). The flow rate was set at 200 nL/min for both sample loading and separation. A shallow gradient, using 0.1 % (v/v) formic acid in water (mobile phase A) and 0.1 % (v/v) formic acid in acetonitrile (mobile phase B), was utilized starting at 2 % B and increasing linearly to 40 % B over 65 min, and then to 90 % mobile phase B over 15 min, and finally isocratic at 90% B for 10 min. Before the next sample was injected, the C-18 column was equilibrated at 2 % B for 30 min at 200 nL/min. Before loading, samples were diluted by mobile phase A and clarified by centrifugation at 10,000 $\times$ g for 5 min to protect the column from clogging. Samples were analyzed in duplicate and blank runs were employed to minimize the carryover between injections.

The mass spectrometer was operated in the data-dependent mode, switching automatically between MS and MS². Briefly, survey full-scan MS spectra with 1 microscan (m/z 400–2000) were acquired in the FT-ICR cell with the mass resolution of 100,000 at m/z 400 (with target ion counts at 2×10^6 ions), and then the 9 most abundant ions were isolated as precursor ions for sequential data-dependent MS² scans. For the data-dependent mode, the dynamic exclusion was utilized with 2 repeat counts, repeat duration of 30 s, exclusion list 200, and exclusion duration of 30 s. The normalized collision energy was 28 % for all MS² scans. Peptide hydrophobicity was approximated by their retention times predicted using Sequence Specific Retention Time Calculator (ver. 3.2 beta available at hs2.proteome.ca/SSRCalc/SSRCalc.html).

Peptide identification via database searching

MS spectra collected using the AB4700 instrument were searched against a UniProt database using the publicly available Mascot server. LC-MS files generated by the LTQ-FT instrument were processed using CPAS ver. 1.6 (KeyLab, Seattle, WA) with the Sequest algorithm (ver. 27 rev. 13). The database search was performed against SwissProt (release 52) consisting of normal and reverse sequences to allow estimation of the false discovery rate. The following peptide filtering criteria were used to select significant identifications: PeptideProphet probability >0.95, XCorr values: 1.9 (1+); 2.2 (2+) and 3.7 (3+), estimated false discovery rate of 1.5 %.

Results and Discussion

We have designed and constructed a prototype centrifugal tube device, which allows the simultaneous and reproducible shredding of polyacrylamide gel sections into uniform pieces with a size distribution in the range of 150–300 μ m, as determined by optical microscopy (see Supplemental information for details). We have compared peptide recoveries from shredded and normal SDS-PAGE bands for various applications including direct MALDI MS analysis as well as LC-ESI MS based analysis and demonstrated that uniform shredding simplifies and speeds up sample processing.

Gel Shredder

The Gel Shredder is a disposable container consisting of an insert into Eppendorf centrifugation tubes with a cross cut to form shredding blades upon the application of centrifugal force, see Fig. 1. The Gel Shredder allows washing and destaining of gel plugs *in situ* prior to gel disintegration with centrifugal force. The device is designed in such a way that the gel band or plug remains in the device while it is spun at low centrifugal force, see Fig. 2, enabling exchange of solvents by centrifugation during the wash and destain steps. Surface tension retains solvents and buffers (up to 80% acetonitrile) in the device unless a low centrifugal force (900 $\times$ g) is applied, which purges the solvent through the device into a

receiving container. Fully destained gel plugs are shredded into a slurry by applying high centrifugal force ($10,000 \times g$) by means of the shredding blades. It is presumed that, upon applying high centrifugal force, the slots between blades widens, allowing the gel pieces to be shredded. Importantly, unlike the irregular gel slurry produced by manual methods, the fragments generated by the Gel Shredder do not interfere with the use of ZipTips or robotic liquid handlers due to their uniform and small size. In addition, it has been found that the shredded gel fragments tend to aggregate during extraction with acetonitrile, which allows aspiration of the solution with conventional gel loading tips. As a result, if desirable, a proteomic robotic system, e.g. Xcise, can be used for processing of samples with the Gel Shredder without significant problems due to tip clogging.

The benefits of uniform gel “shredding” following conventional washing and destaining protocols can be illustrated by a comparison with gel plugs that have been “mashed” manually against the sample tube walls using a pipette tip. Mashing typically leads to a broad distribution of gel pieces, which can cause frequent clogging of the pipette tip during aspiration. In addition, reports of using a razor blade to slice the gel bands into blocks of 0.5–1 mm in size have appeared in the literature (19); however, such an approach is not compatible with moderate throughput proteomics. On the contrary, using the Gel Shredder, multiple samples can be simultaneously processed with a single laboratory centrifuge, resulting in a considerable shortening of time in comparison to the manual procedure, a favorable result for a typical proteomic laboratory. The potential for sample contamination during handling is also greatly diminished since the sample never leaves a single-use device until enzymatic digestion is complete and the peptides are extracted.

