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Pressure Cycling Technology (PCT): a Novel Sample Preparation Approach to Biomarker Discovery and Drug Development in Lipid-Rich Samples

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The United States is in the midst of an obesity epidemic, with levels rising to 33% percent in recent years (1). Obesity is directly associated with an increased risk for a number of serious health conditions, including heart disease, stroke, high blood pressure, cancer, and diabetes. The Centers for Disease Control recently released reports showing that heart disease remains the number one cause of death, affecting over 43% of Americans each year (2). In 2006, the U.S Department of Health and Human Services distributed over two billion dollars for research in diabetes, obesity, and heart disease. Diabetes is also climbing the charts as a global problem, killing millions of people annually worldwide. As the scientific community accumulates more knowledge about these diseases, applied research has become increasingly focused on the underlying cellular and molecular mechanisms thought to be involved in the onset, progression, and treatment of these disorders. While researchers postulate relationships among obesity, obesity-associated diabetes, and heart disease, the interrelationships are complex, and current treatments remain marginally effective against these diseases.

Worldwide pharmaceutical and clinical research organizations are constantly searching for efficient, innovative technologies to accelerate drug discovery, shorten the development cycle, and resolve compounding problems associated with increasingly complex biomedical research. For instance, years of biomarker discovery research has emphasized the need to interrogate tissues in order to understand the origin and nature of potentially relevant proteins that may be present in circulating blood, but at concentrations below the detection limits of current proteomics methods. However, tissue proteomics faces a fundamental issue – the samples are no longer in solution. While considerable investment has been made in the development of high-end analytical instrumentation, frequently forgotten is the success seen in studies of cellular and tissue mechanisms, which begin with sample preparation to release and isolate molecules of interest - such as proteins, nucleic acids, and small molecule metabolites. For decades, researches have relied on manual grinding methods, such as mortar and pestle or Dounce homogenization, or other methods, such as ultrasonic cavitation or French press extrusion, to release cellular constituents. Emerging new methods in sample preparation have recently begun to capture the attention of the traditionally conservative biotechnology and pharmaceutical industries. While a majority of analytical instruments require extracted solutions of biomolecules as an input, most techniques used to release cellular components are inefficient and have other limitations, such as safety issues, potential for contamination, lengthy processing time, and lack of automation. These inefficiencies engender poor reproducibility or limited applicability to just a narrow range of analytes or sample types. Thus, any state-of-the-art downstream analytical method is only as good as upstream processes such as sample preparation.

In particular, protein expression from lipid-rich tissue holds the key to a fundamental understanding of many disease states such as obesity, heart disease, and diabetes. Unfortunately, widespread study of adipose tissue proteomics is hindered by several technical challenges. For example, reliance on detergent-based methods for protein extraction produces disappointing yields that often fail to uncover the breadth of adipocyte proteome in downstream applications. Moreover, the strong detergents used to solubilize high amounts of lipid frequently interfere with downstream analytical methods, such as gel electrophoresis, liquid chromatography, or mass spectrometry. Consequently, the lack of efficient technologies currently available for optimization in protein extraction hinders the understanding of numerous physiological processes that could foster new therapeutic discoveries in the coming years. Researchers from Pressure BioSciences, Inc (PBI) and their colleagues have begun to investigate the

application of a new sample preparation technology for tissue and cell disruption and isolation of proteins from lipid rich tissues. Lipid-rich tissues, such as brown and white adipose tissue, are believed to contain many yet uncharacterized proteins or peptides, particularly those of a hydrophobic nature, or those that are typically localized in plasma membrane and organelles. Such proteins may play crucial roles in regulation of metabolic and secretory activity of the adipose tissue — processes that are essential to our understanding of obesity, heart disease, and diabetes. Once identified and characterized, these proteins may serve as crucial diagnostic and prognostic markers.

Proteomic analysis of lipid-rich tissues can be challenging, due to the fact that proteins, which are bound to lipids by hydrophobic interactions, are frequently lost during the sample preparation steps. Growing demand for better sample preparation prompted several Life Sciences companies to offer a variety of reagent-based sample preparation methods for cell lysis and for the isolation of proteins from complex tissue samples. One of the most common methods to break up lipid-protein interactions incorporates the use of detergents. However, if the lipid content of the sample exceeds 60%, some lipid-protein complexes may be entirely insoluble in the aqueous medium. Subsequently, some complexes are discarded with a pellet as so-called "cell debris"; thus, relevant proteins may not move into the polyacrylamide matrix or end up binding irreversibly to the chromatographic materials. Due to the overwhelming amount of lipid in the sample, detergent-based extraction buffers frequently produce poor protein yields, while many hydrophobic membrane proteins are sequestered into the micelles and escape separation and detection in downstream analysis applications.

As an alternative to traditional detergent-based methods for protein extraction, Pressure BioSciences has developed a novel method that combines pressure cycling technology (PCT) and specific reagents to extract and fractionate proteins from lipid-rich samples without the need for detergents. This new sample preparation approach, used in combination with organic solvents, disrupts cells and tissues and partitions the extracted proteins from the lipids in a single step, thus permitting lipid and protein fractions originated from each sample to be analyzed individually, in a more rapid and efficient manner than conventional methods allow.

