Improved Protein Recovery from Rat Pancreas Tissue Using Pressure Cycling Technology (PCT) and the ProteoSolve-SB Kit

A Comparison of The ProteoSolve-SB Kit and Traditional Urea/Thiourea/CHAPS-based Protein Extraction Buffer

Introduction

Pancreatic cancer has a very high mortality rate, primarily due to the fact that it is usually diagnosed at an advanced stage (Ranganathan 2009). Early diagnosis of this devastating disease could be crucial for improving treatment options and survival rates. The pancreas, as the site of insulin secretion, is also intimately linked to diabetes. A better understanding of the normal and diseased pancreatic proteome might give researchers better insights into pancreatic function and development in health and dysfunction in various disease states (Tonack et al., 2009; Chen et al., 2007).

The pancreas is a highly protease-rich tissue. Endogenous pancreatic proteases can rapidly degrade potential biomarkers and other low abundance proteins of interest. The high rate of proteolysis makes it difficult to extract intact pancreatic proteins for tissue proteomic profiling. Here we describe a method for the efficient extraction of proteins from flash-frozen rat pancreas, using *Pressure Cycling Technology* (PCT) and the detergent-free chemistry of the ProteoSolve-SB Kit. The strong denaturing and chaotropic properties of the ProteoSolve-SB extraction reagent rapidly inactivate endogenous proteases. The amphiphilic ProteoSolve-SB reagent also improves extraction of membrane-associated and other poorly soluble proteins, which are not efficiently recovered by conventional methods.

PCT and ProteoSolve-SB

PCT destabilizes molecular interactions by rapidly and repeatedly raising and lowering pressure in the reaction vessel from ambient to high levels (up to 35,000 psi [240 MPa]). High hydrostatic pressure acts preferentially on the more compressible constituents of the sample. Therefore, selective energy distribution results in destabilization of molecular interactions in the lipid bilayers and other cellular components, but not in the disruption of covalent bonds. Several models of PCT instruments and single-use sample processing containers called PULSE Tubes are currently offered by Pressure BioSciences to suit various sample preparation needs (Figure 1). Pressure BioSciences, Inc. has developed a detergent-free sample preparation kit (ProteoSolve-SB) which allows simultaneous isolation of protein and nucleic acids from a variety of tissues (Gross et al., 2008), including lipid-rich samples such as adipose and brain, as well as protease-rich tissues such as pancreas and prostate. This novel method takes advantage of a combination of sample disruption by alternating hydrostatic pressure and a unique reagent system that rapidly denatures proteins and partitions proteins, nucleic acids and lipids into separate fractions.

Figure 1. Barocycler NEP 2320. This instrument generates cycles of hydrostatic pressure up to 35,000 psi (240 MPa) and accommodates one PULSE Tube (1.5 mL) or up to 12 smaller MicroTubes (50-150 µL) at a time. A larger instrument, Barocycler NEP 3229 is available, which accommodates 3 or 48 samples, respectively.

Methods

Pancreas tissue was harvested from 16 week old rats. The tissue was excised, briefly rinsed in PBS to remove surface blood, flash frozen in liquid nitrogen and stored at -80°C.

To minimize variability between groups, each pancreas (n=7) was divided in half and each half was extracted by one of two methods. To minimize proteolysis, only one pancreas was processed at a time. The frozen tissue was cut into two approximately equal pieces, each of which was rapidly weighed (~170 mg each) and placed into a pre-cooled FT 500 PULSE Tube.

For standard detergent-based extractions, 1.3-1.4 mL of ProteoSolve-IEF reagent [7M urea, 2M thiourea, 4% CHAPS (Pressure BioSciences)], supplemented with protease inhibitor cocktail (Sigma-Aldrich) was added to the PULSE Tube.

For extraction with the ProteoSolve-SB kit, 1 mL of Reagent A and 0.3 mL of Reagent B were added to the sample. All samples were vortexed and subjected to pressure cycling in a Barocycler NEP3229 for 30 cycles at ambient temperature. Each cycle consisted of 20 seconds at 35,000 psi followed by 10 seconds at atmospheric pressure.

Following the pressure cycling treatment, samples were processed according to the ProteoSolve-SB kit manual. The extracts were transferred to 2 mL tubes and centrifuged for 10 minutes at 12,000 g to separate the phases. The solubilized proteins (lower solvent phase) were transferred to clean tubes. A 10% aliquot (0.1 mL) of the solubilized protein solution was dried under vacuum to remove the solvent and rehydrated in ProteoSolve-IEF reagent supplemented with protease inhibitors.

Protein recovery was quantified by Bradford Assay. Protein extracts (150µg per gel) were separated by 2D PAGE: first dimension separation was carried out using 11cm, pH 3-10 ReadyStrip IPG strips (Bio-Rad Laboratories, Inc. Hercules, CA). The second dimension was run on 8-16% Bio-Rad Criterion gels.

A set of four 2D gel images (two pancreas halves extracted in ProteoSolve-SB and ProteoSolve-IEF, respectively) were analyzed using RedFin 3 image analysis software (Ludesi, AB). Normalized spot intensity values were plotted to determine the degree of similarity between samples.

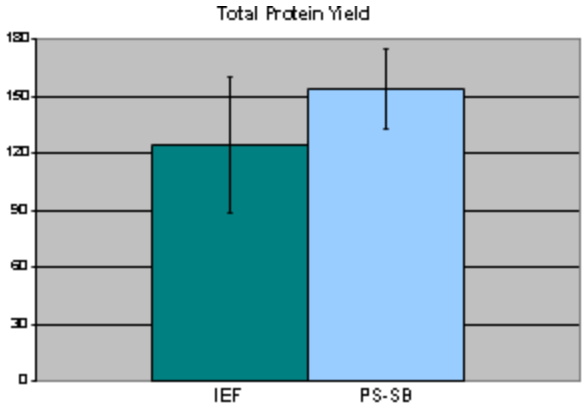

Figure 2. Total Protein Yield. Protein recovery is expressed as micrograms of total protein per milligram pancreas tissue (n=7 for each group). Protein was extracted either in urea/thiourea/CHAPS reagent (IEF), or in ProteoSolve-SB (PS-SB).