In summary, the Gel Shredder simplifies and speeds up washing, destaining and crushing of gel bands in a reproducible manner relative to the typical manual protocol. Moreover, multiple samples can be processed in parallel leading to even faster processing. Based on our experience, processing of a single sample starting with a gel section and including destaining, cutting, protein reduction and alkylation takes roughly 50 minutes. In contrast, the manual protocol requires about 3 to 4 hours. Importantly, about 10 samples can be prepared in parallel without significant increase in the processing time leading to a significant time savings.

In-gel digestion performance

The Gel Shredder offers a semi-automated processing of gel plugs. The next question to examine is the effect of Gel Shredder on tryptic peptide recovery in both MALDI-TOF/TOF and LC-MS/MS experiments from the in-gel digests of gel bands. A sample consisting of four standard proteins was separated using SDS-PAGE and processed using 1) the Gel Shredder, 2) a standard in-gel digestion protocol and 3) in-solution digestion. The results of relative quantitation using intensities of selected peptides are shown in Fig. 3. It can be seen that there is a general trend of higher tryptic peptide recovery from the in-gel digests of gel bands, when the gel is subjected to mechanical disintegration using the Gel Shredder. Compared to conventional in-gel digestion, the Gel Shredder improves the peptide recoveries for standard proteins at least two-fold as compared to the conventional in-gel digestion method.

As expected the relative abundance of peptide ions produced using in solution digestion is higher than for Gel Shredder or in-gel digestion protocols partly due to the fact that the model sample contains only very simple mixture of proteins and virtually no contaminants, and thus presents an ideal sample for in solution digestion. However, for membrane proteins as for human cannabinoid receptor CB-2, a member of the GPCR family (Fig. 3D), the peptide recovery from digest performed using the Gel Shredder is approaching the recovery from a solution digest, potentially due to the concentration effect and purification of CB-2

by SDS-PAGE separation. A higher number of peptides and improved sequence coverage is expected when the Gel Shredder is used with a wide variety of proteins from large SDS-PAGE gel sections [22,23].

Proteomic Analysis of Mouse Adipose Tissue using Gel-LC MS Strategy

In order to evaluate the performance of the Gel Shredder for proteomic applications, protein extract from mouse adipose tissue was used as a model sample. The proteins were separated by SDS-PAGE, and the resulting gel lanes cut into three sections, corresponding to high, medium and low molecular weight regions. Each section was split with one half being processed using the Gel Shredder and the other half by conventional in-gel digestion protocol. The extracted peptides were analyzed in duplicate by LC-MS using the LTQ-FT MS instrument.

Table 1 shows the number of unique protein identifications obtained for samples processed by the Gel Shredder and by the conventional in-gel digestion protocol in duplicate runs as well as combined numbers of unique proteins for specific gel sections. The total number of identified proteins is higher in the case of sample processing using the Gel Shredder (340 proteins), where 89 additional proteins were identified, compared to only 55 additional proteins found using the conventional in-gel digestion protocol (306 proteins). It should be noted that the combined numbers of unique identified proteins do not directly correspond to the sum of unique proteins in individual sections due the fact that some proteins were identified in more than one section. In addition, mouse adipose tissue is known to contain relatively low amounts of protein relative to most tissues [24], thus explaining the generally low number of identified proteins. Next, we examined peptides that were identified exclusively using the Gel Shredder or the traditional in-gel digestion protocol. It is expected that peptide extraction efficiency is related to peptide hydrophobicity and that hydrophobic peptides would be more difficult to recover. For simplicity, the peptide hydrophobicity was approximated by peptide RPLC theoretical hydrophobicity predicted using Sequence Specific Retention Time Calculator [25]. It was found that the distribution of predicted retention times of peptides uniquely identified by the Gel Shredder protocol contained more hydrophobic peptides than in the traditional protocol (Kolmogorov-Smirnov test, one tailed p -value=0.035), see Supporting information for more details. This result indicates that the Gel Shredder improved the recovery for hydrophobic peptides as expected due to smaller gel pieces. Table 1 also summarizes the number of unique peptides found for proteins identified in both the Gel Shredder and standard protocol. Out of total 251 proteins that were found using both protocols, in total 95 proteins were identified by greater number of unique peptides using the Gel Shredder compared to only 40 proteins found using conventional protocol. This result indicates that proteins identified by both protocols are identified by more unique peptides when using the Gel Shredder.

Next, the number of additional proteins found in different MW sections were compared, see Table 1. In the case of the high MW section, the number of additional proteins was almost double for the Gel Shredder, suggesting significantly improved recoveries for this gel section. On the other hand, a comparable number of additional proteins were identified in the low MW section, i.e. 21 compared to 17 using conventional in-gel digestion procedure.