Liquid-liquid extraction methods have been known to chemists for many decades, but they have yet to become standard practice in the proteomic workflow. This is due to several limiting factors, which include (a) the lack of available automated processes, and (b) the variable extraction efficiencies of the diverse protein complement of tissues. This new method of tissue delipidation utilizes the PCT Sample Preparation System (PCT SPS) and the recently released extraction kit ProteoSolveLRS. It is the first automated, reproducible, detergent-free method that has been optimized for protein recovery. This novel method uses alternating hydrostatic pressures ranging between ambient and 240 megapascal (MPa) to modulate mutual solubility of various organic solvents in one another and to promote improved solubilization of sample constituents in these solvents. Tissue samples and appropriate solvents are placed into disposable polypropylene containers called PULSE Tubes™ (Pressure Used to Lyse Samples for Extraction). The PULSE Tubes™ are specially designed to freely transmit hydrostatic pressure to the sample, while keeping the samples fully enclosed to eliminate cross-contamination and to minimize operator exposure to biohazardous materials (3). Pressure is generated in a Barocycler instrument, either the NEP3229, which accepts up to three PULSE Tubes at a time, or the smaller NEP2320, which processes one sample per run. During exposure to cycles of high hydrostatic pressure and rapid de-pressurization, cells are ruptured and dissolved in a solvent mixture, becoming nearly homogenous under pressure. Subsequently, immiscible solvent phases separate, thus separating the sample constituents according to their inherent affinity to either of the solvent phases.

In a recent study conducted by Pressure BioSciences in collaboration with researchers from the Harvard School of Public Health, comparative proteomic analysis of adipose tissue, isolated from several genetically different model animal lines, has revealed significant differences in protein expression that may be useful in mechanistic studies of obesity and an associated disorder such as Type 2 diabetes. Efficiency of protein extraction, both quantitatively and qualitatively, was determined by SDS-PAGE and 2DGE, followed by protein identification with liquid chromatography and high resolution electrospray ionization tandem mass spectrometry. Protein recovery by the PCT/ProteoSolveLRS method was compared to conventional homogenization and solubilization techniques, confirming higher yields in the PCT/ProteoSolveLRS processed sample fractions. The findings of this study where presented at the 55th ASMS Conference on Mass Spectrometry and Allied Topics, held June 3-7, 2007 in Indianapolis, IN (4).

The advantages of this novel, detergent-free protein extraction method for lipid-rich samples is that it allows proteins to be solubilized in high yield, separated, fractionated, and then directly analyzed by high-pressure liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ES-MS). This approach enhances the ability to characterize high molecular weight hydrophobic proteins that are typically lost into detergent-lipid micelles, when conventional protein extraction buffers are employed. In addition, the PCT/ProteoSolveLRS method is a reproducible sample preparation procedure, which minimizes technical variation and accentuates biologically relevant differences. Furthermore, the protein extracts obtained using this novel method may also be used for separation using SDS-PAGE or two-dimensional gel electrophoresis, followed by Western blotting or in-gel tryptic digestion and protein identification by mass spectrometry. By streamlining the sample preparation process and improving recovery of novel proteins, the new method holds promise to accelerate the process of biomarker discovery and drug development.

Applications of novel protein extraction technologies are not limited to studies of adipose tissue. Researchers from Pressure BioSciences are currently working to broaden the scope of pressure-driven liquid-liquid extraction in biological sample preparation. Apart from cardiovascular and metabolic syndromes, some studies of prevalent neurological conditions might also benefit from using the PCT/ProteoSolveLRS sample preparation method due its effectiveness in processing the high lipid content in these tissue types.

In addition to funding research in obesity, heart disease, and diabetes, the NIH also supports a number of research activities in the area of neurodegenerative diseases. This research largely targets proteins thought to be involved in a number of brain disease states. PCT, in combination with detergent-free extraction, may also prove to be effective in comprehensive protein extraction from brain tissue samples. If successful in this area, such research may lead to a better understanding of Alzheimer's and Parkinson's diseases, as well as Multiple Sclerosis, since such conditions appear to exhibit changes in both the protein and lipid content of the brain. For example, myelin - the protective sheath that covers neurons - is comprised of 30% protein and as much as 70% lipid material. Phospholipids and membrane proteins also play a crucial role in the regulation of neuronal activity. Consequently, the molecular basis for mental disorders, such as hyperactivity, depression, and schizophrenia, may be better understood, if tools that improve the characterization of the lipid and protein composition of the brain are developed.

It is clear that the emerging field of proteomics demands new and improved sample preparation technologies capable of generating rapid, safe, and reproducible results. Unfortunately, many proteomic publications give too cursory an overview to the preparatory steps of analysis. The efficient release of proteins from cells and tissues is a critically important first step to discovery, since the quantity and quality of extracted material can profoundly affect the success of downstream applications. To this end, novel sample preparation techniques, such as PCT, need to continue to be developed to help ensure that research in the areas of obesity and its many related diseases progresses with speed, increased knowledge, and success.

References

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