Results and Discussion

Many current methods for protein extraction from human and animal pancreatic tissue rely on mortar and pestle grinding in liquid nitrogen to keep the endogenous proteases from degrading the sample (Sang Woo Kim et al., 2008). The combination of PCT and ProteoSolve-SB is an excellent option for extracting proteins from protease-rich tissues such as pancreas or prostate, without the need for grinding in liquid nitrogen or the use of large amounts of protease inhibitors.

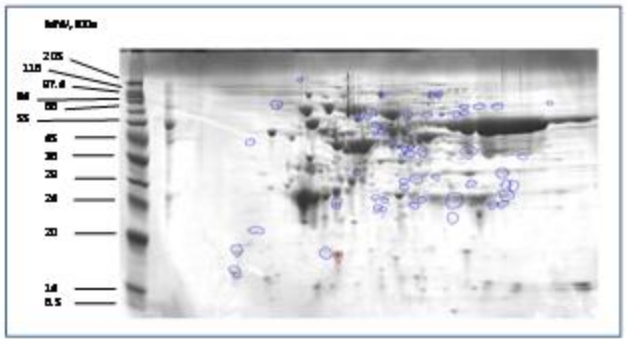

Figure 3. Typical 2D PAGE of pancreatic proteome isolated by pressure cycling and The ProteoSolve-SB kit. Protein spots showing significantly higher recovery in ProteoSolve-SB reagent are highlighted. Some proteolysis (in all samples), is evidenced by the vertical spot smearing and is likely due to tissue handling prior to flash freezing.

Since proteins are rapidly denatured in ProteoSolve-SB reagent, endogenous proteases and other enzymes are rapidly inactivated, leading to better extraction of un-degraded proteins from protease-rich samples such as pancreas. Less spot smearing indicative of proteolysis is observed on the gels extracted in ProteoSolve-SB. In addition, the improved extraction of poorly soluble and/or membrane-associated proteins is made possible by the synergistic action of pressure cycling and unusual chemical properties of the ProteoSolve-SB kit.

However, due to the fact that membrane proteins do not ready separate by isoelectric focusing, 2D-PAGE is not the best method to visualize this enhanced extraction.

Analysis of the 2D gel images detected a total of 457 protein spots, which were matched across all gel images (Figure 4). Of these, only 39 (ca. 8.5%) were found to have significantly (2-fold or more) higher or lower intensity in samples extracted either in IEF or

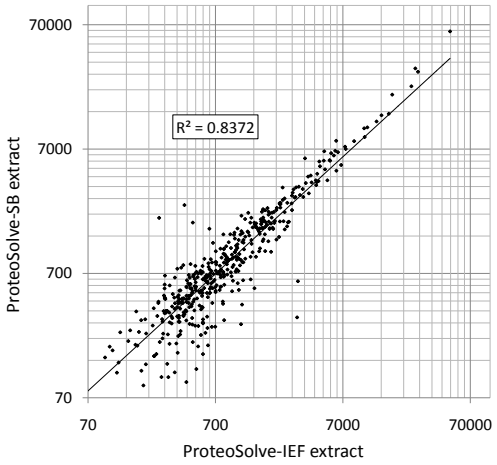

Figure 4. Comparison of average spot intensities for samples extracted by PCT using two reagent systems.

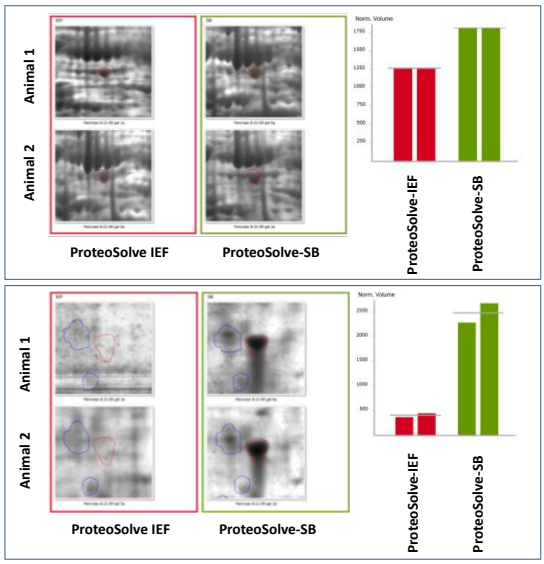

Figure 5. Examples of enhanced recovery of several proteins by the ProteoSolve-SB reagent kit.

ProteoSolve-SB. There was a stronger correlation between the extraction replicates (pancreatic samples from different animals extracted using the same method) than between the samples from the same animal extracted by the two different methods, indicating that both solvent systems reproducibly extract proteins under PCT conditions, while ProteoSolve-SB presents additional advantages such as simplified sample clean-up in the detergent-free reagent, better sample preservation and the ability to recover proteins, lipids and nucleic acids from a single sample.

These results demonstrate that protein extraction by PCT in ProteoSolve-SB is an excellent method for preparation of protein from highly protease-rich samples such as pancreas tissue. In addition, the improved extraction in ProteoSolve-SB may result in the identification of additional proteins that might be missed in samples extracted by more traditional methods (Figure 5).

References

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