In summary, it was concluded that the performance of the Gel Shredder is an improvement over the standard in-gel digestion protocol; however besides providing good peptide recoveries, the Gel Shredder also automates, simplifies and minimizes potential sample losses in the in-gel digestion protocol. First, the selected gel band does not need to be cut manually into small cubes, which is especially advantageous for processing of large gel sections, such as in gel-LC type of proteomic experiments where the entire SDS-PAGE lane is cut into blocks that could be several centimeters long. Second, processing samples in

parallel using a laboratory centrifuge removes a significant amount of time necessary for pipetting of solutions. Third, the processing is accomplished in one closed container, which prevents losses during sample handling. In addition, compared to expensive robotic instrumentation available for in-gel digestion, the Gel Shredder requires only a laboratory centrifuge and provides throughput compatible with requirements of an average proteomic laboratory.

Conclusions

The Gel Shredder has been developed and tested. It was shown that these devices are particularly suitable for processing of large rectangular 1D SDS-PAGE bands but in principle should be also applicable to round 2D-gel plugs typically produced by a variety of proteomic robotic tools. The Gel Shredder appears to be beneficial for hands-free rapid protocol for typical gel LC-MS proteomic experiments.

It has been shown that the benefits of saving time our manual processing of gel bands can be achieved when the tedious steps of washing, destaining and digestion of protein samples in gel bands are performed using the Gel Shredder. The novel devices also help to prevent sample contamination during handling. In addition, it has been shown that peptide recoveries for a simple mixture of standard proteins are higher across a series of proteins with different sizes and hydrophobicity, particularly apparent for a hydrophobic transmembrane protein. The procedure has been also applied to the analysis of an extract of mouse adipose tissue. Improved recoveries were shown. Further enhancement in the performance and reproducibility of Gel Shredder is expected with improved manufacture using, for example, injection molding rather than manual preparation of the Gel Shredder tubes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight mass spectrometry
ES-IT-FTICR	Electrospray Ionization Ion Trap Fourier Transformation Ion Cyclotron Resonance Mass Spectrometry
TFA	trifluoroacetic acid
CHCA	alpha-cyano hydroxycinnamic acid
IEF	isoelectric focusing
SILAC	stable isotope labeling by amino acids in cell culture
MS/MS	tandem mass spectrometry
ES MS/MS	Electrospray Ionization tandem mass spectrometry

DTT	dithiotreitol
ACTH	adrenocorticotrophic hormone
RCF	relative centrifugal force

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Figure 1. Prototype Gel Shredder device

A) Overview of the Gel Shredder. Two angled slots are introduced at the bottom of insert to form 8 sharp shredding blades. B) The bottom of a Gel Shredder device based on 250 μ L microcentrifuge tube (viewed from inside). Note the four leaflets with sharp blades, closed to allow wash steps at low RCF without the loss of gel material.

Figure 2.
A general workflow for sample preparation steps using Gel Shredder.

Figure 3.

Average ratios of peak intensities for four tryptic peaks (as indicated on insets) from each of the model proteins (MYO horse myoglobin, OVA chicken ovalbumin, BSA bovine serum albumin and CB-2 human cannabinoid receptor 2) to intensity of the internal standard peak for traditional in-gel digestion, Gel Shredder and in-solution based protocol. *Absolute protein concentration is not known. Error bars represent standard deviations calculated using 3 measurements.

Figure 4.

Summary of protein identifications from mouse adipose tissue separated by SDS-PAGE to three sections processed using traditional in-gel digestion protocol or using Gel Shredder and analyzed by LC-MS using LTQ instrument.

The number of proteins identified by at least two unique peptides is shown in parenthesis.

Table 1
Summary of protein identifications in three sections of SDS-PAGE gel analyzed by LC MS/MS.

Gel section	Standard protocol ^a			Gel Shredder ^b			Proteins in common ^c	Additional Proteins ^d	
	Run 1	Run 2	Total Number of Unique Proteins ^(e)	Run 1	Run 2	Total Number of Unique Proteins ^e		Std. protocol	Gel Shredder
Low MW	102	105	121	122	125	138	96	25	42
Mid MW	189	175	210	206	208	235	180	30	55
High MW	57	59	75	54	55	71	54	21	17
	Total						251	55	89

Note: Peptide filtering criteria: PeptideProphet >0.9, XCorr values: 1.9 (1+); 2.2 (2+) and 3.7 (3+). Estimated false discovery rate for peptide identifications: 1.5 %

^a Gel sections processed using standard in-gel digestion protocol

^b Gel sections processed using the Gel Shredder

^c Number of proteins found in both ^a and ^b processing

^d Number of protein identified exclusively using either ^a or ^b processing

^e Combined number of unique proteins identified in technical replicates Run 1 and Run 2